THE ESTIMATION OF HEPATIC BLOOD FLOW AND INTRAHEPATIC
SHUNTED BLOOD FLOW BY COLLOIDAL HEAT-DENATURED
HUMAN SERUM ALBUMIN LABELED WITH $^{131}I$

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The measurement of hepatic blood flow based upon the rate of disappearance of colloidal particles from peripheral blood by the Kupffer cells depends upon two basic assumptions: 1) that the colloid is completely removed from the blood in one passage through the liver, and 2) that the liver is its sole source of removal. Dobson and Jones (1) showed in animals that radioactive colloidal chromic phosphate approximated satisfactorily to these theoretical postulates, although the efficiency of hepatic extraction of this colloid has not been determined in man. Radioactive colloidal gold has also been used as an index of liver blood flow, and in the original work of Vetter, Falkner and Neumayr (2) the hepatic extraction efficiency averaged 80 per cent in three subjects. In a later paper (3), however, Vetter and co-workers reported a lower average hepatic blood flow in normal subjects, and subsequently it has been shown that commercial preparations of radiogold are not suitable for measurement of hepatic blood flow, since their particle sizes are not homogeneous (4, 5).

Halpern and colleagues (6) described a heat-denatured albumin colloidal complex labeled with $I^{131}$ (CAI$^{131}$). The particle size could be controlled by the extent of heating and checked by biological standardization. Preliminary results in animals and in man suggested that extrahepatic removal was minimal and hepatic extraction was over 90 per cent (7–9). This colloid might therefore be suitable for measuring liver blood flow and our communication deals with its use for this purpose in control subjects and patients with cirrhosis. The results suggest that the method is valid for measuring liver blood flow in normal subjects. It underestimates total liver blood flow in patients with cirrhosis by the amount of blood shunted through intrahepatic portal-venous anastomoses which are not lined by Kupffer cells and hence do not remove the colloid.

METHODS

Patients. The following groups of subjects were studied: I, 14 control subjects without known liver disease; II, 21 cirrhotic patients with a patent portal vein; III, 11 cirrhotic patients with a portacaval anastomosis (including two patients in whom preoperative studies were made) and 1 patient with thrombosis of the portal vein; IV, 3 patients with an extrahepatic portal vein block and normal liver structure. The state of the portal vein was checked by splenic venography in all patients with liver disease.

CAI$^{131}$. The preparation of CAI$^{131}$ is described in the Appendix.

The estimation of minimal hepatic blood flow without hepatic vein catheterization. A known amount of CAI$^{131}$ containing approximately 0.1 mg per kg body weight of colloidal albumin and 15 to 20 mc of $I^{131}$ in a volume of 1 to 2 ml was injected intravenously, after preliminary thyroid blockade with potassium iodide, 200 mg daily for 3 days. Six-ml arterial blood samples were taken into heparinized tubes from a polyethylene catheter in the femoral artery at 3, 4, 5, 6, 8, 10, 15, 20 and 30 minutes after injection. In some patients, further samples were obtained at 10-minute intervals up to 1 hour after injection, and in one subject blood samples were obtained at 4-hour intervals over 24 hours.

Estimation of hepatic extraction of CAI$^{131}$ and measurement of total hepatic blood flow. The technique was identical with that described for the measurement of minimal hepatic blood flow except that hepatic vein samples were obtained from a no. 9 radiopaque catheter which had been placed under fluoroscopic control in one of the main right hepatic veins. The hepatic venous samples were taken simultaneously with the arterial samples after allowing for the dead space sampling time of the hepatic vein catheter. In certain subjects hepatic blood flow was measured by the Fick principle (10) with a constant infusion of indocyanine green (11–13) at the same time that total hepatic blood flow was measured by CAI$^{131}$.

