# Use of Serum Bile Acids in the Identification of Vinyl Chloride Hepatotoxicity

GARY M. LISS, M.D., M.S.*
RICHARD A. GREENBERG, Ph.D.
CARLO H. TAMBURRO, M.D.
*Louisville, Kentucky*

**Most previous studies proposing serum bile acids as indicators of hepatic function have been performed in hospitalized patients in whom overt symptomatic liver disease was pres nt. Th ability of fasting levels of serum bile acids to identify mild, clinically inapparent chemical liver injury in an occupational setting was compared with that of indocyanine green clearance and routine biochemical liver tests in 67 asymptomatic chemical workers in whom liver biopsi s had been performed for medical indications. Histologically, 15 were found to have chemical liver injury, 27 had nonchemicai liv r disease, and 25 were normal. Two serum bile acids, cholylglycine and conjugates of cholic acid, were determined by radioimmunoassay, using 466 "normal" males from the same worker coh rt as a reference range. The geometric mean concentrations of ch lylglycine in patients with chemical liver injury, patients with nonchemical liver disease, and normal subjects were 47.9, 19.1, and 20.0 μg/dl, respectively ($p = 0.036$ by analysis of variance). Conjugates of cholic acid showed similar differences ($p = 0.027$), as did indocyanine green clearance with mean half-life of 4.2, 3.2, and 3.3 minutes in the three biopsy subgroups, respectively ($p = 0.043$). Such differences were not observed for biochemical liver tests. The fasting level of serum bile acids provided high specificity but lower sensitivity in the detection of all types of liver disease. How ver, s rum bile acids and indocyanine green clearance provided a higher specificity and sensitivity for chemical liver injury than for nonchemical liver disease. An index of average exposure to vinyl chloride was significantly greater in the subgroup with chemical liver injury than in the other two groups, further supporting the association of chemical type injury with impaired anion uptake. These data identify the fasting level of serum bile acids as a clinically usable indicator of early chemical injury in chemically exposed asymptomatic work r populations with liver dysfunction. Further investigation is needed in other occupational hepatotoxic environments to determine if this association is limited to vinyl monomer type injury.**

Occupational and nonoccupational exposure to hepatotoxic agents can lead to acute, subacute, or chronic liver injury. The decrease in acute occupational hepatic injury in recent decades has focused concern that chronic hepatic disease, including malignant neoplasms, may develop as a result of prolonged low-level industrial exposure [1,2]. Currently, there are no specific clinical means to identify hepatic injury from chronic low-level exposure to chemical hepatotoxins.

The inability of standard biochemical enzyme studies to identify the early phases or progression of liver injury has been shown [3–10].

From the Liver Research Center, Division of Gastroenterology/Hepatology, Department of Medicine, and the Divisions of Occupational Health and Epidemiology and Biostatistics, Department of Community Health, University of Louisville School of Medicine, Louisville, Kentucky. This work was supported in part by National Cancer Institute contract NO1-CN-5512 and was presented in part at the American Public Health Association Annual Meeting, November 1982, Montreal, Canada. Dr. Liss was a Resident in Occupational Medicine, Department of Environmental Health, University of Cincinnati Medical Center at the time of this study. Requests for reprints should be addressed to Dr. Carlo H. Tamburro, University of Louisville School of Medicine, Liver Research Center, HSC 55A, Room 119, Louisville, Kentucky 40292. Manuscript accepted July 19, 1984.

* Current address: Special Studies and Services Branch, Ministry of Labour, 400 University Avenue, Toronto, Ontario, Canada.

Serum enzyme studies are of limited value because they primarily reflect acute disruption of cell membrane integrity (liver cell "leaking") rather than the uptake, metabolism, storage, or excretion functions of the liver cell [8]. In subacute, chronic, and end-stage liver disease, the enzymes often return to normal levels after initial elevations [9], and fail to reflect the decreased functional capacity of the remaining parenchyma. Enzyme levels can also be increased in nonhepatic diseases. In severe hepatic injury (fulminant hepatitis or severe toxic necrosis), decreasing enzyme levels may reflect worsening disease [8].

At present, measuring clearance rates of substances primarily or solely removed from circulation by the liver provides the most sensitive and specific indicator of liver function [5]. Such substances include exogenous anionic dyes (bromosulphthalein or indocyanine green) and endogenous metabolites (serum bile acids). However, few studies have examined the ability of these tests to identify early subclinical liver disease in asymptomatic populations.

