特別掲載

DOUBLE ISOTOPE TECHNIQUE OF MEASURING HEPATIC FUNCTION UTILIZING ^{198}AU COLLOID AND ^{125}I-ROSE BENGAL

by

Akiyoshi Kajita

Department of Radiology Kobe University, School of Medicine

(Director: Prof. Kazuyuki Narabayashi)

^{198}Au Colloid 及び ^{125}I-Rose Bengal 同時使用による肝機能検査

神戸大学医学部放射線医学教室（主任　楢林和之教授）

梶　田　明　義

（昭和42年5月25日受付）

従来の放射性同位元素による肝機能検査としては ^{198}Au Colloid 肝摂取率測定による有効肝血流量の推定や，^{131}I-Rose Bengal による肝細胞胆道系機能検査は各々別個に行なわれてきた．しかし同一症例で，これ等の検査を同時におこなうことは，総合的な肝機能判定上望ましいが，^{198}Au 及び ^{131}I の光子エネルギーのピークが接近している為に各々の選別は困難である．^{131}I-Rose Bengal の代りに ^{125}I-Rose Bengal を応用すれば ^{198}Au Colloid との同時測定は可能となり，ひきつづき肝の面シンチグラムも得る事が出来る．著者は，各種肝疾患の鑑別診断を行う目的で^{198}Au Colloid 肝摂取率及び ^{125}I-Rose Bengal 末梢血中消失率を二重追跡法により求めた．基礎実験として，種々の濃度の ^{198}Au Colloid 及び ^{125}I-Rose Bengal 小容積線源を作り，水ファントームを使用し，前者が後者への測定領域に及ぼす影響を調べた．これに基いて較正図を作製し，臨床的応用を行った．

測定は，左大腿股動脈上に固定した検出器より出る信号を ^{125}I 測定域及び ^{198}Au 測定域に各々セットした2個の γ-spectrometer に導き，見かけの ^{125}I-Rose Bengal及び ^{198}Au Colloid 末梢血中消失曲線を同時に記録した．この見かけの ^{125}I-Rose Bengal 濃度曲線を，基礎実験で得た較正図を用いて補正した．

一方，肝上で体外測定された ^{198}Au Colloid 肝摂取率を縦軸に，^{125}I-Rose Bengal 末梢血中消失率を横軸にとり，各種肝疾患の分布図を作成した．正常肝群は左上方に，急性肝炎群は右上方に位置して肝細胞障害を示し，肝硬変症ではその重症度に応じて正常群を扇のかなめとした様な，放射状の分布を示した．又肝癌では上記のびまん性肝疾患の様な分布は認められないが，本法による経時的観察を行えば，腫瘍の進行に伴ってダイアグラム上，特徴ある方向を示し病状を把握する事が出来た．

I. Introduction

Accurate estimations of the hepatic blood flow and hepatobiliary function are the important procedures in the clinical studies of liver diseases.

The measurement of hepatic blood flow was clinically introduced by Bradley[3] in 1945, and his method was based on measuring the hepatic extraction of Bromsulphalein sodium (BSP).

The method, however, requires catheterization of the hepatic vein and unsuitable to repeat on the same patient.

Dobson and Jones[4)] showed a technically simpler procedure. The clearance rate of radioactive colloid from the blood served for the estimation of hepatic blood flow.

Vetter et al.[12)] applied ^{198}Au colloid for the external estimation of hepatic blood flow.

On the other hand, ^{131} I-Rose Bengal was introduced by Taplin[9)] in 1955, for testing liver and biliary tract function.

Nordyke and Blahd[7)] improved the method of radioactive counting, thus detecting the presence of biliary tract obstruction or of borderline dysfunction of the liver.

At present ,widely used biochemical methods of liver functions only show one aspect of the liver functions, therefore, simultaneous measurement of hepatic blood flow and liver functions using double isotope technique with ^{198}Au colloid and ^{125}I-Rose Bengal (^{125}I-R.B) has been devised by the author so that the metabolic functions of the liver could be understood more comprehensively.[6)]

After experimental studies, this technique has been applied for clinical investigation, not only to differentiate various hepatic diseases but also to examine the course as well as prognosis of these disorders.