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**Determination of CAI™ activity.** The amount of CAI™ injected was determined by weighing syringes before and after injection. The volume injected was calculated from the weight and specific gravity of CAI™. The total activity injected was calculated from the activity of a measured standard of CAI™ which had been diluted to a known volume. The activity of the samples was measured in plasma after the blood had been centrifuged at 3,000 rpm for 15 minutes. All samples were counted in a well scintillation counter to a minimum of 5,000 counts. After counting total activity in the plasma (PFA) the protein-bound iodine was precipitated with trichloroacetic acid and the supernatant recounted for free 1™ activity (PFI). This value was subtracted from PFA to give protein-bound activity (P). Free iodine 1™ was not found in any sample before 10 minutes after injection. Plasma volumes were calculated from the initial extrapolated CAI™ disappearance slope (a) and in several patients by the Evans blue dye technique (14). Hematocrits were measured after centrifugation of hepatic venous and arterial blood at 3,000 rpm for 30 minutes and corrected for trapped plasma (15). Blood volumes were calculated from plasma volumes and corrected hematocrit values.

**Calculations**

**Minimal hepatic blood flow (Figure 1).** Peripheral plasma total activity and peripheral plasma protein-bound activity were plotted on a semilogarithmic scale against time. As PFI was not detected before 10 minutes, the initial slopes of PFA and P were identical. The initial slope (a) was extrapolated to zero, and the calculated zero time activity (P) divided into the activity of CAI™ injected, to give the initial volume of distribution of CAI™ (plasma volume). After 10 to 15 minutes, PFA began to rise and coincided with the appearance of free 1™ in the plasma, which latter was assumed to be due to the breakdown of the colloid complex within the liver and subsequent release of free 1™ into the plasma. The subsequent decay of PFA was followed in one subject and represented the resultant of release of free 1™ from the liver and urinary loss, together with the removal of residual protein-bound 1™ (particles of small size).

Pc activity equaled PFA for the first 10 minutes, but after this time it followed a much shallower exponential slope (b); b and Pc decay were extrapolated to zero time and expressed as a percentage of P, giving a percentage activity due to small particles of CAI™; b, assumed to represent the clearance of small particles of CAI™ by the reticuloendothelial system outside the liver, was used as a biological standardization for each preparation of CAI™. In one subject, b was followed for 24 hours, and accurate analysis of its exponential character was possible; b was subtracted from a and the resultant exponential slope c used as the definitive clearance slope of CAI™ by the liver.

The rate constant for c was calculated for the general equation for an exponential function:

\[ P_1 = P_0 e^{-kt} \]  \[ K \text{ min}^{-1} = \frac{Ln2}{t_i} = \frac{0.693}{t_i} \]  \[ \text{where } P_1 = \text{plasma activity of CAI™ at time } t \text{ in minutes, } P_0 = \text{the activity at time zero determined by extrapolation of the slope and } K = \text{fraction of retained plasma activity of CAI™ which was removed from the blood per minute.} \]

If the plasma colloid is removed completely in one passage through the liver then the constant, K, represents the fraction of the circulating plasma or blood volume which perfuses the liver per minute. However, if the hepatic extraction (E) of the colloid is less than 100 per cent, then K underestimates the fraction of blood volume perfusing the liver by the percentage of the colloid not cleared by the liver in one passage.

If E is not known, then the measurement of hepatic blood flow is called "minimal hepatic blood flow" (MHBFS), since it is less than the total hepatic blood flow (THBF), and may be calculated from the equation:

\[ \text{MHBFS (ml/min)} = K \text{ min}^{-1} \times \text{blood volume} \]