The discovery of vinyl chloride-related hepatic angiosarcoma and nonmalignant hepatocellular injury in 1974 [11] provided an opportunity to study chronic occupational chemical liver injury. Recent studies [4,10] have shown that indocyanine green clearance provides the best combined sensitivity and specificity for detecting persons with subclinical hepatic disease. However, this test has certain limitations. It is invasive, involves intravenous injection of a synthetic dye, and requires multiple blood samplings. Further, blood samples are best analyzed the same day and not stored for prolonged periods of time due to indocyanine green degradation.

Serum bile acids, which have been proposed as a clinical test of hepatic function, are natural substances, cleared only by the liver. Bile acid studies have been conducted in the fasting [12–16] and postprandial [12,13,17,18] states and following exogenous loading, either by the intravenous [19–22] or, more recently, the oral [23,24] route. Most of these studies, however, have been conducted in persons with overt disease with liver damage of mixed origins. The objectives of this study were to determine if serum bile acid levels could identify chemical workers who had had chemical liver injury after exposure to vinyl chloride and other vinyl monomers, and to compare the capability of bile acid levels with those of indocyanine green dye clearance and conventional biochemical tests.

## PATIENTS AND METHODS

The study population consisted of 1,200 employees of a vinyl chloride/synthetic rubber manufacturing plant. The occupational surveillance program that monitored these vinyl monomer workers for a seven-year period provided medical data including annual history, physical examination, 45 biochemical and hematologic parameters, urine analysis, chest radiography, liver/spleen scanning, indocyanine green dye clearance tests, and serologic studies for viral infection. Fasting serum samples were obtained from all employees who participated in the screening program. Criteria for participation in this bile acid study included male employees who had worked for one or more years, had undergone the conventional biochemical screening tests, had indocyanine green clearance test performed at the low-dose (0.5 mg/kg) level, and had fasting serum samples available for bile acid determination. The latter two tests had to have been performed on the same day.

A total of 589 employees met these inclusion criteria. At the beginning of the study, prior to any serum bile acid determinations, these employees were divided into three groups on the basis of the results of medical examination, liver/spleen scanning, and enzyme studies. Employees who had undergone cholecystectomy were excluded. None had had bowel resection. Group I included 466 employees who had no clinical, radiologic, serologic, or biochemical evidence of liver disease; Group II included 56 workers who had repeated biochemical test abnormalities consistent with liver injury but in whom no liver biopsies had been performed; and Group III consisted of 67 employees who had liver biopsies performed, 30 because of biochemical abnormalities, 24 because of radiologic abnormalities, and 13 incidental biopsies performed during non-hepatobiliary abdominal surgery. Group III forms the basis for this report.

Serum bile acids, cholylglycine and conjugates of cholic acid, were determined by radioimmunoassay. Radioimmunoassay method used had the same selectivity for cholylglycine as the present-day radioimmunoassay (Diagnostic Kits, Abbott Laboratories, Chicago, Illinois). All assays were performed at one time. In the healthy workers (Group I), the normal range [25] for cholylglycine was 0 to 48 $\mu$g/dl, and 0 to 74 $\mu$g/dl for conjugates of cholic acid. The indocyanine green clearance, expressed as the half-time, was calculated following intravenous injection of 0.5 mg/kg indocyanine green as previously described [26,27]. The normal range in the same standard population is 1.8 to 3.6 minutes [28]. The following biochemical tests were performed in serum using standard methods: alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase. The normal ranges used were also those previously described in this population [28]. All biochemical data from Group I did not significantly differ from generally published norms of similar occupational and general populations [5,6,8].

Biopsy results were interpreted in a double-blind duplicative fashion as previously described [29]. All were classified, a priori into one of three categories: (1) chemical liver injury, (2) nonchemical liver disease, or (3) normal biopsy results, on the basis of known histologic features of vinyl chloride-associated chemical liver injury [3,29–31]. These include focal hepatocellular hyperplasia, mixed focal hyperplasia with focal increased reticulum and/or collagen, and focal sinusoidal dilatation and vascular changes.

