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H. Philip Fortwengler

Univ. of Louisville Sch. Med

Louisville, Kentucky Zip 40201

Telephone no.: Area Code 502 # 588-5251

LYMPHOCYTE TRANSFORMATION TESTS IN VINYL CHLORIDE (VC) WORKERS.

H. Philip Fortwengler*, Michael E. Dever*, Carlo H. Tamburro*, and Enrique Espinosa, Univ. of Louisville School of Medicine, Louisville, KY 40201

Previous reports have shown circulating immune complexes in VC workers and a tumor-associated antigen in VC-related liver angiosarcoma. This suggests immune stimulation by a tissue or plasma antigen induced by or conjugated with VC or a metabolite. In this work, the in vitro lymphocyte reactivity for such antigens was tested in 79 VC workers including 25 with liver abnormalities, and 20 normal individuals having no exposure to VC. The liver angiosarcoma antigen preparation included the tumor-associated antigen and other tissue antigens as shown by immunodiffusion. Stimulation indices (SI) were calculated from cellular incorporation of tritiated thymidine. Mean SI in the normal individuals for antigens of angiosarcoma and normal liver tissues were 4.7 (SE±1.7) and 3.8 (±0.9) and in VC workers, 1.8 (±0.2) and 2.5 (±0.3) respectively. Mean SI for PHA and Con A in the normal individuals were 235 (±35) and 209 (±30) and in VC workers 201 (±23) and 180 (±19) respectively. Thus, these results suggest that VC workers have a decreased lymphocyte response to antigens of liver angiosarcoma and normal liver tissues rather than the hypothesized increased reactivity. This appears to be due to a lower overall lymphocyte responsiveness in these chemical workers.
(Supported in part by the Manufacturing Chemists Association)

All compounds that are designated by code or initial letters must be identified adequately in the abstract, e.g., MJ-1999: 4-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide hydrochloride.

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FACTOR VIII CONTENT AS EVIDENCE FOR ENDOTHELIAL ORIGIN OF VINYL CHLORIDE ASSOCIATED LIVER ANGIOSARCOMA (VCA). H. Philip Fortwengler*, Douglas Jones*, Carlo H. Tamburro* and Enrique Espinosa (SPON: G. Randolph Schrodt). Univ. of Louisville School of Medicine, Louisville, KY. 40232

To ascertain the endothelial cell origin of VCA we have looked for Factor VIII in the tumor since this factor appears to be specific for such cells (Hoyer et al., 1973). Frozen sections of three VCA and one idiopathic case were examined for presence of Factor VIII by indirect immunofluorescence. Sections of angiosarcoma demonstrated a strikingly increased specific fluorescence which lined enlarged sinusoids. This intense fluorescence was easily seen on low power(100x) as an irregular or splotchy pattern. Occasional striations of linear fluorescence which did not follow hepatic cords were also present. A similar pattern of staining was also given by the idiopathic angiosarcoma but was never seen in sinusoids of normal liver. These observations were further supported by the finding that absorption of Factor VIII antiserum with experimental angiosarcoma tissue resulted in complete inhibition of immunofluorescence. These findings demonstrate that VCA and idiopathic angiosarcoma include proliferating cells containing Factor VIII and therefore strongly suppor an endothelial cell origin of this tumor. (Supported in part by the Manufacturing Chemists Association.)

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USE OF DYE CLEARANCE IN DETECTION OF HEPATOCELLULAR INJURY AMONG VINYL CHLORIDE WORKERS. P. Fortwengler* and C. H. Tamburro. Department of Medicine, Digestive Diseases & Nutrition Section, University of Louisville, School of Medicine, Louisville, Kentucky.

Serious hepatic injury associated with exposure to potentially harmful industrial chemicals and the need for a sensitive method of early detection has been previously noted among vinyl chloride workers. (Creech, J. L., et al, Gastroent., 67:786, 1974). Forty-three of the 1,183 employees of this polyvinyl chloride production plant with biochemical, radioisotopic, and histological evidence of liver dysfunction were evaluated using indocyanine green (ICG) dye clearance, at both the 0.5 mg/Kg and 5.0 mg/Kg dose. Percentage disappearance rates (PDR) were determined by both blood and ear densitometry with automatic computer calculation of PDR. Liver histology was normal in 9, minor changes in 10, fat and minimal fibrosis in 14, portal fibrosis in 4, fibrosis and lobular distortion in 2, and angiosarcoma in 4. Individual biochemical tests correctly indicated hepatic status in 80-83% of the cases with 8% false positives and 10-15% false negatives. Radioisotopic studies alone correctly indicated liver status in only 55%, with 45% either false positive or false negative. The 0.5 mg/Kg dose of ICG correctly indicated liver status in 80% with no false positives, but failed to indicate liver dysfunction in 20%. However, at the 5.0 mg/Kg dose, all cases of liver dysfunction were detected. Ear densitometry values were essentially the same as blood values. Ear densitometry with electronic calculator allows a dye clearance determination over 10 minutes without drawing blood. This data demonstrates an effective means for early detection of industrial chemical hepatotoxicity.

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DECREASED GLUCOSE-6-PHOSPHATASE ACTIVITY IN LIVER IN VINYL CHLORIDE EXPOSED RATS. J.T. Du* and C.H. Tamburro* (SPON: M. Fonda) Dig. Dis. & Nutr. Sect., Dept. Med., Cancer Center, Univ. of Louisville Med. Sch., Lou., Ky. 40201.

Increases in key glycolytic enzymes paralleling hepatoma tumor growth (Heinrich, et al., FEBS Letters, 42:145, 1974) and decreases in key gluconeogenic enzymes prior to and with the development of hepatomas (Isok, et al., Voprosy. Med. Khim 19:568, 1973) have been shown. We exposed adult Sprague-Dawley rats to 10,000-20,000 ppm of vinyl chloride (VC), 4-8 hrs./day, 5 days/wk. for 3-4 wks. (40-140 hrs. exposure) to induce liver injury and angiosarcoma formation. Glucose-6-phosphatase, a key gluconeogenic enzyme in the liver microsomal fraction, decreased 25% over control ($P<0.05$ from combined data). Other microsomal proteins and enzymes related to VC metabolism, i.e., P450, NADPH-cytochrome c reductase and mixed function oxidase were unchanged in the same microsomal fraction with no differences in either mitochondrial cytochrome oxidase or blood transaminases. The decrease in glucose-6-phosphatase is similar to the lower gluconeogenic enzyme findings in hepatomas. This may reflect an increase in glycolysis and ribose-5-phosphate production associated with de novo purine biosynthesis prior to tumor development, inhibition in enzyme synthesis, or increased breakdown due to VC exposure. Work is under way to determine the mechanism. The decrease in glucose-6-phosphatase may be an early biochemical lesion usable as an indicator of liver injury associated with the subsequent development of angiosarcoma. (B.F.Goodrich Grant)

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ELEVATED GLUTATHIONE CONTENT, GLUTATHIONE-S-TRANSFERASE AND GLUTATHIONE REDUCTASE IN LIVER OF RATS EXPOSED TO VINYL CHLORIDE Du, J.T.* and Tamburro, C.H.* (SPON: McGeachin, R. L.) Dig. Dis. & Nutr. Sect., Dept. Med., Cancer Center, Univ. Lou. Med. Sch., Lou., Ky., 40232