**FIG. 1. MINIMAL HEPATIC BLOOD FLOW (CONTROL, SUBJECT 2).** Activity of plasma CAI™ is plotted on a semilogarithmic scale against time. Slope (c) t1 = 2 minutes; blood volume = 4,100 ml; K = 0.346 min⁻¹. Minimal hepatic blood flow = 4,100 × 0.346 = 1,420 ml per minute. PFA(O) = peripheral plasma total activity of 1™ up to 10 minutes on slope a and then as (●). PFI = plasma free iodine activity which was not present before 10 minutes; subsequently it was subtracted from PFA(●). Pc(O) = peripheral plasma activity corrected for free 1™ and represented as (O) on slope b. PSS = zero time activity of particles of small size from extrapolation of slope b. Slope c is derived from graphical subtraction of slope b from slope a.
Total hepatic blood flow was then calculated from minimal hepatic blood flow corrected for hepatic extraction $(E)$:

$$\text{THBF} (\text{ml/min}) = \frac{K \min^{-1} \times \text{blood volume (ml)} \times 100}{E} = \frac{\text{MHBF}}{E} \times 100 \quad [5]$$

Statistical analyses were performed by standard methods (16).

RESULTS

The percentage of particles of small size (PSS) averaged $5.72 \pm (1 \text{ SD}) 1.33$ for all preparations of CAI$^{33}$. Any preparation with more than 8 per cent particles of small size was discarded. The plasma half-life of PSS was 13 hours and the
ESTIMATION OF HEPATIC FLOW AND INTRAHEPATIC SHUNT-FLOW

TABLE I

*Minimal hepatic blood flow, hepatic extraction and total hepatic blood flow (CAI III), and estimated hepatic blood flow (indocyanine green) in control subjects and patients with liver disease*

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Diagnosis</th>
<th>CAI III K (min−1)</th>
<th>MHBF (ml/min)</th>
<th>E (%)</th>
<th>THBF (ml/min)</th>
<th>Indocyanine green EHBF (ml/min)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Group I, control</td>
<td>Peptic ulcer</td>
<td>0.396</td>
<td>2,080</td>
<td>95</td>
<td>2,190</td>
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<tr>
<td>2</td>
<td></td>
<td>Peptic ulcer</td>
<td>0.346</td>
<td>1,470</td>
<td>95</td>
<td>1,540</td>
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<tr>
<td>3</td>
<td></td>
<td>Sarcoïdosis</td>
<td>0.308</td>
<td>920</td>
<td>95</td>
<td>980</td>
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<tr>
<td>4</td>
<td></td>
<td>Neurosis</td>
<td>0.386</td>
<td>2,010</td>
<td>95</td>
<td>2,160</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Peptic ulcer</td>
<td>0.346</td>
<td>1,650</td>
<td>92</td>
<td>1,790</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Peptic ulcer</td>
<td>0.250</td>
<td>1,220</td>
<td>95</td>
<td>1,320</td>
</tr>
<tr>
<td></td>
<td>Mean SD</td>
<td></td>
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<td></td>
<td></td>
<td>93.7 ± 1.79</td>
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<table>
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<th>Group II, cirrhosis and patent portal vein</th>
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<td>12</td>
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<table>
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<tr>
<th>Group III, cirrhosis and portacaval anastomosis or thrombosed portal vein</th>
</tr>
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<tbody>
<tr>
<td>7†</td>
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<tr>
<td>8‡</td>
</tr>
<tr>
<td>13</td>
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<tr>
<td>14</td>
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<tr>
<td>15§</td>
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</table>

<table>
<thead>
<tr>
<th>Group IV, portal vein thrombosis and normal liver structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
</tr>
</tbody>
</table>

* = K = rate constant of peripheral plasma slope of CAI III, MHBF = minimal hepatic blood flow, E = hepatic extraction of CAI III in one passage through the liver, THBF = total hepatic blood flow, EHBF = estimated hepatic blood flow.

† ‡ Before and after portacaval anastomosis.
§ Thrombosed portal vein.

Half-life in plasma of $P_{TA}$, after the initial extraction phase, was 24 hours ($P_{C} + P_{ER}$).

There was no significant difference between 14 simultaneously determined plasma volumes using Evans blue and CAI III.