II. Experimental Studies

The effective hepatic blood flow has been calculated through ^{198}Au colloidal clearance which shows the phagocytic ability of the reticuloendothelial cells in the liver and the excretory function of hepatic cells has been analysed by the peripheral disappearance curve of ^{131}I-R.B.

It is important to study these two methods at the same time, so that evaluation of the hepatic blood flow and the liver function can be performed simultaneously.

However, it is rather difficult to identify the main photopeaks of ^{198}Au and ^{131}I separately at the same moment, since each photopeak appears very closely and affects each other.

When ^{125}I-R.B is used instead of ^{131}I-R.B, simultaneous measurement with ^{198}Au colloid can be carried out, because photopeak of ^{125}I shows 27.4 KeV which is markedly apart from the photopeak of ^{198}Au (Fig.1).

1) Experimental device

To measure two radioisotopes, ^{125}I-R.B and ^{198}Au colloid by double isotope technique the devices

Fig. 1 Energy spectra and channels of ^{198}Au colloid and ^{125}I-R·B in air and water phantom.

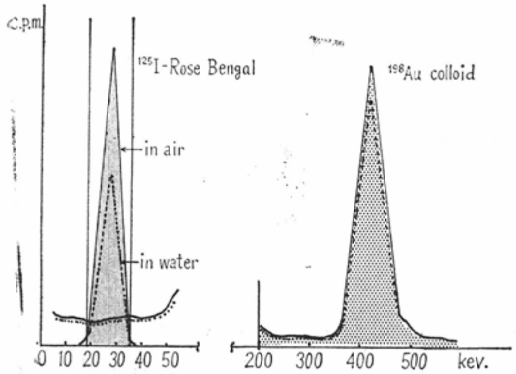
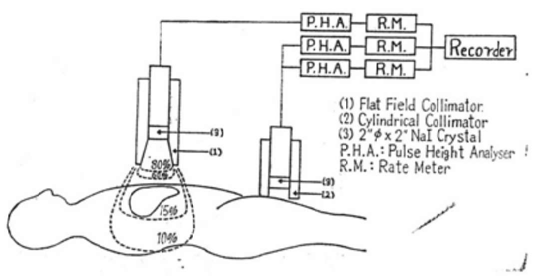


Fig. 2 Blockdiagram of double isotope technique with ^{198}Au colloid and ^{125}I-R·B.



are set as shown in the diagram in Fig. 2.

^{125}I-R.B was analysed by γ-spectrometer Type RSP-101 (Kobe Industries, Kobe), ^{198}Au colloid by Type Es-7 (Shimadzu Seisakusho, Kyoto).

The detector, a NaI crystal 2 $\phi \times 2$ inches fixed with a cylindrical type collimator, was used.

Signals of mixed isotopes from a scintillation probe are fed into two pulse height analysers separately, each of which is attached to a count rate meter in order to distribute between ^{125}I-R.B net counts and ^{198}Au colloid net counts. The outputs of the count rate meters are brought into two channels recorder. For the purpose of calculating the activities of ^{125}I-R.B and ^{198}Au colloid separately, the quantitative relations by equations have to be determined[8].

One of the pulse height analyzers is referred to as the upper channel U (200 KeV discriminated), and the other as the lower channel L (27.4±7.5 KeV). The voltage from the count rate meter attached to U is due to a radioisotope A (^{198}Au colloid), but there is no contribution from ^{125}I-R.B.

Voltage from the count rate meter attached to L is due to a radioisotope B (^{125}I-R.B) and the Compton scatter contribution from ^{198}Au colloid.