All indocyanine green clearance and serum bile acid de-

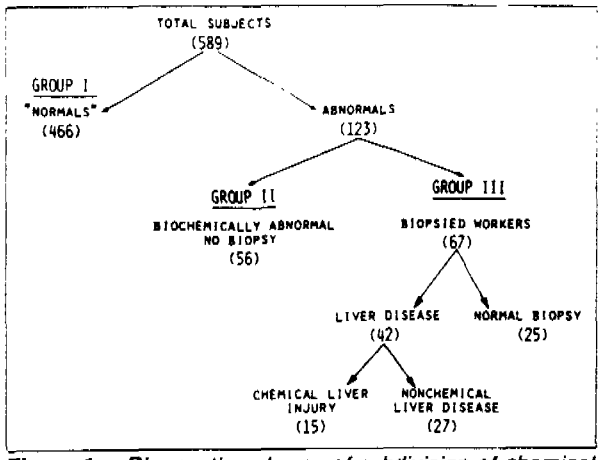


***Figure 1.*** *Diagnostic scheme of subdivision of chemical workers into study groups.*

terminations in a person were from blood samples drawn on the same day. The biochemical blood studies were not performed at the same time as indocyanine green clearance and serum bile acid tests in all cases. In 44 (66.7 percent) of 66 patients, serum samples for indocyanine green clearance, bile acid determination, and biochemical studies were obtained from blood samples drawn on the same day. The remainder were obtained from blood samples drawn within six weeks. The date of biochemical tests chosen for analysis was that closest to the serum bile acids/indocyanine green clearance test date. To assess if this had any effect, the results for those persons with versus those without simultaneous data were compared for each biopsy subgroup. The time interval between liver biopsy and serum bile acid determinations varied, but the median interval was similar in the three biopsy subgroups: six months in patients with chemical liver injury, nine months in patients with nonchemical liver disease, and nine months in normal subjects.

Chemical exposure was assessed through an exposure ranking system [32], which consists of a work and job classification based on an ordered, six-level exposure rating regarding the intensity of exposure to 22 potentially hepatotoxic chemicals, including vinyl chloride. Cumulative exposure rank months and average exposure indexes were determined for each employee.

For analysis, logarithmic transformation was performed when skewed distributions were encountered (serum bile acids, biochemical data). Differences among the three biopsy groups were assessed by analysis of variance on the log-transformed data. The differences in exposure indexes between biopsy groups were assessed as described by Mantel and Haenszel [33] and confidence limits for the odds ratio were calculated with the program of Rothman and Boice [34]. A test for linear trend in proportions of liver function abnormalities with cumulative exposure categories was assessed by the method of Fleiss [35]. The Kruskal-Wallis test was used to compare ages between biopsy subgroups. The Wilcoxon rank-sum test was used to compare results between simultaneously and nonsimultaneously obtained data.

## RESULTS

The derivation of the study group from cohort is summarized in **Figure 1.** Group I laboratory values were used as the reference group for "normal values range" since all tests were performed in an identical manner in the same laboratory. The 67 workers who underwent biopsy (Group III) are further subdivided into 25 with no liver disease (normal biopsy results) and 42 with liver disease on biopsy. This latter subgroup was further subdivided into 15 with chemical liver injury and 27 with nonchemical liver disease, usually of viral origin or alcohol-induced. Biopsy findings in the subgroup with nonchemical liver disease consisted of mild or moderate steatosis and/or portal fibrosis or portal triaditis. Only two patients were described as having "beginning cirrhosis" with steatosis or portal fibrosis. The ages of the workers in the three biopsy subgroups were similar: 45.6 ± 2.7 (mean ± SEM) in patients with chemical liver injury; 46.9 ± 1.9 in patients with nonchemical liver disease; 45.9 ± 1.9 in normal subjects ($p > 0.8$ by Kruskal-Wallis test).

An analysis of the levels of the four enzymes, indocyanine green clearance, and both serum bile acid values revealed no significant differences in results between persons in whom the determinations had been made simultaneously and those in whom it had not. This was observed for all three biopsy subgroups ($p > 0.1$ for all comparisons). The data were thus pooled in further analyses.