Vinyl chloride (VC) is believed to be metabolized to chloroethylene oxide (CEO) and chloroacetaldehyde, and detoxified by way of glutathione. Rats were exposed to 28,000 ppm VC, 7 hrs/day, 5 days/wk for 4 and 6 weeks, the activity of glutathione epoxide-S-transferase (GEST) was elevated 30 to 54% over normal control and air control (9.71± 0.68 vs 7.52± 0.97 and 6.30± 0.68) respectively. However, the activity of glutathione aralkyl-S-transferase (GAST) was not significantly elevated until 6 weeks of exposure to VC. The content of reduced glutathione was also elevated 45% in the VC treated group and the activity of the glutathione reductase, the enzyme to regenerate glutathione from the oxidized form was elevated 50%. These results demonstrate that VC exposed rats have the capacity to maintain glutathione reductase activity and glutathione concentration for detoxification. Further, it suggests that the primary route of VC metabolism is initial oxidation to CEO and then detoxification by GEST directly. With longer exposure and probable saturation of the direct route, there is greater rearrangement of CEO to chloroacetaldehyde, and detoxification with glutathione as supported by the delayed induction of GAST (Supported by a grant from Manufacturing Chemists Association).

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McGeachin, R. L.
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Serum Bile Acids (SBA) Screening For Chemical Hepatotoxicity. Gary Liss* and Carlo H. Tamburro* (SPON: William Waddell). NIOSH Robert Taft Laboratory, Cincin., OH. & Liver Research Center, Div. Occupational Health, Depts. of Medicine & Community Health, Univ. of Louisville, Lou., Ky. 40292.

Standard liver tests have limited ability to detect latent liver disease (Environ. Health Perspect. 1981; 41:117-112). Serum bile acids are more sensitive indicators of liver injury. During the medical surveillance of 1000 chemical workers 67 liver biopsies were investigated for histological evidence of chemical injury (Gastroenterology 1979; 77:A33); 15 had chemical liver disease (CLD), 27 nonchemical liver disease (NCLD) & 25 had normal biopsies (NBX). Fasting SBA-cholylglycine (CG) & conjugates of cholic acid (CCA) were studied by ^{125}I-radioimmunoassay in these groups and 416 "normal" workers. Mean ± S.E.M. for CG in the CLD, NCLD, NBX & normal groups were 95.17 ± 28.75, 27.25 ± 4.43, 34.60 ± 7.13 & 14.9 ± 0.88 ug/ml, respectively. Analysis of variance showed significant differences ($p<0.035$) in the 3 biopsy groups; $p<0.001$ for all 4 groups. The CLD values were significantly different in CG levels of the NBX ($p<0.02$). No significant difference in CG levels in NCLD and NBS groups; CCA showed similar results. Fasting SBA showed promise in detecting latent chemical liver injury.

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Serum Bile Acids (SBA) Screening For Chemical Hepatotoxicity. Gary Liss* and Carlo H. Tamburro* (SPON: William Waddell). NIOSH Robert Taft Laboratory, Cincin., OH. & Liver Research Center, Div. Occupational Health, Depts. of Medicine & Community Health, Univ. of Louisville, Lou., Ky. 40292.

Standard liver tests have limited ability to detect latent liver disease (Environ. Health Perspect. 1981; 41:117-122). Serum bile acids are more sensitive indicators of liver injury. During the medical surveillance of 1000 chemical workers 67 liver biopsies were investigated for histological evidence of chemical injury (Gastroenterology 1979; 77:A33); 15 had chemical liver disease (CLD), 27 nonchemical liver disease (NCLD) & 25 had normal biopsies (NBX). Fasting SBA-cholylglycine (CG) & conjugates of cholic acid (CCA) were studied by ^{125}I-radioimmunoassay in these groups and 416 "normal" workers. Mean ± S.E.M. for CG in the CLD, NCLD, NBX & normal groups were 95.17 ± 28.75, 27.25 ± 4.43, 34.60 ± 7.13 & 14.9 ± 0.88 ug/ml, respectively. Analysis of variance showed significant differences ($p<0.035$) in the 3 biopsy groups; $p<0.001$ for all 4 groups. The CLD values were significantly different in CG levels of the NBX ($p<0.02$). No significant difference in CG levels in NCLD and NBS groups; CCA showed similar results. Fasting SBA showed promise in detecting latent chemical liver injury.

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TISSUE AND URINARY GLYCOSAMINOGLYCANS (GAG) CHANGES IN HEPATIC FIBROSIS C. E. Kupchella, J. O. Jarvis, K. L. Curran, R. A. Greenberg, and C. H. Tamburro Cancer Center and Digestive Diseases and Nutrition Section, University of Louisville School of Medicine, Louisville, Kentucky.

GAG's are felt to play an important role in the collagen fibril formation and collagen bundle stabilization. It has been assumed that hepatic fibrogenesis contributes little to the total body connective tissue and would not be reflected in urinary excretion of GAG degradation or synthetic products. We have previously found, however, that increased urinary GAG's occur in humans with cirrhosis, hepatitis, and hepatic angiosarcoma (Kupchella, C.E., Tamburro, C. H., Clin. Res. 25:329, 1977; Curran, K. L., Kupchella, C. E., Tamburro, C. H., Cancer (in press), 1977). In the present study, changes in urinary and hepatic GAG's were measured in rats with CCL_4-induced hepatic damage. Highly significant increases ($P < .001$) in total GAG's and in the hyaluronic acid (HA), chrondrotin sulfate (CS), and the heparin (H) fractions were found in hepatic tissue after three weeks and persisted through nine weeks of exposure. Histological examination after three weeks showed hepatic necrosis with extensive fatty metamorphosis without histochemical (Trichrome-Alcian Blue-PAS) evidence of fibrosis. Control hepatic tissue GAG levels were 31 ± 5 µg (of uronic acid) per gram of dry defatted liver. CCL_4 treated livers showed a 2 to 4 fold increase in GAG levels; 81.4 µg at 3; 98 µg at 6; and 113 µg/g at nine weeks, respectively. Increases occurred mainly in the CS and H fractions. The urinary CS fraction was significantly ($P < .05$) elevated over the nine weeks of exposure; the HA fraction was elevated during the first two weeks only, and the H fraction excretion appeared to be directly related to CCL_4 injections. These data support our previous observations in humans and demonstrate that urinary GAG excretion pattern changes occur before histochemical evidence of collagen formation and suggest that urinary GAG patterns may be a useful indicator of early hepatic fibrogenesis.

