Serum Bile Acids in Patients with Liver Disease

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By means of a sensitive fluorometric technic, serum bile acids were determined in patients with various liver diseases. Correlations were shown between the bile acid values and those of transaminase and alkaline phosphatase in cases of liver metastases, and bile acid and transaminase values in cases of viral hepatitis. For most clinical purposes, however, the determination does not yield information which cannot be obtained more readily using currently accepted methods.

The synthesis of bile acids and their transport to the intestine are important functions of the liver. In diseases of the liver it would be expected that the serum levels of the bile acids would be altered. However, because of the previous lack of a sensitive assay procedure, relatively few studies are reported of serum bile acid levels in disease states and their correlation with other liver function tests (1, 2). The purpose of this study was to use a rapid and sensitive fluorometric technic (3) for the assay of total bile acids in the serum of patients with and without liver disease, and to correlate these values with serum alkaline phosphatase and glutamic oxaloacetic transaminase activities and the concentration of total serum bilirubin. In addition, several patients were followed sequentially over a period of weeks in order to correlate these various biochemical parameters with the clinical status of the patients.

Methods and Materials

The total bile acid concentration in serum from fasting individuals was determined by the method of Levin and Johnston (3). In this procedure, 2.0 ml. of serum are deproteinized with ethanol, and the resulting alcoholic solution is extracted with hexane and ether to remove

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extraneous lipid material. After evaporation, the residue (from the alcoholic solution) is saponified with sodium hydroxide. Extraction of the acidified hydrolysate with ether yields the bile acids in their free form. Subsequent incubation of suitable aliquots with concentrated sulfuric acid for 60 min. at 65° results in the formation of fluorescent solutions, which are then read in a Farrand filter fluorometer. Total bilirubin was determined by the method of Malloy and Evelyn (4); alkaline phosphatase, by an automated technic (5); and glutamic oxaloacetic transaminase activity, by the method of Karmen (6), except that the reaction took place in a Beckman DU Spectrophotometer equipped with thermospacers to maintain the temperature at 37°. The upper limits of normal (2 standard deviations above the mean) are 1.5 mg./100 ml., 4.0 Bodansky U., and 40 U./ml. for total bilirubin, alkaline phosphatase, and transaminase, respectively.

In a previous study (3), 44 patients free of hepatobiliary disease were found to yield a serum bile acid range of 0–2.5 mg./100 ml. The mean value was 1.2 mg./100 ml. with a standard deviation of 0.6. In the present investigation, a review of records yielded a series of 43 patients who showed no record of hepatobiliary disease and who had no biochemical evidence of liver dysfunction; these were used as a control group. A total of 51 samples from these patients yielded a serum bile acid range of 0.3–3.0 mg./100 ml. serum with a mean of 1.3 and a standard deviation of 0.7, in agreement with previously established values.

Of 22 patients with liver damage used in this study 2 had cirrhosis due to chronic alcoholism; 2, obstructive jaundice; 10, carcinoma metastatic to the liver; 1, primary cancer of the liver; 3, viral hepatitis; 4, toxic hepatitis; and 6, cancer of the small bowel. During the course of the study, each of these patients had 1–12 serum bile acid determinations.

Results

Correlations with Alkaline Phosphatase, Total Bilirubin, and Transaminase

The results of the bile acid determinations for the 22 patients with liver disease are shown in Fig. 1. The values depicted are the highest obtained for each patient during a sequential study. The upper limit of the control values was 2.7 mg./100 ml. serum (2 standard deviations above the mean).

Figure 2 shows the correlations between the values for serum bile acids and the values for transaminase, total bilirubin, and alkaline phosphatase, in 13–20 specimens taken at various intervals from 3 patients with viral hepatitis. Correlations were considered significant when \( p < 0.01 \). The correlation coefficient obtained for the transaminase activity and
serum bile acid concentration was $+0.79$ ($p < .001$). Significant correlations were not obtained between the values of total bilirubin and serum bile acid or between alkaline phosphatase activity and serum bile acid concentration. Figure 3 shows the correlations between these liver function tests in 10 patients with liver metastases and 6 patients with cancer of the small bowel. The latter were included since the liver is the chief site of metastases from carcinoma of the colon and rectum (7). Significant correlations ($+0.76$, $p < .001$) were obtained between serum bile acid and transaminase activity and between serum bile acid and alkaline phosphatase activity.

**Sequential Studies**

Sequential studies, correlating the serum bile acid levels with the clinical status and other parameters, were performed on approximately 10 patients for 2–6 weeks, to determine whether additional information about liver function would be yielded. Of these cases, 2 are presented for illustration. The correlations of transaminase, alkaline phosphatase, and bilirubin with the clinical course of viral hepatitis or metastatic carcinoma of the liver have been considered previously (8–10) and will not be discussed in any detail.

**Case Reports**

**Case I**

A 41-year-old male physician was admitted on Feb. 28, 1963 with malaise, nausea, and mild abdominal pain of 10 days' duration. Scleral icterus had been noted 2 days before, and
viral hepatitis was diagnosed. Our biochemical studies began on the day of admission. As shown in Fig. 4, serum bile acid and total bilirubin values and transaminase activity paralleled one another, reaching maximum levels 13 days after admission (Mar. 11), and then slowly decreased to normal, the bile acid level returning earlier than that of bilirubin or transaminase. The size of the liver increased to a maximum during the period of Mar. 12-16 and then decreased.