Serum bile acid levels differed among the three biopsy subgroups of Group III. Analysis of variance on the log-transformed data revealed significant differences in bile acid levels ($p < 0.05$) among groups. Moreover, considering a priori contrasts, patients with chemical liver injury had the highest mean value, which was significantly greater than that in normal subjects, and therefore in patients with nonchemical liver disease ($p < 0.05$). However, the mean cholylglycine level in patients with liver disease as a whole (chemical liver injury and nonchemical liver disease pooled) was not significantly different from that in normal subjects. Geometric mean level of cholylglycine was 47.9 μg/dl in patients with chemical liver injury, 19.1 in patients with nonchemical liver disease, and 20.0 in normal subj cts. There were similar findings for conjugates of cholic acid (**Table I**).

The sensitivity and specificity were determined by examining individual serum bile acid values in relation to the normal ranges described earlier. For cholylglycine, the sensitivity in chemical liver injury was 40 percent and in nonchemical liver disease was 14.8 percent; the specificity was 80 percent based on the normal group. For conjugates of cholic acid, the sensitivity in chemical liver injury was 26.7 percent and in

**TABLE I Mean Values for Serum Bile Acids and Indocyanine Green Dye Clearance, by Biopsy Group**

| Test (normal range) | Biopsy Subgroup: Chemical Liver Injury (15) | Biopsy Subgroup: Nonchemical Liver Disease (27) | Biopsy Subgroup: Normal Subjects (25) | Analysis of Variance: F | Analysis of Variance: p |
|---|---|---|---|---|---|
| Cholylglycine (0–48 μg/dl) | | | | | |
| Geometric mean | 47.9 | 19 1 | 20.0 | | |
| $Log_{10}$, mean ± SEM | 1.68 ± 0.14 | 1 28 ± 0.09 | 1.30 ± 0.17 | 3.52 | 0.036 |
| Conjugates of cholic acid (0–74 μg/dl) | | | | | |
| Geometric mean | 45.7 | 17.4 | 19.1 | | |
| $Log_{10}$, mean ± SEM | 1.66 ± 0.13 | 1 24 ± 0.08 | 1.2 ± 0.11 | 3.83 | 0.027 |
| Indocyanine green clearance (1.8 –3.6 minutes) | | | | | |
| Mean ± SEM | 4.2 ± 0.6 | 3.2 ± 0.2 | 3.3 ± 0.2 | 3.35 | 0.043 |

nonchemical liver disease, 7.4 percent; specificity was 92 percent.

The indocyanine green clearance half-times revealed similar significant differences among biopsy groups ($p < 0.05$). A priori contrasts again showed that mean indocyanine green clearance was highest in the subgroup with chemical liver injury, significantly greater ($p < 0.05$) than in the normal subgroups or the subgroup with nonchemical liver disease whereas the mean value in patients with liver disease (chemical liver injury and nonchemical liver disease pooled) was not significantly different from that in normal subjects (Table I). The sensitivity of indocyanine green clearance was found to be 77.8 percent in chemical liver injury and 26.1 percent in nonchemical liver disease (40.6 percent for all liver disease). Specificity was determined to be 90.5 percent.

Analysis of the biochemical data (**Table II**) revealed significant differences among the three biopsy subgroups ($p < 0.05$ by analysis of variance) for only one enzyme, aspartate aminotransferase. In this case, the mean value was greatest in the subgroup with nonchemical liver disease, the opposite of the situation found with serum bile acids and indocyanine green clearance.

The relative sensitivity and specificity of serum bile acids (cholylglycine and conjugates of cholic acid) versus standard biochemical tests and indocyanine green clearance in detecting chemical liver injury, nonchemical liver disease, and all liver disease are shown in **Figure 2.** The top panel shows the sensitivity for identifying all types of liver disease; cholylglycine and conjugates of cholic acid provided relatively lower sensitivity whereas gamma-glutamyl transpeptidase had

