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ESTIMATION OF HEPATIC AND PORTAL BLOOD FLOWS BY AN INDICATOR DILUTION TECHNIQUE

A thesis

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Total hepatic blood flow (T.H.B.F.) and portal blood flow (P.B.F.) were measured in dogs using simultaneous indicator dilution curves (I.D.C.) obtained from one or two hepatic veins and from the left branch of the portal vein, after injection of Cr^{51} red blood cells into the cranial mesenteric artery. No difference existed when results obtained using I.D.C. were compared with those obtained by simultaneous measurements of hepatic artery and portal blood flows using electromagnetic flowmeters. In experiments where I.D.C. could be obtained simultaneously from two hepatic veins, no difference was shown between paired T.H.B.F. Similar results were found in animals with ligated hepatic artery. These data, demonstrating uniform mixing of the indicator in the portal vein and within the intrahepatic circulation, validate the use of the indicator dilution method in dogs. Preliminary data in 13 cirrhotics undergoing umbilicoportal and hepatic vein catheterizations indicate that the portal fraction of T.H.B.F. (the ratio of P.B.F. over T.H.B.F.) can be estimated by this method. The portal fraction should be reliable since the portosystemic collaterals resulting in loss of indicator (and overestimation of flows) occurred before the sampling sites.

Le débit hépatique total (D.H.T.) et le débit de la veine porte (D.V.P.) ont été mesurés chez des chiens en utilisant des courbes de dilution d'un indicateur (C.D.I.) obtenues simultanément au niveau d'une ou deux veines sus-hépatiques et de la branche gauche de la veine porte, après injection d'hématies marquées au Cr^{51} dans l'artère mésentérique craniale. Il n'existait pas de différence entre les résultats obtenus par les C.D.I. et ceux obtenus simultanément par la mesure des débits de l'artère hépatique et de la veine porte grâce à des débitmètres électromagnétiques. Lorsque des C.D.I. ont pu être obtenues simultanément au niveau de deux veines sus-hépatiques différentes, aucune différence n'a été trouvée entre les D.H.T. pairés. Des résultats identiques ont été obtenus chez des animaux où l'artère hépatique avait été ligaturée. Démontrant qu'il existe un mélange uniforme de l'indicateur dans la veine porte et au niveau de la circulation intra-hépatique, ces résultats justifient l'application de la méthode de Stewart-Hamilton. Des résultats préliminaires obtenus chez 13 cirrhotiques lors d'un cathétérisme combiné ombilico-portal et sus-hépatique, indiquent que la fraction portale du D.H.T. (le rapport D.V.P. sur D.H.T.) peut être estimée par cette méthode. En effet, la perte d'indicateur par des collatérales porto-systémiques, résultant en une surestimation des débits absolus, a lieu avant les prélèvements.

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I) INTRODUCTION

I. INTRODUCTION

Portal hypertension is the major clinical problem of advanced cirrhosis of the liver. Hemorrhage from esophageal varices is still the main cause of death either from exsanguination or from hepatic failure following the hemorrhagic shock. An average mortality of one half of all cirrhotic patients has been reported one year after the initial hemorrhage. Portal decompression by portosystemic anastomosis has been shown to be efficacious in preventing recurrent variceal bleeding if the shunt remains patent. However, the long term benefits of portacaval anastomosis are not obvious since the reduced incidence of bleeding varices is balanced by the higher mortality due to early or progressive hepatic failure or other undesirable metabolic sequelae such as encephalopathy (1). One of the consequences of standard portocaval shunts is the loss of all portal blood flowing into the liver which has been related to the hepatic failure. However, the importance of portal inflow in hepatic perfusion and function has never been satisfactorily evaluated in awake man because of the double blood supply to the liver and the relative inaccessibility of the portal vein. Therefore, indirect techniques have been devised to measure only total hepatic blood flow, the most widely used being Bradley's method (2). In some cases, during laparotomy for portacaval anastomosis, portal blood flows were measured using electromagnetic flowmeters and showed high values in most patients despite portal hypertension (3,4).

A non surgical method, applicable in the measurement of portal and total hepatic blood flows, which would avoid anesthesia and dissection of vessels, would be of great importance both physiologically and clinically in the evaluation of long term results and the selection of

the surgical procedure most appropriate for each individual case.

The first part of this work deals with an original method for the selective measurement of portal and total hepatic blood flows, the portal fraction of total hepatic blood flow and the sinusoidal fraction of portal blood flow, using indicator dilution curves obtained from portal and hepatic veins after injection of an indicator or a mixture of indicators into the cranial mesenteric artery in dogs. In the second part of this work, data obtained using this method are reported in cirrhotic patients following umbilicoportal catheterization combined with hepatic vein and superior mesenteric artery catheterization.

