

elucidate whether they were able to differentiate intrahepatic and extrahepatic cholestasis. The LP-X concentration was measured by the methods described by Ritland where ( $^3\text{H}$ ) cholesterol was incorporated into lipoprotein of patient's serum in vitro followed by the electrophoretic separation and determination of radioactivity in LP-X. Concentration of LP-X was calculated according to the equation where free cholesterol content in LP-X was assumed 23%.

LP-X (mg/dl) = serum free

$$\text{cholesterol (mg/dl)} \times \frac{{}^3\text{H in LP-X}}{\text{total } {}^3\text{H}} \times \frac{100}{23}$$

The mean LP-X concentration in 6 patients with extrahepatic biliary obstruction was  $374 \pm 207$  mg/dl (mean  $\pm$  sd) and was significantly higher than the mean concentrations observed in the patients with intrahepatic cholestasis. The mean level of LP-X in cholestatic states observed in the patients with acute hepatitis, cirrhosis of the liver and hepatoma were  $46 \pm 36$  mg/dl (9),  $9 \pm 4$  mg/dl (5) and  $4 \pm 1$  mg/dl (5), respectively (mean  $\pm$  sd (no. of cases)). All patients with extrahepatic biliary obstruction revealed LP-X levels exceeding 150 mg/dl, whereas the levels were below 150 mg/dl in the cases of intrahepatic cholestasis.

The LP-X concentrations correlated significantly with free cholesterol concentration in the serum ( $r = +0.881$ ,  $n = 34$ ). But for the purpose of differentiation of intra and extrahepatic cholestasis, the levels of LP-X were more effective than free cholesterol concentrations.

The LCAT activities in the serum were determined according to the method of Stokke and Norum

where ( $^3\text{H}$ ) cholesterol was incorporated into free cholesterol pool of the patients' serum lipoproteins followed by the determination of esterification rate after one hour incubation at  $37^\circ\text{C}$ . The LCAT activities were expressed as n moles of cholesterol esterified/ml of serum/hour.

The mean LCAT activity in 23 normal subjects was  $59.6 \pm 14.4$  n moles/ml/hour (mean  $\pm$  sd). The LCAT values of patients with resolving stage of acute hepatitis, inactive form of chronic hepatitis, active form of chronic hepatitis, cirrhosis of the liver, hepatoma and extrahepatic biliary obstruction were  $46.8 \pm 16.6$  (10),  $58.2 \pm 15.4$  (7),  $41.6 \pm 12.4$  (14),  $22.4 \pm 4.7$  (5),  $22.4 \pm 12.9$  (6) and  $34.6 \pm 21.9$  (3), respectively (mean  $\pm$  sd (no. of cases)). Among those values, the mean activities in the patients with active form of chronic hepatitis, cirrhosis of the liver and hepatoma were significantly lower than the normal controls.

In comparison with the LCAT activities and other kinds of hepatic function tests, the LCAT activities correlated significantly with the serum levels of esterified cholesterol ( $r = +0.819$ ) and albumin ( $r = +0.730$ ). Negative correlations were observed between the LCAT activities and BSP, ICG and TTT. Correlations between the LCAT activities and GOT, GPT, serum bilirubin and alkaline phosphatase were not significant.

In summary, the determination of LP-X levels in cholestatic patients was a useful measure for the differentiation of intrahepatic and extrahepatic cholestasis. The LCAT activity indicated some aspects of hepatic function.

### Measurement of Intra- and Extrahepatic Shunt Rates Using the Procedure for Percutaneous Transhepatic Portography and Macroaggregated Albumin Labelled with $^{113}\text{I}$ and $^{99\text{m}}\text{Tc}$

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It is very important to assess the degree of intra- and extrahepatic portal-caval shunting in the management of patients with cirrhosis. Various methods have in the past been proposed for the measurement of the shunt rates, but none has been adequate in its practicability and accuracy, each having its inherent demerits. Precise measurement of intra-

and extrahepatic shunt rates separately in one procedure has not yet been achieved. Our technique to that end is now described.