2) Phantom test

To determine absolute activities using double isotope technique within internal sourses (left femoral region)from the count rates obtained by external monitoring, corrections must be made as follows[1];

1. Amount of absorber interposed between source and detector.
2. ^{198}Au colloid activity is appeared as Compton scatter over in the ^{125}I-R.B setting.
3. Background activity.

The small bottle sources which have contained ^{198}Au colloid & ^{125}I-R.B solution in various concentration ratio of respective nucleide, were placed into a water phantom at different depths and absorption ratios of these radioisotopes which was equivalent in human femoral ragion were calculated.

3) Results and discussions

^{198}Au colloid which affects the ^{125}I-R.B window is shown by the following formula within a water phantom.

$$U = A + dB \qquad \cdots\cdots\cdots\cdots(1)$$

letting d=0

$$L = B + cA \qquad \cdots\cdots\cdots\cdots(2)$$

Solving (1) and(2)

$$\frac{cU}{L} = 1 - \frac{1}{cR+1} \qquad \cdots\cdots\cdots\cdots(3)$$

Where, A: channel counts of ^{198}Au colloid

B: channel counts of ^{125}I-R.B

U: counts in the upper channel, discriminated at 200 KeV.

L: counts in the lower channel without calibration, differential counting at 27.4 ±7.5 KeV.

c,d: constants to be determined experimentally

R: ratio of channel counts (U/B)

The relationship between the ratio of the two channel counts and calibration rates (cU/L shown in percentage) are plotted in Fig.3, which indicates that when ^{198}Au concentration is increased, calibra-

Table 1 Calibration table

Time in minutes	1— 5	6—10	11—15	16—20	21—30
c%	4± 1	4± 1	5± 1	5± 1	5— 7

^{198}Au colloid peripheral disappearance curves are separated into upper and lower channels. (20 cases)

c = L/U U: Counts in the upper channel
L: Counts in the lower channel

Fig. 3 Calibration Chart.

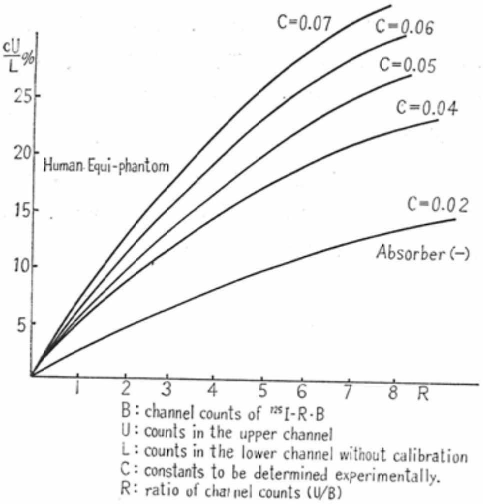
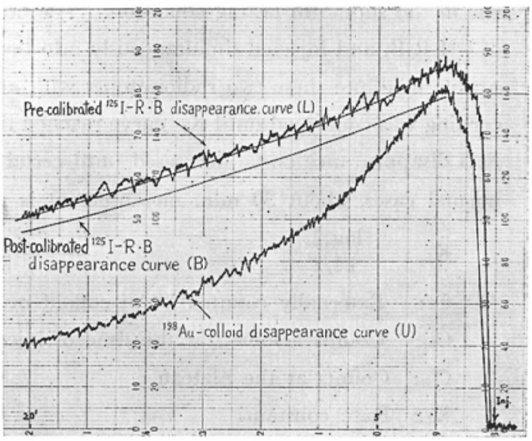


Fig. 4 Example Case. Chronic Hepatitis, K.M. Post-calibrated ^{125}I-R • B disappearanse curve. Range^{198}Au : 200, ^{125}I : 100.



tion rates are also increased.

Utilizing the calibration chart, calculation can be determined simultaneously, despite variable percentage mixture of ^{125}I-R.B and ^{198}Au colloid are found in the peripheral blood.

To prove clinically the calibration rate obtained in Fig. 3,^{198}Au colloid alone was injected i.v. and its signal was separated into U & L channel over the left femoral region and the ratio between L & U was calculated (Table 1, Fig. 3).