**TABLE II Mean Values for Biochemical Studies, by Biopsy Subgroup**

| Test (normal range) | Biopsy Subgroup: Chemical Liver Injury (15) | Biopsy Subgroup: Nonchemical Liver Disease (27) | Biopsy Subgroup: Normal Subjects (25) | Analysis of Variance: F | Analysis of Variance: p |
|---|---|---|---|---|---|
| Alkaline phosphatase (42–109 units/liter) | | | | | |
| Geometric mean | 87.1 | 77.6 | 66 1 | | |
| $Log_{10}$, mean ± SEM | 1.94 ± 0.04 | 1 89 ± 0.04 | 1.82 ± 0.03 | 2.45 | 0.094 |
| Aspartate aminotransferase (11–32 units/liter) | | | | | |
| Geometric mean | 24.0 | 31 6 | 20.9 | | |
| $Log_{10}$, mean ± SEM | 1.38 ± 0.11 | 1.50 ± 0.06 | 1.32 ± 0.04 | 3.42 | 0.039 |
| Alanine aminotransferase (1–36 units/liter) | | | | | |
| Geometric mean | 17 0 | 25.7 | 13 8 | | |
| $Log_{10}$, mean ± SEM | 1.23 ± 0.11 | 1 41 ± 0.09 | 1.14 ± 0.07 | 2.89 | 0.063 |
| Gamma-glutamyl transpeptidase (10–41 mU/liter) | | | | | |
| Geometric mean | 44.7 | 43.7 | 25 1 | | |
| $Log_{10}$, mean ± SEM | 1.65 ± 0.16 | 1.64 ± 0.08 | 1.40 ± 0.07 | 2.55 | 0.087 |

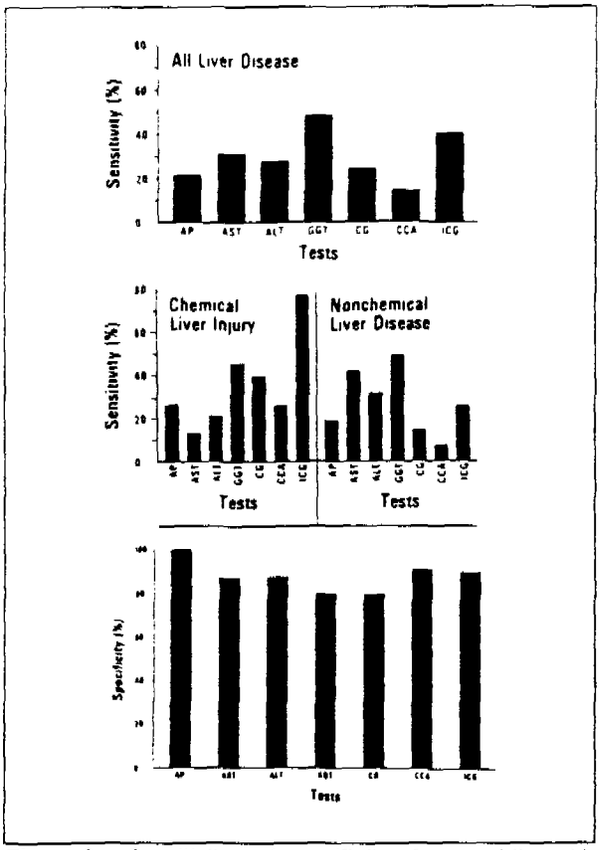


***Figure 2.*** *Sensitivity and specificity of serum bile acids (cholylglycine and conjugates of cholic acid) versus standard biochemical tests and indocyanine green clearances in detecting chemical liver injury, nonchemical liver injury, and all liver disease. AP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyl transpeptidase; CG = cholylglycine; CCA = conjugates of cholic acid; ICG = indocyanine green.*

the highest, followed by indocyanine green clearance. The middle panel shows the sensitivity for chemical liver injury and nonchemical liver disease; indocyanine green clearance was by far the most sensitive in detecting chemical liver injury, followed by gamma-glutamyl transpeptidase and cholylglycine. The bottom panel illustrates the specificity of the tests; specificity increased, in turn, with gamma-glutamyl transpeptidase, cholylglycine, alanine aminotransferase, aspartate aminotransferase, indocyanine green, conjugates of cholic acid, and alkaline phosphatase, the latter showing 100 percent specificity.

The ratio of frequency of positivity of these test results in chemical liver injury relative to nonchemical liver disease was determined (**Figur 3**). Levels of the parenchymal enzymes (aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase) were more frequently elevated in nonchemical liver disease, whereas results of tests that reflected overall hepatic clearance (serum bile acids, indocyanine green) were far more often positive in chemical liver injury.