**Minimal hepatic blood flow, hepatic extraction, and total hepatic blood flow (Figure 3, Table I)**

*Group I.* Minimal hepatic blood flow in 14 control subjects averaged 1,516 ± 362 ml per minute, $K$ (fraction of blood volume perfusing the liver per minute) equaled 0.339 ± 0.044. In 6 of these control subjects $E$ was remarkably constant and averaged 93.7 ± 1.79 per cent in one passage through the liver. Total hepatic blood flow in these 6 subjects averaged 1,633 ± 478 ml per minute (Table I).

*Group II.* In 21 patients with cirrhosis and a patent portal vein, minimal hepatic blood flow averaged 1,139 ± 381 ml per minute and $K$ averaged 0.288 ± 0.0634 min−1. Minimal hepatic blood flow was significantly reduced compared with the control group ($0.001 < p < 0.01$), but $K$ was even more significantly reduced ($p < 0.001$, Figure 3). In 6 of these patients $E$ was...
against time

Arterial and hepatic extraction of CAI\textsuperscript{131} has been expressed as a percentage activity of zero time arterial activity. The patient with a thrombosed portal vein, and the average was 89.6 ± 3.5 per cent. Total hepatic blood flow averaged 726 ± 226 ml per minute (Table I).

In Patients 7 and 8 the hepatic extraction of CAI\textsuperscript{131} and total hepatic blood flow were measured before and after an end-to-side portacaval anastomosis. In Patient 7 (Figure 4) the hepatic extraction of CAI\textsuperscript{131} rose from 65 to 86 per cent after operation, while the total hepatic blood flow was reduced from 1,480 to 1,070 ml per minute. Minimal hepatic blood flow was reduced from 960 to 920 per minute. In Patient 8 the hepatic extraction of CAI\textsuperscript{131} of 90 per cent remained unaltered postoperatively while total hepatic blood flow was reduced from 1,190 to 860 ml per minute. Minimal hepatic blood flow was reduced from 1,080 to 780 ml per minute.

**Group IV.** In 3 patients with normal liver structure and an extrahepatic portal vein obstruction, the minimal hepatic blood flow averaged 837 ml per minute (range, 510 to 1,230) and K averaged 0.193 min\textsuperscript{-1}. In Patient 16 the hepatic extraction of CAI\textsuperscript{131} was 92 per cent and the total hepatic blood flow was 550 ml per minute (Table I).

**Figure 4.** Effect of portacaval anastomosis on hepatic extraction of CAI\textsuperscript{131} (cirrhotic, Subject 7). Arterial and hepatic venous plasma activity of CAI\textsuperscript{131} has been expressed as a percentage activity of zero time arterial activity expressed in TA plasma and after an anaesthesia. Total hepatic blood flow has been reduced by 28 per cent, but minimal hepatic blood flow has been reduced by only 5 per cent. M.H.B.F = minimal hepatic blood flow; T.H.B.F. = total hepatic blood flow; F.A. = femoral arterial plasma; H.V. = hepatic venous plasma; e = definitive slope of CAI\textsuperscript{131} in arterial plasma; e = definitive slope of CAI\textsuperscript{131} in hepatic venous plasma; H.E. = zero time activity of CAI\textsuperscript{131} in arterial plasma; H.E. = zero time activity of CAI\textsuperscript{131} in hepatic venous plasma.

![Diagram](image)

**Figure 5.** Correlation of total hepatic blood flow (CAI\textsuperscript{131}) and estimated hepatic blood flow (indocyanine green IG). ○ = Control subjects (Group I); □ = patients with cirrhosis and a patent portal vein (Group II); × = patients with cirrhosis and portacaval anastomosis or a thrombosed portal vein (Group III); ○ = patient with a thrombosed portal vein and a normal liver structure. The dotted lines indicate the 95 per cent confidence limits about the regression line.
**Indocyanine green**

Hepatic blood flow measured with indocyanine green (IG) was estimated in 10 patients (3 control subjects in Group I, 2 cirrhotics in Group II, 3 cirrhotics with a portacaval anastomosis and 1 with a thrombosed portal vein in Group III, and 1 patient with a normal liver and an extrahepatic block in Group IV). The individual results are shown in Table I. There was a highly significant correlation (R = 0.89) between the IG flow results and total hepatic blood flow (CAI\textsuperscript{131}) results for these patients (Figure 5).