As shown in Table 1, 'c' is $5\pm1\%$ from two to twenty minutes.

The ratio (L/U), however, steadily increases as ^{198}Au colloid is taken up by the liver, and then, the back ground activity towards femoral contribution can not be omitted, but this contribution can be minimized by putting more shields.

Summary

1. The physical aspects of the use of ^{198}Au colloid and ^{125}I-Rose Bengal for the hepatic diseases were discussed.

2. The energy spectra of these two nuclei in air and water phantom have been shown.

3. The relationship between the ratio of ^{198}Au colloid and ^{125}I-R.B channel counts is given as follows:

$$cU/L = 1 - \frac{1}{cR+1} \qquad (c=5\pm1\%)$$

4. ^{698}Au colloid and ^{125}I-R.B are applicable for double isotope technique when used calibration chart.

III. Clinical Studies

In order to investigate the hepatic hemodynamics, simultaneous measurement of hepatic uptake rate constant of ^{198}Au colloid (K_L) and ^{125}I-Rose Bengal peripheral disappearance rate by the double isotope technique were performed to the patients with various liver diseases.

1) Method

As mentioned in the experimental section, the instruments were set as the same as shown in Fig. 2.

In Fig. 2, each signals from the liver and femoral region were supplied into three γ-spectrometeres, one by 412 ± 25 KeV differential counting over the liver, the other by ^{125}I-window and 200 KeV discriminated set of γ -spectrometers over the femoral region. The measurements were taken with the patient in the supine in fasting condition. The administered dose was 50 μ Ci of ^{198}Au-colloid, 100 μ Ci of ^{125}I-R.B, and injected simultaneously into the antecubital vein. According to my experience, it is desirable to dilute these two radioisotopes with about 2 ml of physiological saline. The simultaneous recording was continued until K_L curve reached the plateau over the liver region; on the other hand, ^{125}I-R.B disappearance curve decreased until certain external count level over the left femoral region. In normal cases, about 30 min. measurement is necessary. K_L is calculated as follows;

$$K_L = \frac{\log_e 2}{T1/2}$$

Ct: Externally counted ^{198}Au colloid over the liver at time 't'.

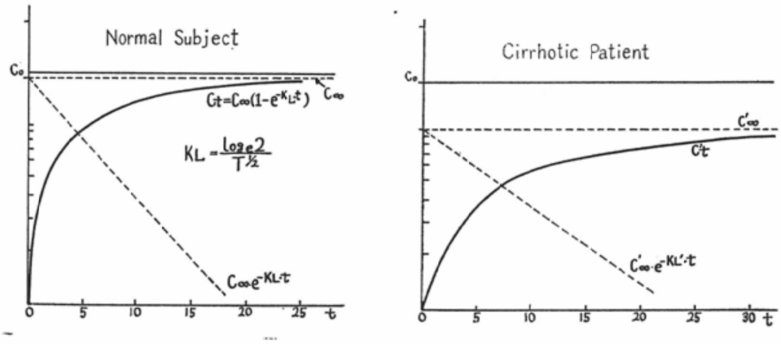
Co: Counts assumed at the time of injection.

C_∞: Counts at the plateau.

K_L: Rate constant.

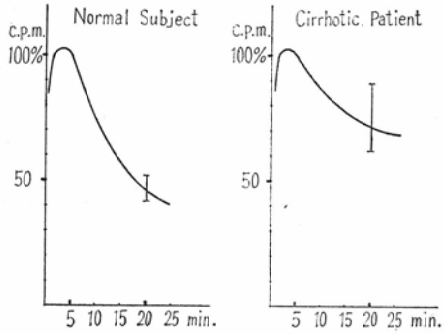
$C_\infty . e^{-K_L . t}$ is replotted on the semilogarithmic scale, and $t = T1/2$ when assumed $C = \frac{Co}{2}$, then K_L is obtained.