II) HISTORICAL REVIEW

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1) Direct flow measurement:

Except for the blood collection techniques (5,6), the earliest methods were based on the use of the mechanical stromuhr (7), the rotameters (8) and the electromagnetic flowmeters (9) by means of an ingoing and outgoing cannula into the proximal and distal section of the divided vessels. These methods had the disadvantages of requiring an extensive operative procedure, an interruption of flows and an artificial resistance to flows. These two last disadvantages were obviated with the introduction of the non cannulating thermostromuhrs (10) and, mainly, the electromagnetic flowmeters in intact vessels (11). To date, the use of the square wave electromagnetic flowmeters has proven to be the most reliable technique for direct measurement of hepatic arterial and portal blood flows (11), a technique which can be applied to man (3,4). However, this method requires laparotomy and results can possibly be modified by anesthesia and surgical manipulations.

2) Indirect flow measurement:

A) Methods based on the extraction of an indicator by the liver.

The development of the bromsulphalein technique by Bradley et al (2) based on the hepatic extraction (or hepatic clearance) of the dye was a great advance in the estimation of total hepatic blood flow. This technique could be applied to conscious man with no operative interference and no circulatory impairment to the liver. Following continuous infusion of the dye at a constant rate, its concentration is determined simultaneously in the hepatic venous blood and in the arterial blood.

Hepatic venous samples were collected by means of a cardiac catheter passed down the inferior vena cava into a hepatic vein. Assuming that the liver is the major organ concerned with bromsulphalein extraction and therefore that the concentration of the dye is identical in the hepatic arterial and portal venous blood, the Fick formula may be applied to the estimation of total hepatic blood flow.

Extrahepatic extraction, conjugation (as a limiting factor for excretion) and enterohepatic circulation with recirculating metabolites have been advocated as sources of errors when using bromsulphalein (12,13,14). However, many workers have found a good correlation between the Bradley's method and direct methods of measurements (15,16). The use of Indocyanine green, rapidly removed by the liver, avoids many of these disadvantages since neither extrahepatic extraction nor enterohepatic circulation are known to occur (17) and there is no hepatic metabolism prior to excretion. To date the Bradley method using continuous infusion of Indocyanine green is the most widely used indirect method for the estimation of total hepatic blood flow (18,19).

Other clearance methods have been described using peripheral plasma disappearance after a single injection of a substance almost completely removed by the liver during one passage, either by the parenchymal cells (hepatocytes) or by the mesenchymal cells (Kupffer cells). Flows measured are underestimated because of incomplete hepatic extraction and therefore are called minimal hepatic blood flows. Estimation of the total hepatic blood flow necessitates correction by the extraction rate which can be calculated by simultaneously performing hepatic venous catheterization. Estimation of minimal hepatic blood flow was first introduced by Dobson and Jones using colloidal particles of chromic phosphate labelled with P_{32} (20). Colloidal radiogold (21,22), Rose Bengal labelled with I^{131} (23)

and Indocyanine green (24) have been similarly employed. However, Indocyanine green clearance method has gained more popularity since it is easy to use and gives more reliable results.

An important modification was the use of colloidal heat-denatured human serum albumin labelled with I^{131} because more than 94% of the labelled albumin was removed by the Kupffer cells during one passage through the sinusoids of the liver (25). Therefore the measurement of minimal hepatic blood flow correlates well with the total hepatic blood flow determined by continuous infusion of Indocyanine green in normal subjects (26). In cirrhotic patients, lower extraction of the colloid exists (25,26). Since development of collateral channels between small portal veins and hepatic veins around the regeneration nodules has been reported in cirrhosis (27), the lowered extraction was related to shunting of some of the blood from the sinusoids lined with Kupffer cells (26). Therefore, assuming that there is no impairment of Kupffer cell function or reduction of the number of Kupffer cells lining each sinusoid, the hepatic extraction efficiency of the colloid would be an estimation of the proportion of blood shunted through intrahepatic portal hepatic venous anastomosis or an index of hepatic sinusoidal blood flow (28). However, the minimal hepatic blood flow could not be used as an index of the sinusoidal blood flow since no correction was done for the possible increased extrahepatic uptake of colloids known to occur in cirrhosis of the liver (29,30).

The indirect methods based on the extraction of an indicator are the most widely used in hemodynamic studies of the liver. However, they are used only to estimate total hepatic blood flow and are dependent on the parenchymal or mesenchymal function of the liver.

B) Indirect methods based on the dilution of an indicator.

Indicator dilution methods are not dependent on the hepatic function and therefore may be more accurate in liver diseases. The application of the Stewart Hamilton method (31,32) for the estimation of total hepatic blood flow was first introduced in man by Reichman et al (33). Flows were estimated following intrasplenic injection of radioactive iodinated serum albumin, from the indicator dilution curves obtained either by continuous sampling from one hepatic vein or by a scintillation gamma counter placed externally over the liver. In dogs, Murray et al and Ballinger and Bartone used injection of Indocyanine green into the portal vein (34,35). Blood was sampled from a hepatic vein or from inferior vena cava after its ligature under the liver (surgical common hepatic vein) through a cuvette-densitometer giving a continuous time concentration curve. In these studies, the hepatic extraction of the dye during one rapid transit through the liver was assumed to be minimal and not sufficient to alter significantly the estimation of flows.