**DISCUSSION**

The CAI\textsuperscript{131} method of measuring liver blood flow without hepatic vein catheterization assumes that the colloid is extracted completely in one passage through the liver. This assumption proved not to be valid, and the flow derived from the disappearance rate constant of CAI\textsuperscript{131} from peripheral blood, uncorrected for hepatic extraction, underestimated liver blood flow. It has been called “minimal hepatic blood flow.” The results obtained for the control group (1,516 ± 362 ml per minute; \(K = 0.339 \pm 0.044\) min\(^{-1}\)) are similar but higher than those reported by Halpern and co-workers (9) (1,445 ± 314 ml per minute; \(K = 0.318 \pm 0.050\) min\(^{-1}\)). However, Halpern did not correct for the slow component of the CAI\textsuperscript{131} peripheral disappearance slope. The probability that the slow component of the plasma colloid disappearance slope represents particles not removed by the liver is suggested by the identical values for the hepatic venous plasma and the peripheral plasma slopes 10 minutes after injection of CAI\textsuperscript{131}. Furthermore, with colloidal radioactive chromic phosphate, the slow component slope is absent in peripheral plasma if the colloid is ultracentrifuged before injection and the smaller particles removed (1). In animals, injection of the smaller particles alone results in a prolonged plasma disappearance slope, and, on sacrifice of the animal, the particles are found principally deposited in the bone marrow (1). The biological estimation of the percentage of small particle size of all preparations of CAI\textsuperscript{131} is an essential prerequisite when this substance is used for estimating liver blood flow. The exclusion of preparations with a high percentage of particles of small size avoids the use of a non-homogeneous colloid. The mean PSS of 5.77 per cent for this series in humans agrees well with Halpern’s figure of 6.4 per cent in guinea pigs.

The hepatic extraction of CAI\textsuperscript{131} was very high in normal subjects, and Halpern and co-workers (9) also reported a 94 per cent extraction in four normal persons. These results suggest that minimal hepatic blood flow will underestimate true liver blood flow by only less than 10 per cent in patients without liver disease, and this may eliminate the need for hepatic vein catheterization in such subjects.

In patients with hepatic cirrhosis the peripheral method is not usually valid, for hepatic vein catheterization showed that such subjects had a much lower extraction of CAI\textsuperscript{131} than had subjects without liver disease. CAI\textsuperscript{131} is removed by the Kupffer cells and the lower extraction could be due to the fact that blood bypasses the sinusoids containing Kupffer cells, to the impairment of Kupffer cell function, or to a reduction in the number of Kupffer cells lining each sinusoid. Morphological evidence favors the first explanation. McIndoe (17) by injection studies showed a very great diminution of the portal sinusoidal bed in cirrhosis. Popper, Elias and Petty (18) have commented on the development of collateral channels between the small portal veins and the small hepatic veins around the regeneration nodules in cirrhosis and have termed these “internal Eck fistulae.” However, it is difficult to discount the possibility of disturbed Kupffer cell function, since there is no certain way of evaluating this.