Fig. 5 Calculation of hepatic uptake rate constant (K_L) of ^{198}Au colloid in normal and cirrhotic subject.



On the other hand, ^{125}I-R.B disappearance curve can not be accurately calculated unless to exclude the effect of ^{198}Au upon ^{125}I-window and after suitable calibration. Therefore, correction was made in the calibration curve which was mentioned in Fig. 3.

Fig. 6 Calculation of RIRB disappearance rate in normal and cirrhotic subject.



In Fig. 6, RIRB (Radioiodinated Rose Bengal) disappearance rate is calculated as a percentage ratio of 20 minutes to 5 minutes after injection.

Regular biochemical liver function tests were also carried out repeatedly on each case, and in the most patients, the diagnosis was confirmed by biopsy, surgery or autopsy.

2) Results

i) K_L

K_L values of the various liver diseases are shown in Fig. 6.

Normal value fell between 0.15-0.23 (mean 0.18 ± 0.024), while chronic liver disorders such as the chronic hepatitis and liver cirrhosis showed low level. In hepatic tumors, however, it decreased in accordance with the progress of the malignant growth. Though, it is possible to differentiate between normal cases and chronic liver disorders by interpreting the K_L value, as shown in Fig. 7; in acute hepatitis and the early stage of hepatic tumor, the results frequently overlapped each other and differential diagnosis can not be made with this method alone.

ii) RIRB disappearance rate

RIRB disappearance rate after serial administration of ^{125}I-R.B and ^{131}I-R.B on the same patient revealed almost identical result.

In Fig. 8, however, these two were used separately on each other.

^{125}I-R-B disappearance rate in normal cases ranged 42-52 %(mean 47 ± 4.2) and it was high in the impairment of the liver cells or biliary tract, since, RIRB retained longer in the peripheral blood in such disorders.

According to the results, it is possible to differentiate the liver diseases from the healthy individuals with the exception of the metastatic tumors. However, it is found difficult to differentiate into the acute hepatitis, liver cirrhosis and hepatic tumors exclusively by this test.

In general, obstructive jaundice has revealed higher level and the hepatic disorder in heart failure showed various higher levels according to the degree of cardiac dysfunction.

iii) Combination test of K_L and ^{125}I-R.B disappearance rate.

To perform double isotope technique, there are two different methods. The first is, to inject and measure ^{125}I-R.B and ^{198}Au colloid separately; the second is, to inject and measure the two radioisotopes simultaneously. The former, usually, takes about twice as long as the latter and put more burden on

Fig. 7 Hepatic uptake rate constant of ^{198}Au colloid (KL) in various hepatic diseases.

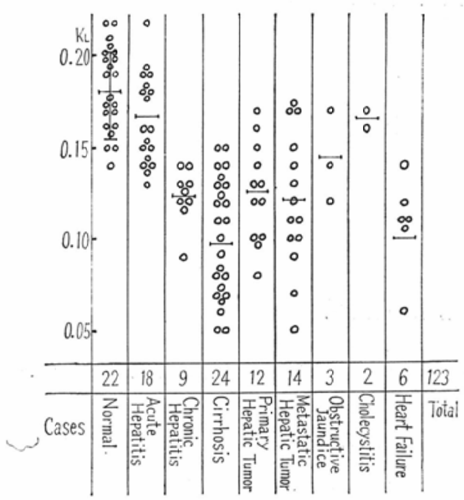
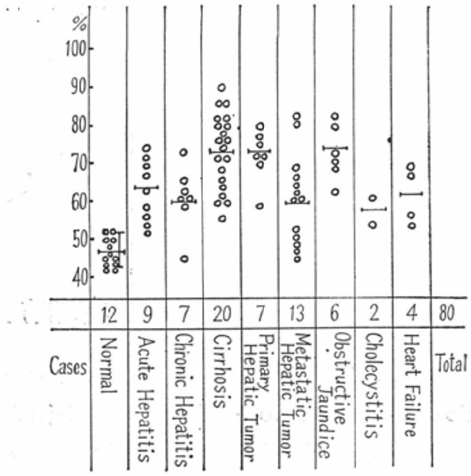


Fig. 8 ^{125}I-R•B disappearance rate in various hepatic diseases.



the patient.