Examining average exposure indexes in the different biopsy subgroups revealed that the highest exposure rankings (4 or greater) were found in seven (50 percent) of 14 patients with chemical liver injury but in only 11 (21.6 percent) of 51 without chemical liver injury, five (19.2 percent) of 26 with nonchemical liv r disease, and six (24 percent) of 25 normal subjects, supporting the relationship between average and cumulative exposure indexes and the specificity of the histologic findings on liver biopsy. The association of chemical liver injury histologic characteristics with high exposure ranking was statistically significant (chi-square 4.4; odds ratio 3.64; 95 percent confidence interval 1.08, 12.2).

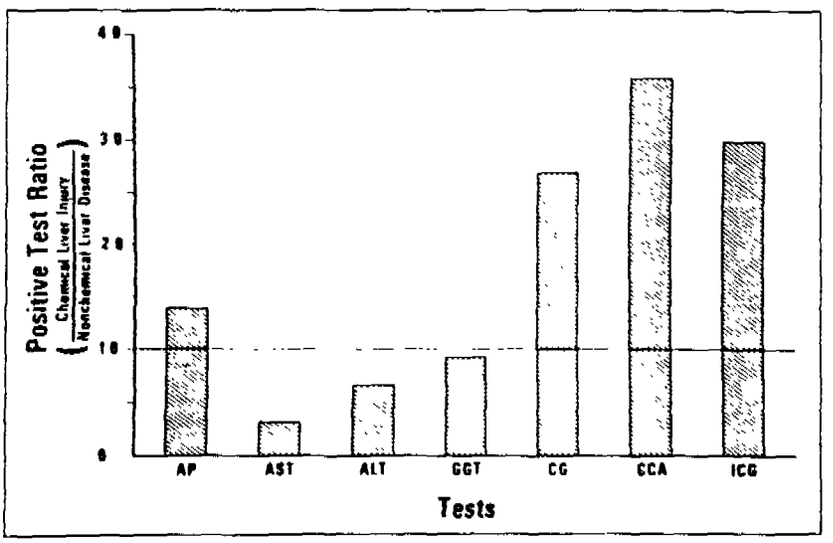


***Figure 3.*** *The ratio of frequency of positivity in chemical liver injury versus nonchemical liver disease. Abbreviations as in Figure 2.*

**TABLE III** **Frequency of Abnormality of Liver Function Test Results in Entire Population by Cumulative Vinyl Chloride Exposure Category**

| Vinyl Chloride Exposure Category | Test | | |
|---|---|---|---|
| | Cholylglycine | Conjugates of Cholic Acid | Indocyanine Green Clearance |
| 20–500 | 22/238 (9.2%) | 15/238 (6.3%) | 4/190 (2.1%) |
| 500–900 | 6/123 (4.9%) | 2/123 (1.6%) | 4/115 (3.5%) |
| 900–1,200 | 5/62 (8.1%) | 5/62 (8.1%) | 3/50 (6%) |
| 1,200–1,600 | 5/47 (10.6%) | 4/47 (8.5%) | 1/57 (1.8%) |
| >1,600 | 4/25 (16%) | 3/25 (12%) | 2/18 (11.1%) |

Finally, the frequency of abnormalities of liver function in the entire population determined for the various cumulative exposure categories is shown in **Table III.** A trend is suggested by the increasing frequency of abnormality with increasing exposure category (dose-response relationship) for both serum bile acids and indocyanine green clearance. The trend was statistically significant ($0.01 < p < 0.025$) for indocyanine green clearance, but did not quite reach statistical significance for cholylglycine and conjugates of cholic acid.

## COMMENTS

This study has illustrated that mean serum bile acid levels are significantly greater in asymptomatic exposed chemical workers with histologically proved chemical liver injury than in those workers with nonchemical liver disease or with normal findings on biopsy. A similar relationship is seen with indocyanine green clearance values but not with the conventional biochemical tests. In severe or massive hepatic injury, all these tests have high sensitivity. This capability, however, is clinically irrelevant when a test's suitability for the identification of early chemical injury among asymptomatic workers is concerned. Therefore, the clinical significance of these results is related to the increasing need for means of identifying the causal agent in chemically exposed persons who are discovered to have persistent liver enzyme abnormalities. Determinations of indocyanine green clearance and bile acids provide a better means of differentiating the underlying causative agent(s) for such abnormalities. Liver enzyme abnormalities in the presence of normal results of bile acid studies are more characteristic of asymptomatic persons with histologically acute-type cellular injury. In contrast, persistent enzyme abnormalities with elevated levels of serum bile acids are shown to be more indicative of persistent subclinical injury associated with increased collagen deposition and vascular changes due to low-grade chemical exposure.