When the portal blood is diverted from the liver either by a surgical portacaval anastomosis or by the development of a collateral circulation after blockage of a main portal vein, a different state of affairs exists. The sinusoids are no longer perfused by portal blood and the portal vein-hepatic vein anastomoses around the nodules are presumably not functioning. The liver is supplied by the hepatic artery which enters the lobe at various levels along the sinusoids (19). On theoretical grounds, therefore, one might expect improvement in the hepatic extraction of CAI\textsuperscript{131} in the cirrhotic patient when the portal blood stream is diverted from the liver, if the initially low extraction of CAI\textsuperscript{131} was due to intrahepatic shunting in “internal Eck fistulae” rather than...
to impaired Kupffer cell function. This proved to be the case, for patients with cirrhosis and a portacaval anastomosis had hepatic extraction of 89.6 per cent, which was significantly higher than that in the cirrhotic group with portal venous blood flowing to the liver. Further confirmation of the hypothesis came with the finding of improvement in the hepatic extraction of CAI131 in two patients after end-to-side portacaval anastomosis. In Patient 7 the hepatic extraction of CAI131 rose from 65 to 86 per cent, although hepatic blood flow was reduced by 30 per cent. This improved extraction occurred in the face of worsening hepatocellular function shown by deepening jaundice and a fall in the serum albumin value. Also in keeping with this thesis is the observation that the two cirrhotic patients (8 and 11), with almost normal hepatic extraction of CAI131, were shown by the method of Iber, Kerr, Dölle and Sherlock (20) to have an extrahepatic portal collateral flow of over 80 per cent of the total portal venous flow. This evidence suggests that the reduced extraction of CAI131 in cirrhosis is not due to impaired Kupffer cell function. If this were so, one would have to postulate an improvement in Kupffer cell function after portacaval anastomosis, and in one subject in the face of deterioration of hepatocellular function. The alternative explanation that the increased extraction of CAI131 in the shunt patients resulted from a reduction in blood flowing along the sinusoids seems less probable, as the highest extractions were seen in control subjects with rates of liver blood flow significantly higher than in cirrhotic subjects. It seems likely, therefore, that minimal hepatic blood flow determined by this method measures blood flowing through functioning sinusoids. In normal subjects and cirrhotic subjects with portacaval anastomosis and patients in whom portal venous blood is totally diverted from the liver, this value correlates closely with total hepatic blood flow. In patients with cirrhosis and a patent portal vein, minimal hepatic blood is less than total hepatic blood flow (Figure 6).

A reduction in total hepatic blood flow after portacaval anastomosis was seen in Patients 7 and 8, but in Patient 7 the minimal hepatic blood flow was unaltered postoperatively. This suggests that in certain patients the reduction in hepatic blood flow after portacaval anastomosis (21, 22) may not affect liver function if the fraction lost was, in fact, bypassing the hepatic sinusoids before operation.

The measurement of total hepatic blood flow by CAI131 correlated well with hepatic blood flow results determined by indocyanine green, and this suggests that the method does indeed measure liver blood flow. The extrahepatic removal of CAI131 has not been checked in man, but in animals it averages less than 10 per cent (7) and over half of this is allowed for by subtraction of particles of small size. Extrahepatic removal of indocyanine green in man is minimal (11). The slightly higher flow by the CAI131 method suggests that its extrahepatic removal may be greater than that of indocyanine green. In patients without a collateral circulation, this factor is not important, since the splenic blood flow is in series with the liver, but in cirrhotics, with an extrahepatic portal collateral circulation, the splenic removal of CAI131 may result in an overestimation of liver blood flow.

With hepatic vein catheterization, the CAI131 method permits an estimation of sinusoidal and intrahepatic shunted blood flow while the constant infusion dye methods (sulfobromphthalein, indocyanine green) measure the sum of these two components. Minimal hepatic blood flow in pa-
patients with cirrhosis may provide a better understanding of the factors responsible for decompensation in such patients, if one assumes that blood available to the Kupffer cells represents blood available to the hepatic parenchymal cells.

SUMMARY

The colloidal albumin I\textsuperscript{131} (CAI\textsuperscript{121}) method for measuring liver blood flow has been investigated in normal subjects and patients with liver disease. In normal subjects the method is valid without hepatic vein catheterization, since the hepatic extraction of the colloid averaged 94 per cent in one passage through the liver.

In patients with cirrhosis the hepatic extraction of the colloid averaged 75.8 per cent when the portal vein was patent, but rose to 89.6 per cent when the portal vein was disconnected from the liver by a portacaval anastomosis. It is suggested that the reduced hepatic extraction of CAI\textsuperscript{121} in patients with cirrhosis is due to intrahepatic shunting of colloid which bypasses Kupffer cells in "internal Eck fistulae." Hepatic vein catheterization permits an estimate of this shunting.