The reaults mainly obtained by the latter method are reported here.

When taking KL value on the ordinate and ^{125}I-R.B disappearance rate on the abscissa, as shown in Fig. 8, it indicates the combined activities of hepatic blood flow and hepatobiliary functions.

These results were plotted after dividing into two main groups, such as diffuse liver diseases and hepatic tumors. In the first group of normals are located on the left upper part of the figure, the group of acute hepatitis are congregated on the right upper, while the cirrhosis on the right lower, and that of heart failure cases are shown on the left lower part, displaying radiate distributione around the group of normals as a pivot (Fig. 9).

Fig. 9 Combination test of hepatic uptake rate constant of ^{198}Au colloid (KL) and ^{125}I-R•B disappearance rate.

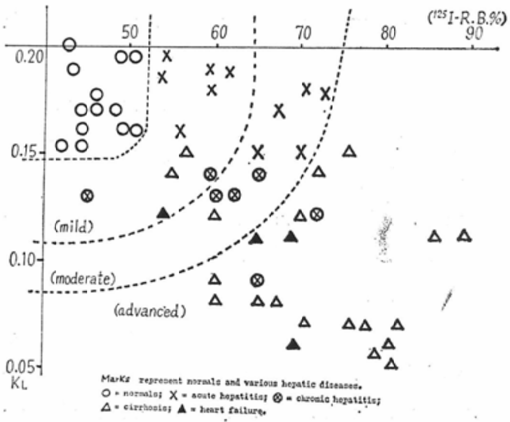


Fig. 10 Combination test of KL and ^{125}I-R•B disappearance rate. Marks represent various hepatic tumors. ( )=Case number.
○ = primary hepatic tumor;
● = metastatic hepatic tumor.

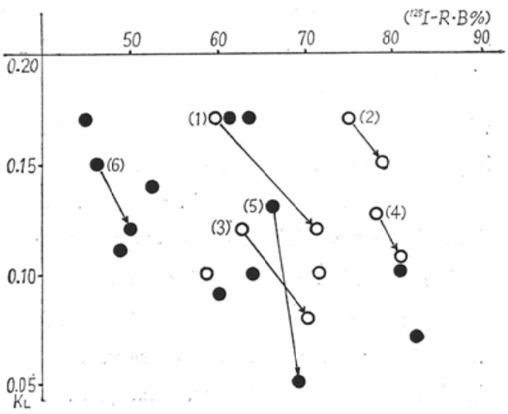


Table 2 Progress of the Hepatic Tumors

Cases No.	Age	Sex	Interval of the serial combination test	Diagnosis
1	23	F.	10 months	Hepatoma
2	52	F.	20 days	Hepatoma
3	70	M.	1.5months	Hepatoma with cirrhosis
4	52	F.	1 month	Cholangioma
5	48	M.	6 months	Extensive liver metastases from carcinoma of the stomach
6	40	F.	2 months	Liver metastases from carcinoma of the colon

In the second group of primary and metastatic hepatic tumor, they do not reveal such characteristic distribution as the diffuse liver disease (Fig. 10, Table 2).

However, the arrow shown in Fig. 10 indicates the trend of disease progress.

3) Discussion

Examination of the hepatic circulation by means of radioisotopes have been tried by a number of methods, such as estimation of effective hepatic blood flow, examination of hepatobiliary function, as referred previously, or by measurement of intra-and extra-hepatic circulatory shunt and the separate evaluation of hepatic arterial and portal blood flow.[7,9,11,13)]

In recent years, by using of better type of apparatuses, introduction of newly developed radioisotopes have been made, the clinical data obtained by these, have offered new diagnostic problems.