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URINARY CHONDROITIN SULFATE FRACTION PATTERNS IN HEPATIC ANGIOSARCC

C. E. Kupchella, K. L. Curran, and C. H. Tamburro. Cancer Center, University of Louisville, Louisville, Kentucky.

This study was undertaken to evaluate the usefulness of urinary glycosaminoglycan patterns in the detection of hepatic angiosarcoma and in monitoring the course of this disease. Glycosaminoglycans e tracted from 24 hr urines from 2 patients with angiosarcoma of the liver and from normal controls were separated as cetylpyridinium cc plexes into "hyaluronic acid," "chondroitin sulfate," and "heparin' fractions. These fractions were further purified by anion-exchange chromatography. The "chondroitin sulfate" fraction isolated fro angiosarcoma patients consistently demonstrated an increase in the amount of glycosaminoglycan eluted in 1.25 M NaCl and a concomitant decrease in the 1.5 M NaCl elution peak. This "shift" was apparent related to the course of the disease and was unaffected by chemotherapy. As indicated by the following results, the ratio of 1.25 to 1.5 M elution peaks may be of value in monitoring the course of angiosarcoma: 2 normal controls, .364 and .316; angiosarcoma (prechemotherapy), .843; angiosarcoma (post-chemotherapy), .971; angiosarcoma (advanced), 5.00. Characterization of the 1.5 M and 1.25 M NaCl elution peaks by hyaluronidase susceptibility and comparison w elution patterns reported in the literature suggest that the observe shift was from chondroitin 4 and/or chondroitin 6 sulfate to heparar sulfate. Preliminary data indicate that the excretion pattern in vinyl-chloride-associated liver injury other than angiosarcoma is characteristically different from patterns associated with hepatiti cirrhosis, or metastases to the liver. These observations may be related to progressive connective tissue proliferation in angiosarc

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URINARY GLYCOSAMINOGLYCAN EXCRETION PATTERNS IN CHEMICALLY INDUCED LIVER INJURY AND CANCER C. E. Kupchella*and C. H. Tamburro**, Cancer Center, University of Louisville, Louisville, Kentucky.

Glycosaminoglycans (GAG) are essential compounds of connective tissue (CT) matrix and are increased with CT proliferation. Urinary GAG patterns were studied in 9 individuals with vinyl chloride (VC) induced chemical injury, 2 VC induced angiosarcomas, 8 viral hepatitis, 6 alcoholic cirrhosis, 7 non-hepatic cancers, and 9 controls. Hepatic histological and electron microscopic (EM) studies were obtained in all but normal controls which were studied biochemically, radioisotopically, and physically. Urines were analyzed for creatinine, total GAG, and uronic acid content. GAG levels (ug uronic acid/mg creatinine) in controls were 3.2 $\pm$ .4, in VC exposed 4.1 $\pm$ 4, and in alcoholic liver disease 5.1 $\pm$ 0.8. In contrast, in angiosarcoma they were 7.6 $\pm$ 1.6 and hepatic metastasis, 13.8 $\pm$ .9. Total GAG's were further separated into hyaluronic acid (HA), chondroitin sulfate (CS) and heparin (H) fractions. Although no significant differences were found in total GAG's 78% (7/9) of VC exposed had positive CS and negative HA and H fractions in contrast to 9% (3/32) of the other cases. Further characterization by anion-exchange chromatography showed a shift in the CS fraction composition. Total GAG's eluted at 1.25 M NaCl, were increased and at 1.50 M NaCl decreased. These changes became more pronounced as the disease developed. Enzyme digestion indicates that 1.25 M fraction is heparan sulfate (HS). EM studies showed increased sinusoidal collagen. Since HS is associated with blood vessels, its increased urinary excretion in early VC injury and angiosarcoma (vascular injury and sinusoida cell cancer) may be used as an early indicator of chemical injury and liver cancer formation.

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TISSUE AND URINARY GLYCOSAMINGLYCANS IN TRANSPLANTABLE HEPATOMAS. C. E. Kupchella, K. L. Curran, E. Drake, J. Kennedy, and C. H. Tamburro. Cancer Center, and Division of Digestive Diseases and Nutrition, University of Louisville, School of Medicine, Louisville, Kentucky.

The purpose of this investigation was to evaluate: a) the glycosaminoglycans (GAGs) in different behavioral/histological types of intermuscularly transplanted hepatomas, b) GAG patterns in tumor tissue in relationship to degrees of fibrosis and necrosis, c) the GAG changes in t livers of tumor-bearing animals, and d) urinary GAG excretion as a function of tumor growth. Three types of Morris hepatomas, 7777, 5123tc, and 9618A, which differ i rates of growth, metastatic potential, fibrosis, and necrosis, were studied. Urinary and tissue GAGs were extracted as cetylpyridinium complexes and measured as uron acid. Tissue GAGs were also evaluated histochemically using alcian blue staining with and without enzyme pretreatment. Tumor tissue exhibited four- to six-fold greater GAG levels in the hyaluronic acid (90 ± 10, 91 ± 12, and 111 ± 9 vs. 25 ± 3 μg uronic acid/g dry liver, respectively) and chondroitin sulfate (261 ± 29, 217 ± 51, and 208 ± 22 vs. 47 ± 7 μg uronic acid/g, respectively) fractions than normal liver; the heparin fractions did not differ significantly (31 ± 7, 17 ± 4 and 67 ± 11 vs. 39 10). The livers of tumor-bearing animals exhibited slightly greater hyaluronic acid levels than normal livers Moreover, increased urinary GAG excretion was evident afte two weeks in animals bearing fast-growing tumors. The GAC tissue levels in fast vs. slow-growing tumors were not sig nificantly different. This further supports our previousl reported studies of urinary GAG excretion in human hepatic angiosarcoma (Curran, K. L., et al., Cancer 40 (6): 3050-3053) in suggesting that urinary GAG analyses may be usefu in the detection, screening and diagnosis of hepatic cance.

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URINARY GLYCOSAMINOGLYCAN PATTERNS IN HUMAN HEPATIC ANGIOSARCOMA, HEPATOMA, AND IN WORKERS AT RISK FOR ANGIOSARCOMA.

K. L. Curran, C. E. Kupchella, J. Sandoz, and C. H. Tamburro. Cancer Center and Division of Digestive Diseases and Nutrition, University of Louisville, School of Medicine, Louisville, Kentucky.

A previous study reported an abberation in glyccsaminoglycans (GAGs) eluted from anion-exchange columns with 1.25 and 1.5 M NaCl in the urine of patients with hepatic angiosarcoma. A controlled pilot study examined urinary GAG patterns in workers at risk for angiosarcoma. Six individuals with a history of high vinyl-chloride exposure and documented liver disease were paired with individuals with a high exposure index to vinyl-chloride but no clinical liver disease. Similarly six persons with low exposure but abnormal liver function were paired with low exposure/normal liver function individuals. A 24-hour urine was collected from each individual and the GAGs analyzed by anion-exchange chromatography. Individuals with clinically active liver disease at the time of this study were found to have urinary GAG excretion patterns which were similar to those described in angiosarcoma. No significant differences were found between high vs. low vinyl-chloride exposure or between those with inactive liver disease and those with normal liver function. GAG excretion was also studied in an additional hepatic angiosarcoma and human hepatoma and confirm the reported urinary changes. These findings support the concept that urinary GAGs are increased only in active hepatic disease and may be useful in evaluating the degree of activity at the various stages f liver disease in humans.