Total hepatic blood flow may be calculated from the peripheral colloid blood flow (minimal hepatic blood flow) corrected for hepatic extraction. Total hepatic blood flow measured by colloidal albumin correlated well with hepatic blood flow measured with indocyanine green. Minimal hepatic flow results (uncorrected for hepatic extraction) may be used as an index of hepatic sinusoidal blood flow and as a measure of the functional blood supply to the cirrhotic liver.

APPENDIX

Preparation of heat-denatured human serum albumin labeled with radioactive iodine I\textsuperscript{131}

The procedure was carried out under sterile conditions. All reagents were sterilized prior to use.

1. Denaturation of albumin. The albumin was 97 per cent pure with no added stabilizing agents.
   a) The albumin was diluted with normal saline to make a 1 per cent solution containing 8 g of albumin.
   b) The solution was brought to pH 7.2 with NaOH.
   c) The optical density of the solution was checked in a Unicam spectrophotometer at 500 nm. Optical densities of 0.056 to 0.112 were considered satisfactory.
   d) The whole solution was then heated in a flask in a water bath while the flask was shaken continuously. The temperature of the water bath was controlled to bring the solution to 70° C, and this temperature was maintained for 15 minutes.
   e) The optical density of the solution was read and, if it had risen by 0.168 U above the original reading, heat denaturation was stopped. If not, the flask was heated to 71° C for a further 2 minutes and the optical density rechecked. The maximal permissible temperature for denaturation was 75° C.
   f) After satisfactory denaturation, the solution was cooled to 45° C under running water and allowed to cool at room temperature until the solution was at 30° C.
   g) N HCl was added to obtain a precipitate at pH 4.7 to 5.5. The solution was left to stand for 1 hour.
   h) It was then centrifuged for 15 minutes at 3,500 rpm and the supernatant fluid was removed and discarded.
   i) The solution was washed twice with acidified normal saline solution (HCl, pH 5.5) and centrifuged at 3,500 rpm for 15 minutes between washings. The supernatant fluid was checked after the second washing for protein content by the addition of trichloroacetic acid. If a protein precipitate resulted, washings were repeated until the supernatant fluid was free of protein.
   j) The precipitate was redissolved in normal saline and brought to pH 7.6 with N NaOH and made up to a final volume of 200 to 250 ml.
   k) The solution was stored at 4° C for 24 hours and centrifuged at 3,500 rpm for 15 minutes; the precipitate was discarded.
   l) Two ml of supernatant fluid was removed for protein determination and the remainder tyndallized for 1 hour daily for 3 days at 60° C. The protein was stored at 4° C and was suitable for iodination for up to 3 months.
   m) Protein content of the denatured solution was estimated by weighing the protein precipitated from solution by the addition of trichloroacetic acid. The precipitate was washed with water and acetone and dried to a constant weight.
   n) Particle size: Attempts to measure the particle size of the preparation were unsuccessful, since both electron-microscopy and ultracentrifugation altered the particle size by further denaturation of the colloid. The single component exponential phase of the fast disappearance slope suggested a uniform particle size. The rate of disappearance in control subjects was similar to that of colloidal chromic phosphate (P\textsuperscript{32}) with a mean particle size of 200 A (1).

2. Iodination of colloidal albumin
   a) One hundred mg of colloidal albumin (determined from protein estimation) was mixed with 0.8 ml carbonate buffer (pH 10) keeping the solution in ice.
   b) Twenty to 30 mc of carrier-free NaI\textsuperscript{131} was made up to a volume of 3 ml, and 0.15 ml of KI solution (1,845 g per 100 ml of I\textsubscript{2} and 2.3 g of KI) was added; a light brown color appeared.
   c) The iodine solution was then added drop by drop to the colloidal albumin while the solution was kept in ice.
   d) The solution was left at 4° C for 2 hours and the activity per unit volume estimated in a well scintillation counter, from a diluted aliquot.