The first experimental evaluation of the indicator dilution technique for the measurement of total hepatic blood flow was performed in dogs by Shoemaker et al (36), using autologous red blood cells labelled with Cr^{51}. They stated that similar flows have to be recorded simultaneously from two independent hepatic veins if the following requirements are fulfilled: "1) that the indicator used be not metabolized by the liver; 2) that the known amount of indicator which is injected into the portal vein is not directed into the systemic venous system prior to its passage through the liver; 3) that the injected substance be uniformly mixed in blood at the site of injection; 4) that the hepatic arterial-portal venous flow maintains a constant ratio of contribution to the

total inflow of all parts of the liver during the period of observation; 5) that the volume of blood contained within the liver remains constant during the period of observation." In chronic experimental dogs (24 to 48 hours following surgery), similar flows were obtained from two different hepatic veins, validating the indicator dilution method, although results were less reproductible in acute experiments (just following surgery). However, in their experiments, splenectomy was performed and catheters were placed in hepatic veins through direct puncture of the anterior wall of the thoracic inferior vena cava. These surgical manipulations could induce vascular changes similar to "outflow block" of the liver reported in the perfused canine liver (37) mainly due to contraction of the muscular sphincters located on hepatic veins in dogs.

Goresky has evaluated, in dogs, the liver sinusoidal and extravascular volumes from multiple indicator dilution curves using a vascular reference substance (Cr^{51} labelled red blood cells) and one or more diffusible indicator substances (T1824, C^{14} labelled inulin, tritium enriched water, I^{131} albumin, and $Na^{24}Cl$) (38).

To avoid the portal vein injection, Cohn and Pinkerson used injections of labelled indicator into the hepatic artery and found no significant difference in total hepatic blood flows values when compared with those obtained after portal injection in dogs (39). A similar technique has been applied in men, where total hepatic blood flow was estimated after injection of the labelled indicator into the hepatic artery, the splenic artery and the superior mesenteric artery (40). No difference was found when flows recorded from the indicator dilution curves were compared with results obtained with the Indocyanine green disappearance method in 7 normal subjects. In some cases nearly identical flows were

calculated simultaneously from two hepatic veins. These findings suggest that adequate mixing of the indicator is achieved in the portal vein as well as within the hepatic circulation after instantaneous injection of the indicator in the splenic artery and superior mesenteric artery (42).

This method has been applied in cirrhotic patients to evaluate portosystemic shunting from the splenic and mesenteric beds by comparing curves obtained after injections in the hepatic artery and in the splenic and mesenteric arteries (41).

The estimation of total hepatic blood flow by this method using hepatic arterial injection would be advantageous since it is independent of hepatic function and does not need portal (or splenic) injection. However, selective hepatic arterial catheterization, distal to any extrahepatic collateral such as the gastroduodenal artery, is required and results are not reliable when the arterial supply to the liver is derived from more than one common hepatic artery, as it frequently occurs in man (43).

These methods using the Stewart Hamilton principle were devised to measure only the total hepatic blood flow until Chiandussi et al (44) described an original method to evaluate simultaneously portal and total hepatic blood flows in cirrhotic patients. Following intrasplenic injection of I^{131} labelled serum albumin, indicator dilution curves were recorded simultaneously from the portal vein, cannulated through the umbilical cord, and one hepatic vein. The portal curve was used to estimate portal blood flow and the hepatic curve to estimate total hepatic blood flow. Flows were overestimated because of loss of indicator through portosystemic collaterals; however, the same amount of

indicator was analysed from portal and hepatic veins validating the ratio of the portal blood flow over the total hepatic blood flow. Intrasplenic injection was subsequently obviated by the use of a double lumen umbilicoportal catheter: the distal opening serving as an injecting site and the proximal opening as portal sampling site (45). These approaches have not been controlled in an experimental model by a direct method of measurement such as using electromagnetic flowmeters.

Another method based on the Stewart Hamilton principle was introduced by Marleau et al (46) for the estimation of the hepatic arterial or portal fraction of the total hepatic blood flow. Following injection of a labelled indicator into the abdominal aorta above the coeliac artery, the indicator dilution curve recorded from the hepatic vein is found to be biphasic: the early component is related to the part of the indicator flowing through the hepatic artery and the late component to the part of the indicator flowing through the splanchnic arteries and the portal vein. The ratio of the early component area over the total area of the biphasic curve is equal to the ratio of the hepatic arterial flow over the total hepatic blood flow. High arterial fractions of total hepatic blood flow were obtained and these data have to be confirmed by further experimental studies.