Concerning the caliculation of K_L, this value theoretically forms straight line on the semilogarithmic scale as shown in the equation (Fig. 5). It does not follow this theoretical line, however, deviates with progress of the time, since the size of ^{198}Au colloid particles (made by Dainabot Lab.) were not of homogeneous type when observed under electron microscope. In consequence, its theoretical line is represented by the sum of exponential function of more than two.

If 2 to 8 min. on the abscissa would be chosen when the liver extraction efficiency of ^{198}Au colloid reaches almost constant, straight line could be obtained on the semilogarithmic scale and calculation of K_L value becomes easier[5)]. When ^{198}Au colloid is taken up only by the liver and if this extraction efficiency can be measured, it is possible to estimate the hepatic blood flow. To measure hepatic extraction efficiency of ^{198}Au colloid, however, catheterization of the hepatic vein would be necessary. As estimation of total blood volume, methods with ^{131}I-HSA or ^{51}Cr are currently thought to be accurate but calibration is required when ^{198}Au is used at the same time. This simultaneous measurement with ^{131}I-HSA or ^{15}Cr and the catheterization of hepatic vein is technically much more complicated and may cause errors.

Where F= hepatic blood flow (ml/min.)

V= total blood volume (ml)

E= hepatic extraction efficiency

K_L= rate constant

$$K_L = E \cdot \frac{E}{V}$$

Letting E to be constant, K_L can be considerd to be the ratio of the hepatic blood flow to the total blood volume.

The RIRB disappearance rate may be a more sensitive liver function test than BSP and thought to be even superior than BSP on the following points; first, it does not need photometry, therefore, more accurate result can be obtained even in severe jaundice. The tracer dose of RIRB test (total dosis of within 1 mg) is much smaller than that of BSP (5mg/kg body weight), thus the burden to the liver is assumed to be small. It does not require blood sampling and continuous estimation of the peripheral blood concentration can be made.

According to the multicompartmental analysis with digital computer by Berman et al.[2], RIRB disappearance curve is represented as the sum of three exponential functions. When each rate constant is culculated, the 'kinetics' of RIRB, that is the transport from intravascular compartment to hepatic compartment and to biliary compartment, can be evaluated accurately. Definite calculations of these rate constant are almost impossible without computer. Turco et al.[10] calculated ^{131}I-R.B peripheral disappearance curve 'kinetics' by digital computer, and reported that so long as the initial phase of the disappearance curve was used, even the ratio of twentieth minute to fifth minute which was described before, represented the hepatocellular and biliary functions; clinically, sufficient information could be obtained by calculating RIRB disappearance rate alone.

Although external counting of ^{131}I-R.B over the liver region is frequently performed, this method is strongly affected by radioactivity of intrahepatic circulating blood and of biliary tract at the same time. Therefore, it is useful as a monitor, but is not so suitable to measure the kinetics of ^{131}I-R.B accurately.

Concerning the meaning of the results obtained by double isotope technique, as shown in Fig. 8, it represents the disturbance of the liver or biliary tract if plotted on the right part and if plotted on the left lower part, decreased effective heaptic blood flow; in other words, general cardiovascular disorder or proliferation of the liver stroma are suggested.. The more it is away from the pivot, the more it shows marked hepatic disorder. When the hepatic disturbances become more chronic, the distribution of each disease on the figure overlaps each other, although differential diagnosis is not easy, it is possible to obtain fairly accurate idea of hepatic hemodynamic changes by performing serial tests. Plotting the distribution of the hepatic tumors, it does not show such characteristic distribution as seen in the diffuse hepatic diseases. The presence of coexisting liver cirrhosis or hepatitis can be substantiated, so that the diagnostic value is greatly enhanced when combined with area scintigram.

IV Summary and Conclusions

Simultaneous measurement of hepatic uptake rate constant of ^{198}Au colloid and peripheral disappearance rate of ^{125}I-Rose Bengal (Double Isotope Technique) was made possible by means of three channeled γ-spectrometer system and a newly developed calibration chart after suitable corrections.