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GLYCOSAMINOGLYCAN CHANGES ASSOCIATED WITH HEPATIC TUMORS: THE CONTRIBUTIONS OF REGENERATION AND NECROSIS. C. E. Kupchella, E. M. Secskas,* J. S. Kennedy,* and E. Espinosa*. Cancer Center and Department of Pathology, University of Louisville, School of Medicine, Louisville, Kentucky.

Although glycosaminoglycans (GAGs) have been shown to be elevated in many types of animal and human tumors including hepatic tumors, the cause and significance of these changes in neoplasia are still open questions. Regeneration and necrosis are operative in hepatic cancer and the purpose of this investigation was to evaluate the GAG changes associated with hepatic regeneration and hepatic necrosis. Regeneration was induced in male Sprague Dawley rats by partial hepatectomy and hepatic GAGs were evaluated at 4, 8 and 12 days post operatively. Necrosis was induced by: a) ligating the medium lobe, b) by resecting and placing median lobes in the peritoneal cavity and c) by resecting median lobes and incubating them in vitro in sterile saline. Analyses were carried out after 5 days of treatment. While regenerating livers exhibited GAG levels that were not statistically different from sham operated controls or non-operated controls, in vivo necrosis was accompanied by 3-4 fold increases in tissue GAGs. These data suggest that necrosis may make a substantial contribution to the elevated GAG levels found in some tumors.

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Early Detection of Disease in Individuals Exposed to Vinyl Chloride.

Richard Greenberg, MD. and Carlo Tamburro, MD. University of Louisville, Louisville, Kentucky

This is a collaborative comparison study of the effectiveness of ultrasound, nail bed capillary study and urinary glycosaminoglycan excretions in vinyl chloride workers with biochemical dysfunction or histopathologically documented injury.

It represents an effort to detect early morbidity in vinyl chloride workers. Included is Dr. Maricq's method of investigating capillaries of the middle and distal phalanges of the fingers, including the nailfold, as a screening test for early changes from vinyl chloride exposure. Another new method is Dr. Taylor's "grey scale" ultrasonographic method which has not yet been widely adopted for diagnosis of liver disease. The University of Louisville group reported that the first indicator of liver changes occurred in the vascular sinusoids and was reflected by the appearance of mucopolysaccharides in the urine.

The results of the massive clinical study in Louisville should soon be able to indicate whether more sensitive criteria of early disease may be forthcoming, especially from the liver function testing. The special value of the capillary and ultrasound method remain doubtful at this time for early detection of the effects of exposure to vinyl chloride.

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Computer Assisted Morphologic Quantitation of Collagen in Human Liver Biopsies.

Name(s) of authors, institutional affiliations and addresses:
G. H. Barrows, M.D., M.J. Joyce, G.R. Schrodt, M.D., R. Greenberg, Ph.D., C.H. Tamburro, M.D. Departments of Pathology, Epidemiology and Biostatistics, and Medicine, University of Louisville, Louisville, Kentucky 40202.

Underline name of author to notify and give Telephone (Area) 502-588-5341 Ext.
Address: Department of Pathology, University of Louisville School of Medicine,
P.O. Box 40232, Louisville, Kentucky Zip 40232

Abstract -- Double space.

Because unfixed liver is rarely available for analytical studies, appraisal of the extent of collagen must be made from appropriately stained sections of liver biopsy material leading to judgemental as well as sampling error. We have developed a computer assisted morphologic technique to evaluate the distribution of collagen in liver biopsy material. We examined multiple biopsy samples from patients dying suddenly with no history of liver disease to establish normal values. A Hewlett-Packard 9864-A digitizer and 9815-A micro computer were used to quantitate areas of trichrome stainable collagen within the biopsy samples. Random area selection and statistical analysis proved to be important considerations in determining the experimental model and these factors were readily incorporated into the calculator program. Stainable collagen estimates in normal liver varied from 0 to 6.1% (mean = 1.25%) in patients with no evidence of liver disease and showed considerable variation even in different biopsies from the same patient. As anticipated, subcapsular biopsies had more collagen than deep biopsy; however, the specimens from several deep biopsies often had significant variation in collagen content. Differences in central, mid-zonal and portal collagen could be obtained using this method. The computer assisted morphological technique for evaluating hepatic collagen appears to be readily adaptable to clinical studies. This study of normal liver implies considerable care must be taken before the clinical diagnosis of significant fibrosis can be made.

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EARLY HEPATIC HISTOLOGICAL ALTERATIONS AMONG CHEMICAL (VINYL MONOMER) WORKERS. C.H. Tamburro, L. Makk, and H. Popper, Division of Digestive Diseases and Nutrition and St. Anthony Hospital, University of Louisville, Louisville, Ky., and Stratton Laboratory for Study of Liver Disease, Mt. Sinai School of Medicine, New York, N.Y.

Industrial vinyl chloride exposure is associated with hepatic subcapsular, portal, and perisinusoidal fibrosis and hyperplasia of both sinusoidal cells and hepatocytes. Earlier histological studies, mainly on autopsy material, indicated that focal mixed hyperplasia (of hepatocytes and sinusoidal cells) is the early histologic alteration indicative of exposure. To substantiate this observation and its potential use in screening workers, liver biopsies from 55 persons were investigated in double blind duplicative fashion; 34 were workers exposed to chemicals with hepatic biochemical abnormalities, and 21 were a comparison group composed of 8 non-exposed persons and 13 exposed workers without hepatic abnormalities who had biopsies for non-liver related reasons. Of the exposed workers with abnormalities, 12 (35%) had a hepatic lesion consistent with exposure; 6 (18%) had focal hepatocytic hyperplasia; 6 (18%) had focal mixed hyperplasia. In contrast, only 5 of the comparison group (2 exposed and 3 non-exposed persons) had similar findings; 1 exposed and 2 non-exposed had only focal hepatocytic hyperplasia. The other exposed worker had focal mixed hyperplasia as did the other non-exposed individual who, subsequent to the biopsy, was found to have angiosarcoma. Thus, only 19% of the comparison group demonstrated hepatic lesions with chemical exposure, half of whom had worked with chemicals. Of the 55 biopsies, 22 were re-read double blindly 2 to 4 times over a 2-year period. In addition, 9 of these individuals had second or third biopsies also read double blindly. Duplicate readings were identical in 86%, 9% had 1 positive and 1 questionable reading, and only 5% were read differently. Focal hepatocytic hyperplasia, in addition to the previously described mixed hyperplasia, appears to be the earliest identifiable changes consistent with chemical exposure and seem to be precursors of angiosarcoma. They are useful in the screening of chemical workers.

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Carlo H. Tamburro
(Signature of Principal Author)

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1/1979

Chloroacetaldehyde-induced Damage to Bacillus subtilis. A.D. Laumbach, U.N. Streips* and J.L. Wong. Bureau Foods, FDA and U. Louisville, Louisville, Ky.

Chloroacetaldehyde (CAA), a proposed metabolite of vinyl chloride metabolism, has been shown to be mutagenic in the Salmonella assay system, and to specifically inhibit the growth of a recombination-deficient mutant of Bacillus subtilis (Ellmore, et al BBA, 442: 405, 1976).