Other dilution methods, utilizing Fick's principle, have been developed using indicator substances not excreted or metabolized by the liver but almost completely removed during one passage through the kidneys or the lungs. First used in sheep (47,48) and dogs (48), para aminohippuric acid (normally removed by the kidneys) was continuously infused into a distal mesenteric vein and its concentrations determined in portal and hepatic veins and in one peripheral artery. The Fick formula was used to estimate the portal and total hepatic blood flows and results correlated

well with those obtained using Bradley's method for total hepatic blood flow. However, sources of errors could result from high para aminohippuric acid concentration in the arterial blood because of an incomplete extraction by the kidney, even in normal dogs, resulting in a high recirculation.

This high arterial concentration was obviated with the use of Xenon, a substance almost completely removed by the lungs during a single passage, the extraction efficiency being over 95%. In two experimental studies, known amount of radioactive Xenon 133 was injected into the stomach (49) or the small bowel (50) and its concentration was determined simultaneously in the portal and hepatic veins and in one peripheral artery. Stone et al (49) compared simultaneous concentration of radioactive Xenon and used a derivative of the Fick formula to evaluate the portal flow as per cent of the total hepatic blood flow in normal dogs. Results correlated well with those obtained simultaneously using electromagnetic flowmeters. Shizgal and Goldstein (50) found that the disappearance of the radioactive Xenon 133 from portal and hepatic veins plotted against time was exponential and used the slope of the concentration decay in the determination of flows. Assuming that all Xenon injected in the small bowel was absorbed and passed up the portal vein, absolute flows were estimated using the Fick formula.

These methods can be easily applied to awake men using portal catheterization via the umbilical cord combined with hepatic catheterization (51). Strandell et al (52) described, in a preliminary report, a modification of these methods using umbilicoportal catheterization with multiple catheters. Following continuous infusion of radioactive Xenon 133 distally into the splenic or mesenteric vein, portal blood flow was estimated using samples collected proximally from the right and/or the left branches

of the portal vein. Simultaneously, total hepatic blood flow was estimated using samples collected in hepatic veins.

However, these studies did not demonstrate that adequate mixing of radioactive Xenon is already achieved into the portal vein after a single injection into the lumen of the intestine or after continuous perfusion into one tributary of the portal vein. In our laboratory we failed to obtain reproducible identical radioactive Xenon concentration in two different hepatic veins after continuous infusion into the cranial mesenteric artery in dogs suggesting that, under these experimental conditions, poor mixing of the Xenon occurred in the portal vein and/or the intrahepatic circulation (B. Millette, unpublished data).

Some dilution methods have been devised to measure only the portal blood flow in men. Reichle et al (53) used lipiodol droplets injected into the portal vein through an umbilicoportal catheter. Portal blood flow was estimated using the velocity of the droplets recorded on high speed cinefluorography and the cross-sectional area of the portal vein assessed by biplane portography. Dencker et al (54) estimated portal blood flow using indicator dilution curves from the portal vein after injection of indocyanine green into the superior mesenteric artery. These methods are of limited interest since no information could be obtained simultaneously concerning the total hepatic blood flow.

Other non surgical dilution methods have been described to evaluate the hepatic arterial or portal venous fraction of total hepatic blood flow in men. Ueda et al (55) used the hepatic uptake of intravenous radiogold measured by external scintillation detector. They found that the hepatic accumulation of radiogold is biphasic with time: the first peak activity was related to removed radiogold flowing through the hepatic artery, and the second to removed radiogold flowing through the portal vein.

Hepatic arterial flow was calculated as per cent of total hepatic blood flow using the ratio of the first peak activity (hepatic arterial inflow) over the second (hepatic arterial plus portal venous inflows). Nakamura et al (56) used radioactive iodinated serum albumin continuously infused into the pulmonary artery. Portal blood flow as per cent of total hepatic blood flow was calculated from "plateau" concentration in the peripheral arterial and hepatic vein radioactivity curves. However, the determination of either the peak activities or the plateau were difficult and these methods require further experimental controls before being used clinically.

III) PART I: EXPERIMENTAL STUDIES IN DOGS

1) MATERIAL AND METHODS

A) Material:

One hundred and twenty four (124) adult mongrel dogs of either sex and weighing more than 10 Kg. were used. Animals were anesthetized with intravenous pentobarbital (20 mg/Kg). Endotracheal intubation was performed and the animals breathed atmospheric air. In prolonged experiences, anesthesia was maintained with additional injection of 10% of the initial anesthetic dose per hour and in some cases, respiration was assisted by a ventilator using nitrous oxide (70%) and oxygen (30%).

B) Methods:

a) Experimental design to validate the use of the indicator dilution method.

