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 When an intrahepatic portal branch is entered, the needle is withdrawn, and the catheter is advanced following insertion of a guide-wire, into the portal trunk and further into the splenic vein.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 veen made, 5 mCi of ^{99m}Tc-MAA is instilled at the splenic hilum, the catheter is pulled back as far as the hepatic porta before the bifurcation and 200 μCi of ^{131}I-MAA is instilled.

After scanning for ^{99m}Tc and ^{131}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}Tc) and intrahepatic shunt index (^{131}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 therepeutic 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

Akira YACHI, Akiko ARAKI and Makoto KAWAHARADA

Department of Internal Medicine, Division I, Sapporo Medical College

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}I-CEA (50uCi/ug, 15,000 cpm/0.1 ml). Usually the absorbed antiserum 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 heterogenous 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

tentatively decided to be 1 u/ml.

Diagnostic significance of serum CEA determination was evaluated in neoplastic and non-neoplastic diseases. The upper limit was serum CEA in healthy control was 10 u/ml. A slight elevation of the level was found in the aged. In malignant diseases, elevation was found in 33/50 (66%) for colorectal cancer, 80/200 (40%) for gastric cancer and in 25 to 60% for other cancers. The classification of stages of gastric carcinoma was possible in 88 patients. In early gastric cancer, stage I, the levels were elevated in 3/22, while in the advanced stages II, III and IV an increase was observed in 1/10, 10/32 (37%) and 27/39 (69%) of the cases, respectively. An especially high level was noted in 16/18 (89%) of patients of the stage IV with liver metastasis. In primary hepatoma, slightly elevated values were found in 11/20 (55%) of the cases. This incidence was higher than 20 to 32% seen in patients with hepatitis and liver cirrhosis.

It is, however, obvious that the simultaneous determinations of CEA and AFP during the clinical course are useful for the differential diagnosis of primary and metastatic liver carcinomata. In addition, despite the fact that CEA assay may not be valuable for the early detection of cancer, it may be useful for monitoring cancer patients during therapy and, especially, for predicting the metastasis to the liver.

Radioimmunoassay of α-Fetoprotein

Shinzo NISHI
Department of Biochemistry, Hokkaido University School of Medicine

Radioimmunoassay (RIA) of α-fetoprotein (AFP) was developed in 1971 and many findings have been accumulated during these five years. Among them I reported about the following three subjects.

1) *SOME PATHOPHYSIOLOGICAL CONDITIONS WITH INCREASED SERUM AFP:* Among malignant diseases, abnormal values (> 20 ng/ml) were found in hepatoma (>10,000 ng/ml 70%, 10,000 ng/ml-20 ng/ml 20%), teratoblastoma (>10,000 ng/ml 40%, 10,000 ng/ml-20 ng/ml 20%) and digestive tracts cancer mostly accompanied with liver metastasis (<10,000 ng/ml very rare, 10,000 ng/ml-20 ng/ml rare). AFP positive teratoblastoma tissues contained yolk sac like elements.

Among non-malignant diseases, moderate elevations of AFP (<1,000 ng/ml-20 ng/ml) were frequently observed (40-50%) in hepatitis and liver cirrhosis and the appearances were transient and related to liver cell regenerations. In some congenital diseases such as ataxia teleagiectasis and tyrosinemia, serum AFP were observed to increase frequently. During the pregnancies of abnormal fetuses with such as open neural tube defects, esophagus atresia and, etc, maternal serum AFP were found to elevate abnormally.

2) *PROPERTIES OF AFP IN NORMAL ADULTS:* AFP of normal adults or patients with liver cirrosis has been suggested to have a mobility of γ-globulin in electrophoresis (C.C.A. 71, 343, 1976). We purified seum AFP from normal adults and it migrated between α_1-globulin and albumin indicating that it was not different from AFP of fetus or hepatoma concerning the electrophoretic behavior as well as immunoreactivity.

3) *DEVELOPMENT OF A NEW RIA:* RIA in current uses are based on the competitive binding reaction of radioiodinated antigen and antigen in sample to a limited amount of antibody. We developed a more sensitive and simpler method based on sandwich technique using filter paper discs coated with antibody to AFP and the radioiodinated antibody. Filter paper disc with a diameter of 5 to 6 mm was activated with BrCN and coupled with the antibody. Antibody dis was incubated with serum specimen and after washing with saline reacted with the radioactive antibody. The radioactivities of the discs were proportional to the amounts of antigen adsorbed on the discs and AFP values were estimated from the radioactivities. The method covered a range as wide as 1 ng/ml-1,000 ng/ml and was sensitive 1 ng/ml of AFP being able to be measured with a satisfactory reproducibility.