We have examined further the biological activity of this compound. First, CAA caused a significant increase in induced mutations to streptomycin resistance. Using B. subtilis 168, the relative mutation frequency (treated 5mM CAA/control) was 13.6, while using an her mutant of B. subtilis the frequency was 14.2. Secondly, a survey of the available repair-deficient mutants of B. subtilis revealed, that the only strains sensitive to CAA were of the rec-genotype. Furthermore, the several rec-mutants, show three types of response to CAA. Strong killing by CAA (inhibition of growth of 10 mm or more) was exhibited on the recA, recB, rec-4 (GSY1616), and recD27 (GSY1627) strains. Intermediate killing (3mm-8mm) was shown with strains bearing the recE, recF, and rec-13 (BD246) loci. All other strains examined (recC, recH, mtc-41, uvr, her, polA, as well as several repair-proficient cultures) grew in the presence of 1.1M CAA.

These studies are part of an ongoing program sponsored by the MCA to determine the molecular basis for vinyl-chloride induced carcinogenesis.

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These studies are part of an ongoing program sponsored by the Manufacturing Chemists Association to determine the molecular basis for vinyl-chloride induced carcinogenesis.

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INHIBITION OF INTERFERON INDUCTION AS A SCREEN FOR THE CARCINOGENIC POTENTIAL OF CHEMICALS. Gerald Sonnenfeld, Mary Carol Barnes, and Uldis N. Streips, Department of Microbiology and Immunology, Univ. of Louisville School of Medicine, Louisville, Kentucky 40232.

Type I interferon was produced after challenge of mouse embryo fibroblasts with Newcastle disease virus. This production of interferon could be sharply reduced by pretreatment of the cells with the known carcinogens benz-α-pyrene, aflatoxin, 2-aminofluorine, and the #4 fraction of tobacco smoke condensate. This finding suggested that inhibition of interferon induction (I-I-I) may serve as an indicator for the carcinogenic potential of chemicals. We tested the screening potential of this assay by testing two chemical analogs, methylmethanesulfonate (MMS) a carcinogen and mutagen and ethylmethanesulfonate (EMS) a non-carcinogenic mutagen. Both of these chemicals are active in the Ames Salmonella assay but can be separated in "SOS" induction assays such as the Comptest. In the I-I-I assay, MMS was highly positive while EMS showed no inhbition of interferon induction. Thus, the I-I-I assay appears to have the potential to screen for carcinogens. Preliminary results using the carcinogen chloracetaldehyde and its non-carcinogenic analogs chloroethanol and chloroacetic acid support this idea. Following further verification of the discrimination potential of the I-I-I assay, this test could become an integral part of a comprehensive battery of tests for chemical carcinogenicity.

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Properties of 14 Week Maintenance Cultures of PLC/PRF/5 Cells. P.B. JOHNSTON*, E. Espinosa, S. Chia and S. Caple. Univ. of Louisville, Louisville, Ky 40232

This established cell line was originally described as being short lived within a given passage, with considerable cell loss by day 6 to 8. However, after 80 passages, we found that culturing in glass roller bottles at 0.25 RPM allowed maintenance for at least 14 weeks with cells exhibiting excellent morphology without sloughing. After treating monolayers with Hoechst 33258 DNA stain, intense cytoplasmic fluorescence was seen in a proportion of the cells, possibly a function of the subviral hepatitis B genome. HBsAg of culture supernates was monitored and solid phase RIA assay revealed 31 to 47 ratio-units/0.2 ml after 2 to 14 weeks in medium with 10% fetal calf serum. The 14 week old cultures were extremely well adapted to maintenance in serum free medium for 14 additional days when supplementing with 100 ng/ml of insulin, in contrast to 1 week cultures which survived well for only 5 days without serum. The HBsAg titers of supernates from these 1 week or 14 week cultures, were very similar to the previous harvests in 10% serum. In addition, both young and 14 week cultures were similar in their rate of acid production; and in that both elaborated serum albumin, fibrinogen, transferrin and alpha-2 macroglobulin, the proteins tested to date.

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LIVER-SPECIFIC F ANTIGEN IN TRANSPLANTABLE HEPATOMAS HAVIN(DIFFERENT GROWTH RATES. Enrique Espinosa, Sue Chia*, Sam Caple* and Charles Kupchella*. Dept. Pathology and Cancer Center, Univ. of Louisville School of Medicine, Louisville KY 40232

In the course of studies of antigenic changes associate with liver neoplasia we measured the relative concentratio of liver-specific F antigen in fast (7777), slow (9618A) a medium (5123tc) growing Morris hepatomas in comparison wit its concentration in normal adult rat liver. F antigen wa solubilized from each tissue by aqueous homogenization. T supernatant containing the antigen was then tested by a double diffusion dilution assay capable of detecting a min mum of 1-2% the concentration of antigen present in normal rat liver. The F antigen antiserum used was prepared according to Fravi and Lindenmann by immunizing CBA mice wit BALB/c mouse liver extract. F antigen was undetectable the fast growing hepatoma and ranged from less than 2% to 10% of the normal liver concentration in the slow growing one. The medium growing hepatoma showed similar or higher concentration than normal rat liver. Thus, the level of F antigen in these tumors does not appear to correlate wi their rates of growth. (Supported in part by the Manufacturing Chemists Association)

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LIVER-SPECIFIC F ANTIGEN IN TRANSPLANTABLE HEPATOMAS HAVIN DIFFERENT GROWTH RATES.

Enrique Espinosa, Sue Chia*, Sam Caple* and Charles Kupchella*. Dept. Pathology and Cancer Center, Univ. of Louisville School of Medicine, Louisville KY 40232

In the course of studies of antigenic changes associate with liver neoplasia we measured the relative concentratio of liver-specific F antigen in fast (7777), slow (9618A) a medium (5123tc) growing Morris hepatomas in comparison wit its concentration in normal adult rat liver. F antigen wa solubilized from each tissue by aqueous homogenization. T supernatant containing the antigen was then tested by a double diffusion dilution assay capable of detecting a min mum of 1-2% the concentration of antigen present in normal rat liver. The F antigen antiserum used was prepared according to Fravi and Lindenmann by immunizing CBA mice wit BALB/c mouse liver extract. F antigen was undetectable in the fast growing hepatoma and ranged from less than 2% to 10% of the normal liver concentration in the slow growing one. The medium growing hepatoma showed similar or higher concentration than normal rat liver. Thus, the level of F antigen in these tumors does not appear to correlate wi their rates of growth. (Supported in part by the Manufacturing Chemists Association)

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TWO LIVER ANTIGENS UNDETECTABLE IN A FAST GROWING LINE OF TRANSPLANTED HEPATOMA (MORRIS HEPATOMA 7777). Enrique Espinosa, Sam Caple*, Charles Kupchella* and Sue Chia*. Dept. Pathology and Cancer Center, Univ. of Louisville School of Medicine, Louisville, KY 40232