In 54 dogs, a left and a right hepatic veins were cannulated via the external jugular veins using open ended polyethylene catheters (No. 8F, 100 cm. length, Reno Micro Precision, Montreal) with lateral holes. Following laparotomy, a close ended polyethylene catheter (No. 8F, 100 cm. length, Reno Micro Precision, Montreal) with lateral holes was positionned in the portal vein at the junction of the splenic and superior mesenteric veins via a cannulated splenic tributary. The cranial mesenteric artery was cannulated using a polyethylene catheter (No. 6.7F, 65 cm. length, Cook Incorporated) via one femoral artery (fig. 1). All catheters were positionned under fluoroscopic visualization (Medical T.V. Chain, Philips; type 67104/00/01) using radiopaque material (Renographin 60, Squibb). The other femoral artery was cannulated by direct puncture with a small arterial cannule (Cathon IV, Jelco) to

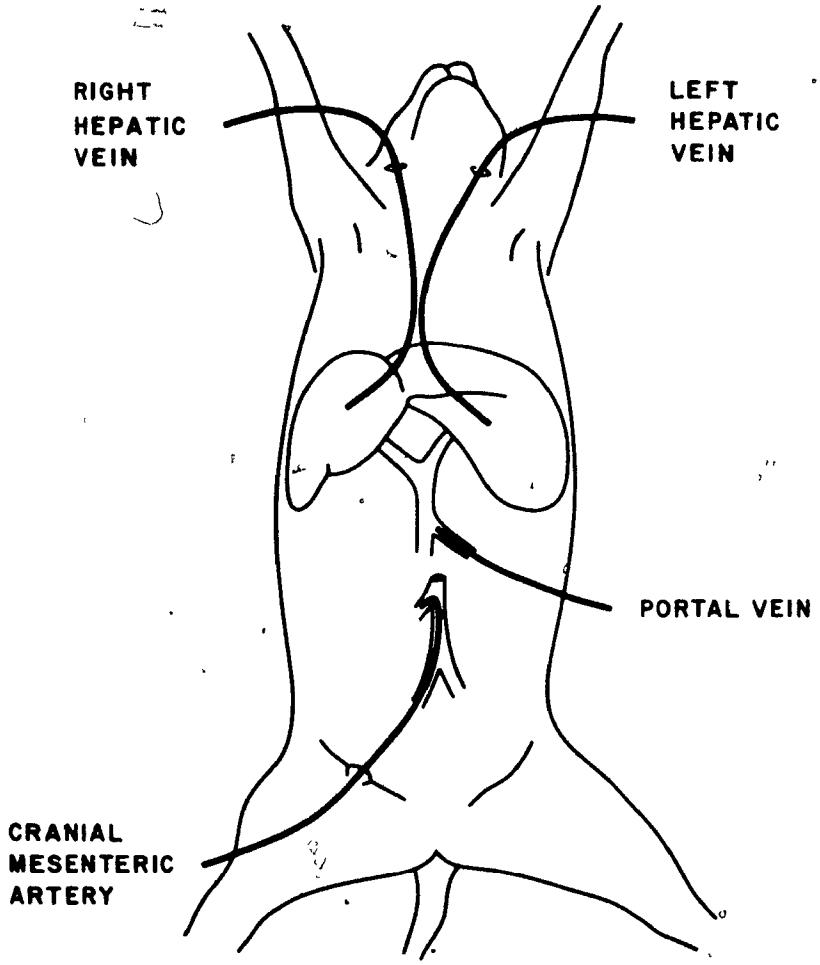

FIGURE 1: SCHEMATIC DIAGRAM ILLUSTRATING THE POSITION OF THE INJECTING AND SAMPLING CATHETERS IN DOGS (GROUPS A AND B).

record blood pressure using a Sanborn pressure transducer. After positioning of the catheters perfused with normal saline (1 to 2 ml/min.), heparin (10,000 units/hr) was given intravenously to all dogs.

The indicators used were Evans Blue (E.B., T1824, 5 mg/ml, Warner-Chilcott) and Indocyanine Green (I.C.G., 1.25 mg/ml, Hynson, Wescott and Dunning). Each single injection of the indicator (1.26 ml) was performed using a dye dilution glass tube with a one way valve (Becton-Dickinson) and flushed by 10 ml of blood or saline. Blood was withdrawn via the sampling catheters through two cuvette densitometers (Waters densitometers, model XC250) using a Harvard pump (model 932) at a constant rate (22.9 ml/min.). The resultant curves of the dye concentration were photographically recorded on a multiple channel physiograph (Sanborn, model 350) at a paper speed of 5 mm/sec. Simultaneous estimations of flows were obtained from cardiac output computers (Lexington Instruments Company, model C.O.C.) (fig. 2). The system was calibrated using heparinized blood standards with increasing dye concentrations (0, 2.5 and 5 mg/l).