The predictive valu s of such tests are greatly dependent upon the prevalence of the disease they are to detect. We can provide an approximation of their predictive value only by using a hypothetic model since the actual prevalence of chemical liver injury cannot be determined without obtaining a liver biopsy specimen in all exposed workers with biochemical abnormalities. If the assumption is made that our biopsy group has identified *all* cases of chemical liver injury (the lowest prevalence possible), then the prevalence rate would be 13 per 1,000 exposed workers. If our biopsy group reflects the distribution of disease throughout the cohort and the biochemical screening tests have identified all cases (reasonable assumption since we have three to seven-year follow-up on 90 percent of all workers), then the highest prevalence rate for chemical liver injury would be 30 per 1,000 exposed workers. The positive predictive values for alanine aminotransferase, gamma-glutamyl transpeptidase, conjugates of cholic acid, cholylglycine, and indocyanine green clearance were 5, 7, 9, 6, and 21 percent, respectively, in a working population with the aforementioned assumption regarding prevalence of chemical liver injury. It can be seen that conjugates of cholic acid bile acid test and indocyanine green clearance have better predictive value than the most sensitive (gamma-glutamyl transpeptidase) and most specific (alanine aminotransferase) of enzyme tests.

The positive predictive values of these tests are lower than is usually accepted for clinical use. It must be kept in mind, however, that we were screening for latent subclinical liver injury in an asymptomatic population at high risk of exposure. These persons were not the customary symptomatic patients with clinically overt liver disease seen in the hospital.

This is an important difference. Physicians are often called upon to rule out occupationally related hepatic injury in an asymptomatic person incidentally discovered to have persistent enzyme abnormalities. A positive screening result will either direct the patient to further medical workup for nonoccupational disease or indicate a need to remove the patient from a toxic environment until the exact nature of the hepatic dysfunction is determined. Serum bile acids, especially

conjugates of cholic acid, provide a suitable substitute for indocyanine green clearance as a means of differentiating these types of causes.

Essentially all previous studies examining serum bile acids considered hospitalized or ill patients or at least persons being assessed for overt clinical liver disease. Moreover, comparisons between these studies are limited by the heterogeneous types of hepatic disease considered and the methods of serum bile acid determination (enzymatic and gas-liquid chromatography) that preceded radioimmunoassay.

Although it is not certain that vinyl chloride monomer exposure alone is accountable for all the differences observed, several pieces of evidence suggest it. First, the subgroups were formed on the basis of "a priori" identification of pathologic features shown to be characteristic of vinyl chloride-type chemical liver injury. Second, greater mean cumulative and average exposure ratings for vinyl chloride (but not for any other of 22 chemicals assessed) were observed in the group with chemical liver injury. Third, in this analysis, tissue documentation was used as a criterion. The alcohol-related type injury was diagnosed in over half the patients with nonchemical liver disease and in none of the subgroups with chemical liver injury. Thus, alcohol is not a likely explanation for the observed differences. Fourth, there is a strong suggestion of a dose-response relationship between the frequency of abnormality of serum bile acid and indocyanine green clearance test results and increasing cumulative exposure category. However, we have observed but do not yet have a definitive explanation for the relatively high proportion of abnormalities in the low exposure category in the overall worker population. Future sequential studies of these abnormalities may provide an explanation.

The differences in the frequency with which results of clearance tests versus enzyme tests were positive in the two groups with liver disease (chemical liver injury/nonchemical liver disease ratio less than 1) probably reflect the more cytotoxic nature of the cellular injury seen in nonchemical liver disease, due to alcohol, drugs, and/or viral hepatitis. On the other hand, long-term, low-level chemical injury to the liver tends to have less observable cytotoxicity and more evidence of chronic injury with fibrotic repair. The less frequent enzyme elevations and more frequently abnormal serum bile acid levels and indocyanine green clearance (chemical liver injury/nonchemical liver disease ratio greater than 1) better reflect the type of overall functional impairment of the hepatocytes.