During investigations of antigenic changes in liver tumors we examined fast (7777), medium (5123tc) and slow (9618A) growing Morris hepatomas for presence of liver antigens. Analyses of normal liver and hepatoma tissue saline extracts were performed by immunodiffusion methods using rabbit antiserum to rat liver extract absorbed with pooled normal rat plasma. Hepatoma 5123tc and 9618A gave immunoelectrophoretic patterns similar to normal liver. In contrast, hepatoma 777 showed 2 arcs of precipitation missing. This was confirmed by the finding that after absorption of antiserum with hepatoma 7777 two lines of precipitation given by normal liver extract persisted whereas absorption with extract of normal liver, hepatomas 5123tc or 9618A abolished all lines of precipitation. The liver antigens found to be absent in hepatoma 7777 were characterized as proteins relatively unstable to heating and acid pH and unrelated to liver-specific F antigen. In Sephadex G-200 and Bio-Gel A-5m columns these antigens eluted as proteins of approximately 51,000 and 240,000 daltons respectively. The fact that 7777 is the fastest growing and least differentiated of the tumors studi suggests a possible functional relationship between the absent antigen and these properties. (Supported in part by the Manufacturing Chemists Association)

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OXIDATIVE AND DETOXIFYING ABILITY OF LIVER MESENCHYMA PARENCHYMAL CELLS IN THE METABOLISM OF XENOBIOTICS

Du, J. and Tamburro, C.H., Liver Research Laboratorie Dept. of Medicine, University of Louisville, Louisvil

The metabolism of xenobiotics, by the liver most ofte volves oxidation via mixed function oxidase (MFO) anc hydryl detoxification with glutathione. Hepatic toxi and carcinogenicity are modified by these two pathway Vinyl monomer (e.g. vinyl chloride) exposure is assc with liver parenchymal cell (hepatocytic cell, HC) tc and mesenchymal cell (sinusoidal cell, SC) carcinoger even though the hepatocyte is the main cell for xenob oxidation. The metabolic ability of these two cell g was assessed by isolation of SC using pronase digesti and HC using collagenase profusion. Viability assess 90% by trypan blue exclusion and cross contamination mated (< 1%) by pryuvate kinase activity. MFO activi (demethylation benzphetamine), glutathione transferas (E) using (1,2, epoxy-(P-nitro nitrophenoxy) propane substrate, glutathione transferase A,C and E (A) us nitrobenzyl chloride) as a substrate and glutathio tase (GR), were determined in subcellular isolated HC SC fractions. Results based on protein content (N mo min/mg protein) were:

	E	A	GR	MFO
SC	42.9 ± 17	18.5 ± 6	47.4 ± 2	7.49 ±
HC	82.5 ± 11	215.0 ± 61	65.0 ± 7	16.0 ±

and activity in mole/10^6 cell were:

	E	A	GR	MFO
SC	1.16	0.5	1.28	0.026
HC	65.6	220.	52.	4.7

These results demonstrate both the greater average, as as total, cellular ability of HC to oxidize xenobiotic More importantly, it demonstrates an increased abilit detoxify the xenobiotic's toxic metabolites. This gr ability may account for less severe HC injury and pla important role in preventing HC malignant transformat In contrast, SC ability to oxidize but its lesser abi to detoxity xenobiotic may account for SC's potential ~~malignant transformation.~~

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Toxic, Carcinogenic & Safe Exposure Levels in Vinyl Chloride Induced Hepatic Angiosarcoma. Carlo H. Tamburro* and Richard A. Greenberg* (SPON: William Waddell). Depts. of Medicine & Community Health, Univ. of Louisville, Lou., Ky. 40292.

Vinyl chloride (VC) induced hepatic angiosarcoma (HA) has rapidly decreased since 1974. The relationship of latency & duration of exposure was studied in 21 North American (NA) cases and 62 worldwide (WW) reported cases; 92% of NA & WW cases had long exposures (LE) (10-33 yrs); 7 cases (8%) had short exposures (SE) (3.5-7 yrs). The mean duration in both LE & SE groups was 18.5 yrs. with 2 peaks at 15 & 25 yrs. Deaths from HA began in 1955, peaked in 1976 & rapidly fell thereafter. HA peaked in Canada (CA) in 1973 and USA 1974, France (FR) 1976, & Germany (GE) 1978. Operations began in CA in 1941 & USA 1939-44; FR 1941-57, & GE 1952-60. Exposure levels reported in 1940's of ∿2000 ppm; in 1950's ∿200-500 ppm, in early 1960's ∿50-500 ppm, late '60's & early '70's ∿50-250 ppm, & 1-10 ppm after '74. No relationship was found in total average exposures (TAE) range of 50 ppm; 200-1000 ppm range showed a direct relation of lower TAE to shorter latency, while higher TAE (1000-2000 ppm) had longer latencies. The data suggests VC has a biological threshold level, an optimum carcinogenic level and subcarcinogenic toxic level for occurrences of HA.

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The reproducibility in identifying these histological lesions is quite high. Under double blind conditions there was consistent histological identification of chemical injury in both duplicate readings of single biopsies as well as single readings of multiple biopsies in the same individual. Inconsistency appears to occur more frequently among the duplicate readings of wedge biopsies and between wedge and needle biopsies than between duplicate readings of needle biopsies or readings of multiple needle or wedge biopsies in the same individual. This may be due to the increased time and attention needed for wedge section review in order to determine the absence or presence of these lesions anywhere in the specimen. There are, however, some pitfalls that require caution. The quality of the histological preparation is of vital importance. Technical artifact, excessive thickness, torn or fragmented specimens will greatly reduce the ability to correctly identify these lesions. The use of silver impregnation for assessment of the reticular structure is very useful in verifying focal areas of hepatocellular hyperplasia. The presence of fatty infiltration and/or chronic disease (hepatitis, granuloma, etc.) may prevent the identification of these lesions. We were, however, able to correctly identify the presence or absence of these early lesions in the six individuals who also had a concomitant granulomatous process (four individuals with granuloma were repeatedly read as negative and two individuals with granuloma were repeatedly read as positive for chemical liver injury).

The more frequent elevation of alkaline phosphatase in those individuals with histological evidence of chemical injury may provide some guidance in sequential uses of laboratory screening tests. During the first phase, tests with the greater sensitivity for identifying liver injury, such as the ICG and ALT can be used. Positive results can be followed up by using tests of greater specificity, such as the alkaline phosphatase, to further identify those at high risk of chemical injury.

of acute or chronic injury, appear to be the earliest change associated with chemical exposure. Subsequently, there occurs perisinusoidal fibrosis associated with a proliferation of fibroblasts and/or its precursor cell, the Ito cell. In later stages sinusoidal cell activation occurs involving both the macrophagic and endothelial lining cells. These changes are associated with focal sinusoidal enlargement. In the late stage mixed hyperplasia (hepatocyte and sinusoidal cells) becomes prominent, followed by sinusoidal cell dysplasia with palasading of cells, and finally malignant transformation.

Our data demonstrates that focal hepatocytic hyperplasia is the earliest consistently identifiable histological finding in industrial workers exposed to vinyl monomer chemicals. The very limited occurrence of these findings in non-chemical workers and the fact that focal mixed hyperplasia, a more advanced lesion, was only found in chemically exposed workers and in a non-chemical worker with vinyl chloride exposure (via hair spray), adds further confirmation that these lesions are related to prolonged or repeated periods of chemical exposure.