The animals were divided into two groups: one group of 35 dogs (group A) where Evans Blue was injected either into the portal vein (20 dogs) or into the cranial mesenteric artery (15 dogs) and another group of 19 dogs (group B) where Indocyanine Green was injected either in the portal vein (6 dogs) or into the cranial mesenteric artery (13 dogs). In all dogs, blood samples were withdrawn from one right and one left hepatic veins. The surface area of the dye dilution curves obtained were corrected for recirculation by extrapolation of the downslope to the baseline on semilogarithmic paper. Calculation of flows from the corrected surface area was computed by an Olivetti Underwood calculator (Programma 101) using the formula:

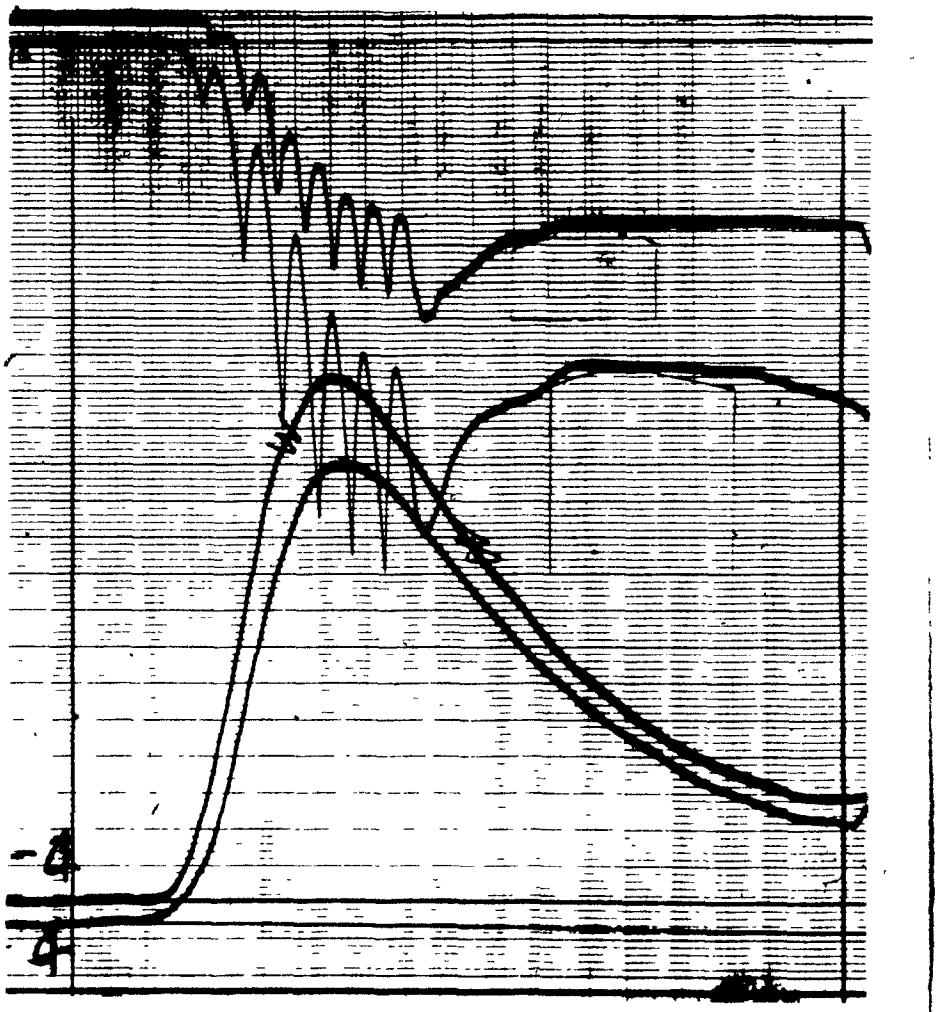

FIGURE 2: SIMULTANEOUS INDICATOR DILUTION CURVES RECORDED ON PHOTOGRAPHIC PAPER FROM ONE RIGHT AND ONE LEFT HEPATIC VEINS AFTER INJECTION OF EVANS BLUE INTO THE CRANIAL MESENTERIC ARTERY. AT BOTTOM, DEFLEXION RECORDED BY THE CARDIAC OUTPUT COMPUTER.

$$F\ (L/min.) = \frac{60 \times Q}{\Sigma C}$$

Q = quantity of dye injected (mg)

ΣC = sum of the dye concentration at one second interval (mg/L/sec.)

At the beginning of each experimental protocol the reliability of the electronical set-up was tested by comparing flows recorded serially by the two densitometers from one hepatic vein. Sixty four (64) serial flows were obtained using Evans Blue and 37 using Indocyanine Green. No significant difference was found between paired flows and the mean difference was 5.0% (0 to 21.8%).