According to this double isotope technique, the disturbance of the hepatic hemodynamics could be evaluated after separating into the decrease of effective liver blood flow and into the hepatobiliary dysfunction .When the results of the combined tests were plotted on same diagram, each hepatic disease shows the characteristic distribrtion, and these results not only provide the means to make the differential diagnosis, but also suggest the progress of various hepatic diseases.

Basic problems concerning the simultaneous use of ^{198}Au colloid and ^{125}I-Rose Bengal and their clinical applications were also discussed.

Acknowledgments:

The author wishes to express his sincere appreciation to professor Kazuyuki Narabayashi for his continuous guidance and advice during present study.

I am also grateful to Professors T. Tomomatsu, T. Yamori and Drs. I. Hasegawa, A. Tominaga for their suggestions and criticisms, and indebted to Drs. T. Maeda, S. Okaya, S. Nishiyama for thier generous cooperation and assistance.

This work was supported by a grant for Fundamental Scientific Research Program of the Ministry of Education.

References

1) Akcay, M.M., Johnson, D.E., Taplin, G.V. & Liu, C.K.; A new quantitative external monitoring technique. Int. J. appl. Radiat. Isotope. 17, (1966), 261—268.

2) Berman, M., Shahn, E. & Marjory, F.W.: The routine fitting of kinetic data to models: A mathematical formalism for digital computers. Biophy. J. 2, (1962), 275—287.

3) Bradley, S.E., Ingelfinger, F.J., Bradley, G.P. & Curry, J.J.: Estimation of hepatic blood flow in man. J. Clin. Invest. 24, (1945), 890—897.

4) Dobson, E.L. & Jones, H.B.: The behaviour of intravenously injected particulate matter; its rate of disappearance from the blood stream as a measure of liver blood flow. Acta med. scand. Suppl. 144 (1952), 1—71.

5) Matsuyama, M.: Studies of hepatic hemodynamics with ^{198}Au colloid in hepatobiliary diseases. The Japanese Journal of Nuclear Medicine 2, (1965), 99—110. (Japanese)

6) Narabayashi, K., Matsumoto, I., Tominaga, A., Kajita, A., Maeda, T., Okaya, S., Nishiyama, S. & Inoue, Y.: The hepatic scinti-scanning and its diagnostic significance. Shinryo 20, (1967), 15—26. (Japanese)

7) Nordyke, R.A., Bland, W.H.: The differential diagnosis of bilary tract obstruction with radioactive rose bengal. J. Lab. & Clin. Med. 51, (1958), 565—579.

8) Spencer, R.P.: Simultaneous use of two radioisotope by scanner plus analogue computer coupling. J. Nuclear Med. 6 (1965), 844—846.

9) Taplin, G.V., Meredith, O.M., & Kade, H.: Radioactive I-131 tagged Rose Bengal uptake excretion test for liver function using external gamma ray scintillation counting techniques. J. Lab. & Clin. Med. 45, (1955), 665—678.

10) Turco, G.L., Ghemi, F., Molino, G. and Segre, G.: The kinetics of ^{131}I Rose Bengal in normal and cirrhotic subjects studied by compartmental analysis and a digital computer. J. Lab. & Clin. Med. 67, (1966), 983—999.

11) Ueda, H., Unuma, T., Iio, M. & Kameda, H.: Measurement of hepatic arterial and portal blood flow and circulation time via hepatic artery and portal vein with radioisotope. Jap. Heart J. 3, (1962), 154—166.

12) Vetter, H., Faulkner, R. & Neumayr, A.: The disappearance rate of colloidal radiogold from the circulation and its application to the estimation of liver blood flow in normal and cirrhotic subjects. J. Clin. Invest. 33, (1954), 1594—1602.

13) Wagner, H.N. Jr., Iio, M., Hornick, R.B., et al.: Studies of the reticuloendothelial system (RES). II. changes in the phagocytic capacity of the RES in patients with certain infections. J. Clin. Invest. 42, (1963), 427—434.