Case 2

A 36-year-old man had a biopsy in January 1962 for a tumor in the neck, and histological examination indicated reticulum cell sarcoma. The growth was excised but 11 months later, in December, roentgenograms showed the presence of vertebral lesions. Radiation to the spine was given between Dec. 28, 1962 and Jan. 11, 1963. The patient was readmitted on Jan. 24 because of extreme weakness, loss of weight, and hepatosplenomegaly. The liver enlargement was thought to be due to reticulum cell sarcoma involvement, and Prednisone* (100 mg. per day) was administered. The clinical picture rapidly improved and on Feb. 8, the Prednisone dosage was lowered to 25 mg. per day. The spleen and liver began to enlarge and the Prednisone dose was increased to 50 mg. Hepatosplomnemegaly increased in spite of a further increase in Prednisone dosage.

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**Fig. 2 (left).** Correlation between serum bile acids and other biochemical parameters in viral hepatitis showing, for transaminase, n (number of samples), 19, t, 5.46, p < .001; for total bilirubin, n, 20, t, 2.50; and for alkaline phosphatase, n, 13, t, 0.39. **Fig. 3 (right).** Correlation between serum bile acids and other biochemical parameters in liver metastases showing, for transaminase, n, 17, t, 4.52, p < .001; for total bilirubin, n, 18, t, 1.80; and for alkaline phosphatase, n, 15, t, 4.23, p < .001.
Our biochemical observations began on Feb. 22. On Mar. 1, the serum bile acid value was elevated substantially, to 8.0 mg./100 ml., and the alkaline phosphatase to 24 U., but the transaminase activity was 52 U./ml., only slightly above normal. On the previous day the total bilirubin value had been elevated to 10.5 mg./100 ml. Radiation to the liver was begun on this day and continued for 1 week. Prednisone was increased to 150 mg. per day on Mar. 3. The size of the liver began to decrease and the patient became more comfortable. During this period, the total bilirubin and serum bile acid levels decreased (Fig. 5). Alkaline phosphatase was not determined during this interval. The transaminase activity remained unchanged, slightly above normal. From Mar. 6 to 7, the patient had severe pain over the liver, and it was the clinical opinion that he probably had hemorrhaged or suffered an infarction of the liver. The bile acid level rose to 8.0 mg./100 ml. on Mar. 7 and then decreased. Radiation to the liver was reinstituted Mar. 13–20, but the clinical state of the patient continued to deteriorate. The transaminase activity, which had been elevated only slightly above normal was unresponsive. The patient was discharged on Mar. 27; his condition worsened and he died on Apr. 18.

**Discussion**

Many attempts to measure the bile acids in blood are cited in the literature (11). In several of these studies, only serum cholates were determined because of the relative specificity of the Pettenkofer reaction and its modifications (11). The total bile acid concentration in blood has also

![Fig. 4 (left). Results of sequential study of serum bile acids and other biochemical parameters in Case 1 (viral hepatitis). Dashed lines indicate upper limit of normal. Fig. 5 (right). Results of sequential study of serum bile acids and other biochemical parameters in Case 2 (intrahepatic metastases). Dashed lines indicate upper limit of normal.](image-url)
been measured by various ultraviolet spectrophotometric technics. The values for total bile acids obtained with these several methods have not been in very close agreement, normal values ranging from 0 to 40 mg./100 ml. (11). The probable reason for such a wide range of values has been discussed previously (3). Osborn et al. (12), using a method not described in detail but stated to involve countercurrent distribution, paper chromatography, and fluorometric determination, reported that the normal serum could not contain more than 1–2 mg. of total bile acid per 100 ml. The values which they obtained in patients with liver dysfunction could not be related to the severity of the diseases. The preceding methods (1, 12) are characterized by poor recoveries (30–80%) of added conjugated bile acids. In contrast, the method used in the present study (3), has been shown to yield 99% (S.D., 12%) of added conjugated bile acids.

The mechanisms of elevation of the 4 biochemical parameters considered in this study have been explored by several investigators (9, 10, 13–16). Although the hepatocellular and obstructive components of liver damage are rarely found independently, the former is characterized by high values of transaminase activity, ranging from 500 to 2500 U./ml. (13), slight or moderate elevations of alkaline phosphatase (13), and average maximal values of about 7 mg./100 ml. for bilirubin (10). In obstruction due to intrahepatic or extrahepatic metastases, alkaline phosphatase activity may be quite high, up to 60–80 U. (15, 16), but transaminase activity is elevated in only about 50% of the cases, and then usually to not more than approximately 200 U. (16). The bilirubin level may be quite low in many cases of intrahepatic obstruction (9) but may range up to 20 or 30 mg./100 ml. in other cases, particularly in extrahepatic obstruction (9). Josephson (14) has indicated that elevations of serum bile acid may occur in either hepatocellular damage or in obstructive disease of the liver.

Our results, showing elevations of bile acid in viral hepatitis, agree with those in the literature (11, 14), and in addition, demonstrate a significant correlation between such elevations and those of transaminase. However, the latter determination is much more responsive, showing elevations 12–60 times the upper limit of normal. With respect to liver metastases, our results agree with those in the literature in showing substantial elevations of alkaline phosphatase (16); in addition, a significant correlation between this criterion and the elevations of serum bile acids is demonstrable. The correlation between transaminase and serum bile acids is also significant, but it may be noted that, as reported in the literature (16), the elevations in transaminase are quite modest, most of them ranging only up to 100 U.

The present study shows that even when a sensitive quantitative meth-
od for determinations of bile acids is available, it adds little as a diagnostic acid to methods currently available.

References