In group A, using Evans Blue, 62 experiments were recorded after injection into the portal vein and 54 after injection into the cranial mesenteric artery. In group B, using Indocyanine Green, 41 experiments were recorded after injection into the portal vein and 64 after injection into the cranial mesenteric artery.

b) Estimation of the portal and total hepatic blood flows by electromagnetic flowmeters and Cr^{51} red blood cells dilution curves.

Experiments were performed in 34 dogs. The cranial mesenteric artery, the left branch of the portal vein, a left hepatic vein, and when possible, a right hepatic vein, were catheterized under fluororsopic visualization using radiopaque material. The hepatic veins were cannulated via the external jugular veins, using open ended polyethylene catheters (No. 8F, 100 cm. length, Reno Micro Precision, Montreal) with lateral holes. The left branch of the portal vein was then cannulated through the pancreaticoduodenal vein by laparotomy using an open ended polyethylene catheter (No. 8F, 100 cm. length, Reno Micro Precision, Montreal) with lateral holes (fig. 3). All catheters were perfused with saline (1 to 2 ml/min.).

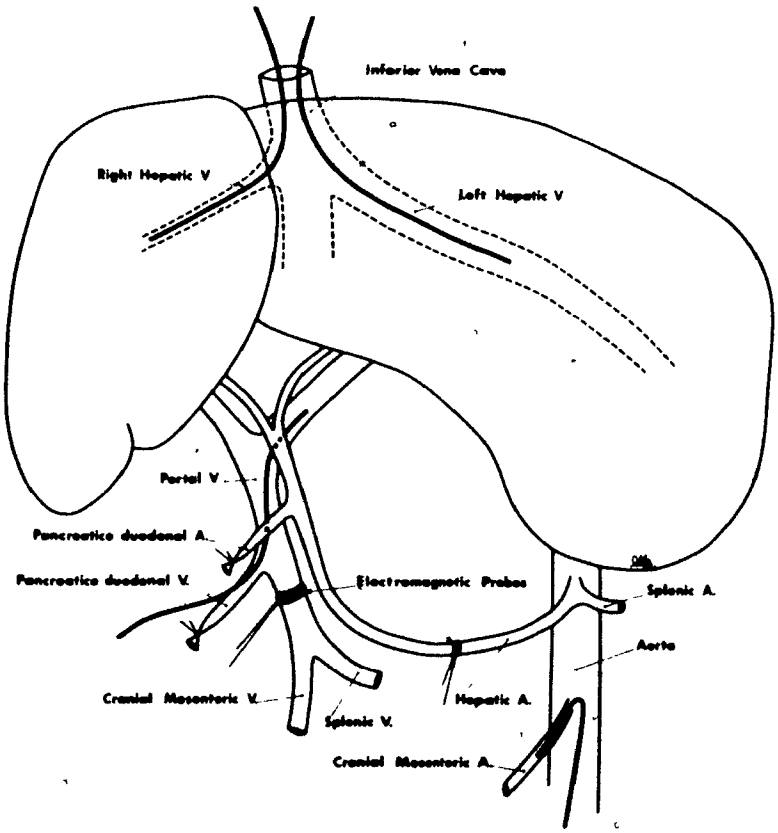

FIGURE 3: SCHEMATIC DIAGRAM ILLUSTRATING THE POSITION OF THE INJECTING AND SAMPLING CATHETERS AND OF THE ELECTROMAGNETIC FLOW PROBES.

The hepatic artery was isolated along the lesser curvature of the stomach, and the nerve plexus was carefully preserved for the positioning of a calibrated flow probe (2 or 3 mm, Micron Instruments). The pancreaticoduodenal artery was then ligated to insure that the liver total arterial flow was measured. The portal vein was then isolated before its junction to the pancreaticoduodenal vein, for the positioning of a calibrated flow probe (6 or 7 mm, Micron Instruments (fig. 3). No accessory hepatic artery was found in any dog. All dogs received intravenous heparin (10,000 units/hr) after positioning of the catheters and probes.

i) Electromagnetic flowmeters.

Flow probes measurements of hepatic arterial and portal flows were analysed by two square wave electromagnetic flowmeters (Micron Instruments, RC 1000) and recorded on the multiple channel physiograph. The zero levels, directly obtained in flowmeters without occluding the vessels, were validated at the end of each experiment either by occluding the vessels or by taking measurements after cessation of flows at death. The error of the flowmeters was within 6%, as verified by the blood collection method. Total hepatic blood flow was calculated by the sum of the simultaneous recording of hepatic arterial and portal venous blood flows.

ii) Indicator dilution method.

Portal and total hepatic blood flows were calculated by the Stewart Hamilton method after a single injection of an indicator into the cranial mesenteric artery. Portal blood flow was calculated using the indicator dilution curve (I.D.C.) obtained from the left branch of the portal vein and total hepatic blood flow was calculated by simultaneous I.D.C. from hepatic vein(s). The same amount of indicator was

first analysed in the portal vein and then in one or two major hepatic veins.