Whether these lesions should be viewed as precursors to the development of cancer or only as an early reflection of the degree and duration of chemical exposure cannot be determined at this point in time. The unchanging and stable status of screening studies over a seven year follow-up period and the histological similarities among the serial biopsies of these workers suggest that these early lesions are non-progressive when the affected individuals are removed from exposure (7).

The presence of sinusoidal cell dysplasia, however, has been associated with the later development of angiosarcoma as illustrated in one of our cases and may represent a true precancerous or a non-reversible lesion.

this worker cohort had shown that all the cases of angiosarcomas had an average vinyl chloride exposure ranking (AER) of 3.5 or greater. AER's of 3.5 or greater occurred in 45% of the CLI group. In contrast, those with liver disease (LD) alone, only 22% had a vinyl chloride exposure rating of 3.5 or greater and in the chemically exposed workers with no evidence of liver disease, 32% had a rating of 3.5 or greater.

Biochemical Studies

A study of the biochemical abnormalities among the two histological groups of chemical workers with evidence of liver disease is illustrated in Figure 7. The five screening tests with the best degree of sensitivity and specificity in identifying latent liver injury were reviewed. Indocyanine green clearances, at the 0.5 mg/kg dose, provides the most sensitive and the most specific of the laboratory screening study, followed by the alanine amiontransferase and aspartase aminotransferase, gamma glutamyl transpeptidase and alkaline phosphatase (6). However, specificity for chemical injury appeared to be associated more with abnormalities in the alkaline phosphatase, which was far more frequently abnormal in workers with chemical liver disease than in those with non-chemical liver injury. In contrast, the ALT, AST, GGT, and ICG were more frequently abnormal in those workers with liver disease of a non-chemical origin.

DISCUSSION

Figure 8 provides the most likely progression in the development of vinyl chloride and other vinyl monomers hepatic injury and cancer development.

These findings confirm those previously reported by Thomas and Popper (2) in their original pathological studies of vinyl chloride associated hepatic angiosarcoma and hepatic injury. Focal hepatocytic megalocytosis associated with focal increases in reticulum structure, in the absence of other evidence

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A comparison of the reproducibility of the biopsy readings is shown in Tables 3, 4 and 5. Table 3 illustrates the high degree of consistent readings between multiple biopsies in the same individual. In only one individual was one of the four biopsies read differently--the wedge was read differently from the three needle biopsies.

Table 4 makes a comparison between readings of multiple needle biopsies or multiple wedge biopsies from a single individual. In these cases there was consistent agreement in all readings.

Table 5 illustrates the consistent readings of the same biopsies over the three-year period. All the needle biopsy duplicate readings and 12 of the 14 wedge duplicate readings were the same.

At least 96% (43/45) of all readings, whether duplicate or from multiple biopsies, provided reproducible and consistent findings.

Correlation of Histological Lesions With Exposure

Biopsies from all the chemical workers were divided into three categories on the basis of final diagnosis:

1) individuals with no evidence of histological liver disease (N)
2) individuals who had liver disease but no evidence of chemical injury (LD)
3) individuals who had chemical liver injury identified by the histological criteria specified (CLI).

A comparison was made between the average vinyl chloride exposure ranking of each worker and his/her final histological diagnosis. The exposure rankings were not known to those making the final histological determination at any time during the three year period. As illustrated in Figure 6 the group of workers with evidences of chemical liver injury had the highest percentage of individuals with the highest ranking of exposure to vinyl chloride. Previous studies of

for each job for each year based on the best available information and industrial expertise. Each worker's cumulative exposure to each of the 22 chemicals was based on the various job classifications he/she held during their employment. A detailed explanation of this system and an actual in-the-field demonstration of the ability of this system to identify work-related liver injury (angiosarcoma) has been published elsewhere (4,5).

RESULTS

Thirty-seven percent (13/35) of the exposed workers with screening test abnormalities had hepatic lesions consistent with chemical exposure. Among the exposed workers without biochemical abnormalities 23% (3/13) had hepatic lesions consistent with chemical exposure. In the non-worker comparison group none of those with normal biochemical screening tests and 17/19 (89%) with abnormal biochemical screening tests had hepatic lesions consistent with chemical exposure. Of the remaining two, one had focal hepatocellular hyperplasia; the other had focal mixed hyperplasia and early peliosis hepatis. This latter individual was later found to have angiosarcoma possibly from vinyl chloride hair spray exposure. The former individual had no history of chemical exposure and/or alcohol consumption, but did have hepatobiliary tract disease in the form of biliary stones. Table 1 lists the hepatic lesions found in those with and without biochemical abnormalities for the entire group.

Reproducibility

An evaluation of the reproducibility of these biopsy readings was carried out in 17 individuals who had 32 biopsies. Seven individuals had only one biopsy (2 needle, 5 wedged) and 10 individuals had their biopsies read in duplicate. Four had needle biopsies with 9 readings and 6 had wedge biopsies with 14 readings (Table 2).

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hepatocellular hyperplasia; b) focal increased reticulum associated with the hepatocytic hyperplasia; c) perisinusoidal fibrosis; d) focal hyperplasia; e) focal mixed hyperplasia associated with increased reticulum deposition; f) sinusoidal cell activation; g) sinusoidal dilitation; h) portal and capsular fibrosis; i) sinusoidal dysplasia; and, j) hepatic angiosarcoma.

The presence of focal hepatic cellular hyperplasia with focal increases in reticulum, in the absence of other evidences of hepatocellular disease or steatosis, were considered the minimum presumptive evidence of chemical exposure. The presence of focal mixed hyperplasia with increased reticulum and/or perisinusoidal fibrosis was considered evidence of chemical injury of a more advanced stage. Sinusoidal cell activation and dilitation of the sinusoids were considered evidences of chemical injury of an even further stage of advancement. The presence of subcapsular fibrosis, (on wedge biopsies only) and/or increases in both portal and sinusoidal fibrosis, with sinusoidal cell activation and sinusoidal dilitation was considered the most advanced stage of chemical injury characteristic of vinyl chloride or vinyl monomer exposure. Finally, sinusoidal cell dysplasia and/or evidence of malignant transformation, constituted definitive evidence of chronic vinyl chloride exposure and the terminal stages of injury.

Work and exposure histories were available for all the chemical workers. The work histories provide a total and average exposure data for 22 chemicals based on rank order exposure estimates. These exposure work histories consisted of an estimate of exposure to each of 22 different chemicals for all of the 350 job classifications used since the start of the chemical plant. Each of the chemicals exposure was ranked on a scale of zero to six for each of the 22 chemicals. These exposure ranking estimates were done separately

nucleoli with a moderate amount of cytoplasm which reacted on PAS staining and persisted after diastase reaction. These cells are interpreted as activated fibroblasts, are associated with an increase in fat storing sinusoidal cells and augmented perisinusoidal fibrosis tissue (Figure).

3. Fibrotic changes consisting of an excell of irregular connective tissue in the periportal zone, frequently arranged around proliferating bile ductules, and focal thickened subcapsular accummulation of corrective tissue into the parencyma (Figure 7).

These histological lesions are believed to be the three earliest hepatic findings associated with vinyl monomer chemical injury. The hepatocytic changes are referred to as focal hepatocytic hyperplasia or FHH. The sinusoidal cell changes (sinusoidal cell hyperplasia) associated with the hepatocytic changes are referred to as focal mixed hyperplasia or FMH.