Although there were no significant differences in the biochemical studies between the subgroup with nonchemical liver disease and the normal subgroup, the mean value for all tests was greater in the subgroup with nonchemical liver disease than in the normal subgroup. The absence of significance is not unusual considering the mild nature of the liver injury in the subgroup with nonchemical liver disease and the variability in enzyme test values. It is not unexpected that the sensitivity of serum bile acids found in our present study is below that found in various studies in the literature [12]. The subjects in those studies were, for the most part, symptomatic hospitalized patients with more cytotoxically severe and acute disease. A few studies have attempted to look primarily at anicteric subjects or those with minor hepatic dysfunction. In 16 anicteric subjects with chronic liver disease (chronic active hepatitis, alcoholic cirrhosis, and primary biliary cirrhosis) studied by Gilmore and Thompson [24], fasting concentrations of bile acids by the enzymatic method were abnormal in 10 (63 percent). Kobayashi et al [36] investigated 70 patients with histologic evidence of liver disease but with normal results of routine liver function tests. They found a sensitivity of 24 percent for fasting serum bil acid concentrations (enzymatic method), which compares closely with that in our study. Their patients included 10 with minimal methotrexate-induced liver injury, 13 with hepatic sarcoidosis, seven with resolving viral hepatitis with bridging necrosis, 12 with fatty infiltration of unknown cause, and 28 with miscellaneous hepatic lesions. Douglas et al [20] examined fasting and postprandial levels of serum bile acids by radioimmunoassay in 20 patients with anicteric liver disease who had only minor abnormalities on conventional liver function tests. The diagnoses included alcoholic hepatitis in six, alcoholic cirrhosis in eight, and one case each of nonspecific reactive hepatitis, alcoholic h mosiderosis, cirrhosis with hemosiderosis, alpha$_1$-antitrypsin deficiency cirrhosis, and mild alcoholic liver disease. Bilirubin level was abnormal in nine of 20, alanine aminotransferase level abnormal in four of 20, aspartate aminotransferase level abnormal in seven of 20, alkaline phosphatase level abnormal in four of 20, and gamma-glutamyl transpeptidase level abnormal in 10 of 20. The authors found abnormal fasting cholate levels in seven of 20 and abnormal postprandial cholate levels in 10 of 20. These studies illustrate the higher frequency of liver disease detection by functional type tests (bilirubin and bile acids) when the disease process is mild in activity and/or chronic and cumulative in its severity. Comparisons between studies, however, are limited because of the heterogeneity in the types of liver injury and the various pathologic stages. Consistent with our findings is Berk et al's [37] observation of abnormal cholylglycine clearance as the sole liver dysfunction present in a case of persistent vinyl chloride-induced liver injury.

A potential limitation to this prospective study might

be the time relationship between the performance of the liver biopsy, biochemical studies, and the serum bile acid determinations and the indocyanine green clearances. Three factors mitigate against the time differences influencing the results. First, there is the nonprogressive nature of these early histologic lesions associated with vinyl monomer chemical injury. Second, the microcirculatory rather than the hepatocellular nature of the injury, which has already been described [8,9], is highly correlated with increased collagen deposition and appears to be persistent. Third, the permanent nature of the histologic lesion has been documented by serial biopsy in the same person [28,29]. Finally, the results for each test were similar in each biopsy subgroup between simultaneous and nonsimultaneous determinations as shown before.

In conclusion, fasting serum bile acid levels, especially conjugates of cholic acid, provided a high degree of specificity (i.e., very few false-positive results) in persons with normal biopsy findings—a highly desirable charact ristic for a diagnostic test to be used in asymptomatic populations. Fasting serum bile acid levels, because of their lower sensitivity in detecting acute but mild cytotoxic disease, are not appropriate for use as a screening test by themselves. They do, however, when used in combination and in the proper sequence, identify those among the exposed with a very high probability of having low-grade, chronic, or progressive chemical liver injury and needing more extensive clinical investigation. The low sensitivity may be improved by use of an exogenous load, preferably by the oral route [23,24], but this remains to be demonstrated.

Finally, the serum bile acid determinations provide a clearance-type liver test with greater acceptability with regard to time, cost, storage of blood samples, and ease of performance than does indocyanine green clearance. This diagnostic use of bile acids should continue to undergo further validation in other distinct types of hepatotoxic liver injury.

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