

follows;

20 $\mu$ C of  $^{131}\text{I}$  RB in 1 cc of physiological saline solution was injected into the marginal ear vein of adult male rabbit, weighing ca. 2 Kg. After the injection of  $^{131}\text{I}$  RB, radioactivity in the liver was measured by  $\gamma$ -spectrometer with the lead collimator for rabbit liver measurement.

Uptake and excretion curves of  $^{131}\text{I}$  RB of the rabbit liver consisted of two phases, the initial phases by  $^{131}\text{I}$  RB uptake and the followed phase by excretion of  $^{131}\text{I}$  RB as described by Lowenstein (1956).

The excretion curve consisted of the first phase curve ( $E_1$ ) and the second phase curve ( $E_2$ ) in any case.

The  $^{131}\text{I}$  RB uptake curve were differentiated into three exponential curves, uptake line (U), the second phase excretion line ( $E_2$ ) and the excretion line of the difference between the first and second phase ( $E_1-E_2$ ).

On the non-irradiated rabbits, the mean

time of TU,  $TE_2$  and  $T(E_1-E_2)$  were 5.0, 62 and 16 minutes respectively.

The mean excretion time of the second phase  $TE_2$  of liver irradiated animals was clearly prolonged after 200 R irradiation and the excretion time recovered to the normal state in 6 hours after irradiation. By 500 and 1000 R irradiation, the  $TE_2$ , was markedly prolonged and returned to normal state in 24 hours after irradiation. TU and  $T(E_1-E_2)$  were independent of the irradiation dose. The second phase excretion time ( $TE_2$ ) and its recover time depended on the dose irradiated.

The uptake curve (U) related to the translocation of the  $^{131}\text{I}$  RB from blood to liver and the ( $E_1-E_2$ ) curve was concerned with the excretion of the  $^{131}\text{I}$  RB from blood to the excretion systems, such as urine secretion. The second phase ( $E_2$ ) of the excretion curve showed the excretion function of the liver cells.

### Diagnosis of the Infantile Jaundice by $^{131}\text{I}$ -Rose Bengal Test

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The differential diagnosis between the congenital biliary atresia and the infantile hepatitis with obstructive jaundice is perplexing only by usual laboratory diagnostic manouver.

In case of the atresia, the clear-cut differential diagnosis from the infantile hepatitis is essentially cardinal, because the delay of the diagnosis gives serious damage upon the progress of the disease.

In this report, we discussed the value of the clinical application of the  $^{131}\text{I}$ -Rose Bengal test to the cases of the infantile jaundice admitted to Dept. of Keio Univ. Hospital.

Comparing with the other diagnostic procedures such as usual liver function test, the liver biopsy, the diagnostic operation or the clinical pictures,  $^{131}\text{I}$ -Rose Bengal method is verified to have a most advisable diagnostic value to attain a clear-cut diagnosis.

### Clinical Studies with $^{131}\text{I}$ -Labeled Rose Bengal in Hepatobiliary Diseases

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Besides the external counting over the liver, the disappearance curve of intravenous-

ly administered  $^{131}\text{I}$ -Rose Bengal from blood was determined in patients with hepatobiliary diseases to examine the activity of hepatic uptake and excretion of the dye. From this curve the rates of hepatic uptake and excretion were calculated, and were compared with those obtained by the external counting and with the results of other liver function tests.

From the recorded hepatic uptake-excretion curve the rates of hepatic uptake and excretion were determined by two methods: one was the method by Miwa et al, the other was by Lowenstein. The rate of hepatic uptake determined by the former method was demonstrated by UR and the rate of excretion was by ER. By the latter they were demonstrated by Ku and Ke respectively.

Simultaneously the radioactivity of peripheral venous blood samples drawn serially was measured. From the blood disappearance curve thus determined, the rate constants from blood to liver (hepatic uptake rate, KL), from liver to blood (KL') and from liver to bile (hepatic excretion rate, KB) were calculated with the method by Araki et al.

As to the rates of hepatic uptake, all three levels were paralleled with jaundice index and S-GOT and S-GPT levels. They were favourable levels when the liver was damaged slightly and were decreased when it was damaged seriously. In patients with gallstone who accompanied jaundice, they were decreased. Among three levels, the levels of KU and KL had a good correlation.

As to the rates of hepatic excretion, both levels of ER and Ke were paralleled with jaundice index and with serum alkaline phosphatase level, but the level of KB revealed no correlation with these levels of liver function tests and with ER or Ke.

The level of KL' revealed no correlation with the results of other clinical liver function tests either.

Consequently, the level of KL appeared to provide reliable evaluation of hepatic uptake and agreed with the external counting, but the levels of KB and KL' appeared to be unable to evaluate hepatic excretion and reentry. Therefore, further studies are considered to be necessary.

### Labeling of Bilirubin with Tritium

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The necessity for RI labelled Bilirubin has been marked recently. However, Bilirubin does not contain an element which emits suitable  $\gamma$  ray, and preparation of Bilirubin- $^{14}\text{C}$  is very troublesome, and so instead of it we tried to label bilirubin with  $^3\text{H}$ . 200 mg of Bilirubin was exposed with 10 curies of  $^3\text{H}$  gas for 15 days. After exposure labile tritium was removed from the Bilirubin- $^3\text{H}$  by equilibrating twice in chloroform for 2-3 hrs at room temperature and twice in single-phase solution consisting of chloroform/methanol/water (5:5:1) under the same con-

ditions. To minimize oxidation solvents were removed in the dark by a brisk jet of nitrogen. Bilirubin- $^3\text{H}$  was dissolved in chloroform by boiling 10-20 seconds under reflux and cooled to room temperature. This solution was run down a column of anhydrous sodiumsulphate shielded from light. More than half of the chloroform was then removed by distillation until crystallization starts. To chloroform solution of Bilirubin- $^3\text{H}$  was added ether and cooled to  $-20^\circ\text{C}$ . The precipitate was collected by centrifugation, washed with ethyl ether several times. The