For purposes of this study, early fibrotic changes were not classified since the majority of liver biopsies were of needle type, which could not be evaluated for subcapsular changes.

The liver biopsies were coded and read blindly to identify (1) histological evidence of hepatic disease; (2) any characteristics of non-chemical injury excluding steatosis; (3) steatosis with or without fibrosis and, (4) evidence of chemical injury identified by the following histological findings: a) focal

A group of 30 individuals who were not chemical workers but had undergone abdominal surgery with liver biopsies, were used as a comparison population. All of the comparison population's liver biopsies were performed during the same three-year period, at the same hospital, by members of the same medical-surgical team, with the informed consent of the individuals. This group had similar hepatic biochemical studies with the exception of the GGT and ICG clearance. None had a history of extensive exposure of working with halogenated hydrocarbons, although one patient had a history of household contact with vinyl chloride via commercial home products.

Pathology

Earlier experiences with autopsy and animal material from vinyl chloride associated angiosarcoma have recognized certain hepatic lesions in the non-tumorous area of the liver parenchyma ( ). They include:

1. Hepatocytic changes consisting of foci of enlarged hepatocytes with increased amount of cytoplasm, and large hyperchromatic nuclei (Figure 1). These cells are intermixed with hepatocytes of normal and smaller sizes and create focal, indistinct nodular areas (Figure 2a). These areas are often associated with very slight increased sinusoidal dilitation and focal increase in the reticulin framework illustrated best by silver impregnation (Figure 2b).
2. Sinusoidal lining cell changes consisting of an increase in cell number associated with nuclear changes, particularly conspicuous in areas of sinusoidal widening. This proliferation of sinusoidal cells involves (a) Kupffer cells (macrophages) with PAS-positive granules; (b) fibroblasts with elongated, almost rectangular nuclei with loose vesicular chromatin patterns and inconspicuous

INTRODUCTION

Industrial vinyl chloride and other vinyl monomer exposure is associated with various hepatic histological abnormalities. These include subcapsular, portal, and perisinusoidal fibrosis as well as hyperplasia of both hepatocytes and sinusoidal cells. Early histological studies, mainly on autopsy material, had shown focal mixed hyperplasia (hyperplasia of hepatocytes and sinusoidal cells) to be an early histological alteration associated with vinyl chloride exposure (1,2,3). To substantiate this observation and determine its potential use in medical surveillance screening of the exposed workers, liver biopsies from 78 individuals were studies in duplicate blind fashion to determine 1) if these early histological findings were associated with extensive vinyl chloride exposure, and 2) if these histological findings occur in non-exposed populations or were associated with other diseases.

MATERIALS AND METHODS

Liver biopsies from 48 vinyl monomer chemical workers with and without hepatic biochemical abnormalities were investigated. All 48 chemical workers had had hepatic biochemical studies performed on an annual or semi-annual basis. These included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), total bilirubin (TB), gamma glutamyl transpeptidase (GGT), prothrombin time (PT), and indocyanine green clearance (ICG). In addition, history and physical examinations, liver-spleen scans, and abdominal plain films had been performed annually. Thirteen of the chemical workers had hepatic biopsies for non-liver related reasons while 35 had biopsies performed because of screening biochemical and/or liver-spleen radioisotopic scan abnormalities.

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ABSTRACT

Earlier histological studies of industrial vinyl chloride exposure, obtained mainly on autopsy material, indicated that focal mixed (hepatocytes and sinusoidal cells) hyperplasia is the earliest histological alteration indicative of exposure. To substantiate this observation and its potential use in screening workers, 93 liver biopsies from 78 persons were investigated in double blind duplicative fashion; 35 were exposed chemical workers with hepatic screening tests abnormalities, and 13 were exposed workers without hepatic abnormalities who had liver biopsies for non-liver related reasons. A comparison group consisted of 30 individuals who were not chemical workers, but had liver biopsies for non-hepatic related reasons during the same time period. Of the exposed workers, 23 (48%) had a hepatic lesion consistent with exposure; 17 (35%) had only focal hepatocytic hyperplasia; six (13%) had focal mixed hyperplasia or more advanced lesions. In contrast, only five of the comparison group had similar findings; four (13%) had only focal hepatocytic hyperplasia and one (3%) had focal mixed hyperplasia and sinusoidal dilitation. On a subsequent biopsy, this individual was found to have angiosarcoma and a history of using hair spray containing vinyl chloride as propellant. Ten individuals had 28 multiple biopsies also read double blindly, and 10 individuals had 23 readings of the same biopsy; 21/23 (91%) duplicate readings and 27/28 (96%) multiple biopsy readings in the same individuals were identical. Only 18% of either duplicate and/or multiple biopsy readings had disagreements in their biopsy assessment. Focal hepatocytic hyperplasia, in addition to the previously described mixed hyperplasia, appears to be the earliest identifiable change consistent with chemical exposure and both lesions are present prior to the development of angiosarcoma. These lesions can be consistently identified and are useful in the screening of chemical workers for evidence of exposure.

EARLY HEPATIC HISTOLOGICAL ALTERATIONS AMONG CHEMICAL (VINYL MONOMER) WORKERS

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The reproducibility in identifying these histological lesions is quite high. Under double blind conditions there was consistent histological identification of chemical injury in both duplicate readings of single biopsies as well as single readings of multiple biopsies in the same individual. Inconsistency appears to occur more frequently among the duplicate readings of wedge biopsies and between wedge and needle biopsies than between duplicate readings of needle biopsies or readings of multiple needle or wedge biopsies in the same individual. This may be due to the increased time and attention needed for wedge section review in order to determine the absence or presence of these lesions anywhere in the specimen. There are, however, some pitfalls that require caution. The quality of the histological preparation is of vital importance. Technical artifact, excessive thickness, torn or fragmented specimens will greatly reduce the ability to correctly identify these lesions. The use of silver impregnation for assessment of the reticular structure is very useful in verifying focal areas of hepatocellular hyperplasia. The presence of fatty infiltration and/or chronic disease (hepatitis, granuloma, etc.) may prevent the identification of these lesions. We were, however, able to correctly identify the presence or absence of these early lesions in the six individuals who also had a concomitant granulomatous process (four individuals with granuloma were repeatedly read as negative and two individuals with granuloma were repeatedly read as positive for chemical liver injury).

The more frequent elevation of alkaline phosphatase in those individuals with histological evidence of chemical injury may provide some guidance in sequential uses of laboratory screening tests. During the first phase, tests with the greater sensitivity for identifying liver injury, such as the ICG and ALT can be used. Positive results can be followed up by using tests of greater specificity, such as the alkaline phosphatase, to further identify those at high risk of chemical injury.

CONCLUSIONS

Focal hepatocellular hyperplasia, in addition to the previously described focal mixed hyperplasia, appear to be the earliest identifiable changes consistent with chemical exposure. These histological lesions are useful in identifying high risk exposed chemical workers as part of a medical screening surveillance program. Biochemical studies may help identify which individuals warrant a liver biopsy for further identification of the cause of the liver abnormalities.

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