*Material and method.* A total of 16 patients — 2 cases of liver steatosis, 2 of chronic hepatitis and 12 cirrhosis — have been studied. The diagnosis was based on laparoscopy and biopsy. Under TV con-

trolled fluoroscopy, a 27 cm sheathed needle (catheter) with an outer diameter of 1.35 mm, is inserted from the right flank as is done in transhepatic cholangiography. When an intrahepatic portal branch is entered, the needle is withdrawn, and the catheter is advanced following insertion of a guide-wire, into the portal trunk and further into the splenic vein. After portography has been made, 5 mCi of  $^{99m}\text{Tc}$ -MAA is instilled at the splenic hilum, the catheter is pulled back as far as the hepatic porta before the bifurcation and 200  $\mu\text{Ci}$  of  $^{131}\text{I}$ -MAA is instilled.

After scanning for  $^{99m}\text{Tc}$  and  $^{131}\text{I}$  with a window at  $140 \pm 20$  Kev and  $364 \pm 50$  Kev, respectively, the areas for the liver and both lungs are set on a  $64 \times 64$  matrix display and counts are taken. The total shunt index ( $^{99m}\text{Tc}$ ) and intrahepatic shunt index ( $^{131}\text{I}$ ) are calculated in per cent by the formula: lung counts/lung and liver counts. The extrahepatic shunt index (%) is then given:

Total shunt index—Intrahepatic shunt index  
100—Intrahepatic shunt index

*Results.* The intrahepatic shunt index was 4.2 and

5.4%, respectively, in 2 cases of hepatic steatosis, and 4.5 and 15.9% in chronic active hepatitis. It varied from 1.6 to 78.4% in cirrhosis. It tended to increase with the progress of chronic liver disease leading to cirrhosis and eventually to hepatic failure. One patient with an intrahepatic shunt index of 78.4% died from hepatic failure within a half year. The intrahepatic shunt index was correlated well with the degree of liver function impairment. The extrahepatic shunt index varied from 0 to 49.9% in patients with cirrhosis. It was above 14% in patients with esophageal varices and was generally correlated with the absence and presence, and the degree of varix. In a few patients with demonstrable extrahepatic shunting and without esophageal varices, other routes of collateral circulation were suggested.

These data indicate that our procedure for separate measurement of intra- and extrahepatic shunts is worthwhile, providing important information necessary for the assessment of the prognosis and determination of the appropriate therapeutic measure to take.

## S-5 Application of Radioimmunoassay for Diagnose of Liver Disease

### Basic Studies on the Radioimmunoassay of Serum Carcinoembryonic Antigen and its Diagnostic Significance

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A two antibody system for the radioimmunoassay (RIA) of carcinoembryonic antigen (CEA) was established in our laboratory and the specificity of the method was examined with regards to the crossreactivity of non-specific cross-reacting antigens (NCA and NCA2).

The purification of CEA, NCA and NCA2 and the specificity of anti-CEA sera prepared were reported elsewhere (Jap. J. Gastroenterol. 73 (4), 384, 1976). The method of RIA was Egan's double antibody technique modified by Laurence except that 0.2 ml serum samples without perchloric extraction were used. The titer of anti-CEA serum was set to achieve a 50% binding of  $^{125}\text{I}$ -CEA (50  $\mu\text{Ci}/\mu\text{g}$ , 15,000 cpm/0.1 ml). Usually the absorbed anti-serum was diluted from 1:4,000 to 1:10,000.

In this RIA, an amount of NCA even greater than 10,000 ng failed to inhibit significantly the antibody binding of the hot CEA, indicating that the influence of NCA in the practical assay of serum CEA may be negligible. On the other hand, the amount of NCA2 to achieve 50% inhibition on antibody binding of the hot CEA was 15,000 ng. This corresponded to 7 ng of the standard CEA used. Such crossreactivities of NCA and NCA2 were noted in Roche, Dainabot and CS RIA systems.

Analyses by gel filtration and isoelectrofocusing showed that serum CEA may be composed of heterogeneous molecules which are "immunoreactive" in the RIA used. Therefore, we would express CEA levels as unit/ml of the CEA immunoreactivity; 1 ng/ml of the standard CEA of our laboratory was