In these experiments, Indocyanine Green was used to control the position of the catheters by direct recording of curves on photographic paper as described in paragraph B (fig. 4), but was not chosen as indicator because of its metabolic interaction with liver cells.

Therefore, a chromium 51 labelled autologous red blood cells (Cr^{51} R.B.C.) suspension (250 µc/15 ml.), prepared according to Wagner (57) (with three washings) was chosen as the ideal indicator as it gives, after sudden injection, a sharp peak, making it easily dissociable from recirculation (38). After instantaneous injections of known volume (2.5 to 5 ml) of Cr^{51} R.B.C. into the cranial mesenteric artery, the catheter was flushed with 10 ml. of blood or saline. Known volume (0.4 ml) of the same suspension counted on an automated gamma counter (Nuclear Chicago, model 300) were used as standards. The use of identical catheters for all samples prevented distortion (58), as well as the use of a peristaltic pump (Harvard apparatus) at a fixed flow (30 ml/min.). Samples were collected into three serial collection racks running at a constant speed of 1 tube per second (fig. 5). The standard volume (0.4 ml) was withdrawn from each tube, counted separately, and results were plotted on semilogarithmic paper (fig. 6). Extrapolation of the downslope to the baseline, correction for the background, expression of c.p.m. (counts per minute) per ml and calculation of flows were computed by a Wang calculator.

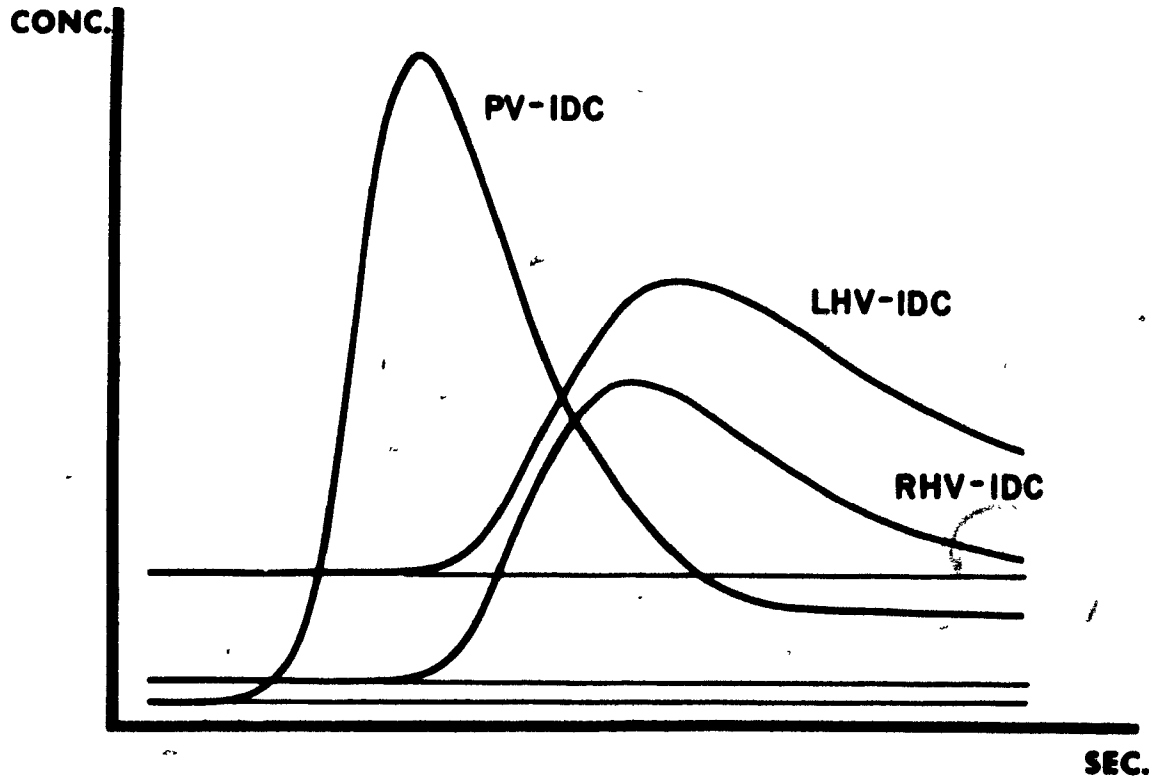

FIGURE 4: SIMULTANEOUS INDICATOR DILUTION CURVES RECORDED FROM THE PORTAL VEIN (PV-IDC), A LEFT HEPATIC VEIN (LHV-IDC) AND A RIGHT HEPATIC VEIN (RHV-IDC) AFTER INJECTION OF INDOCYANINE GREEN INTO THE CRANIAL MESENTERIC ARTERY.

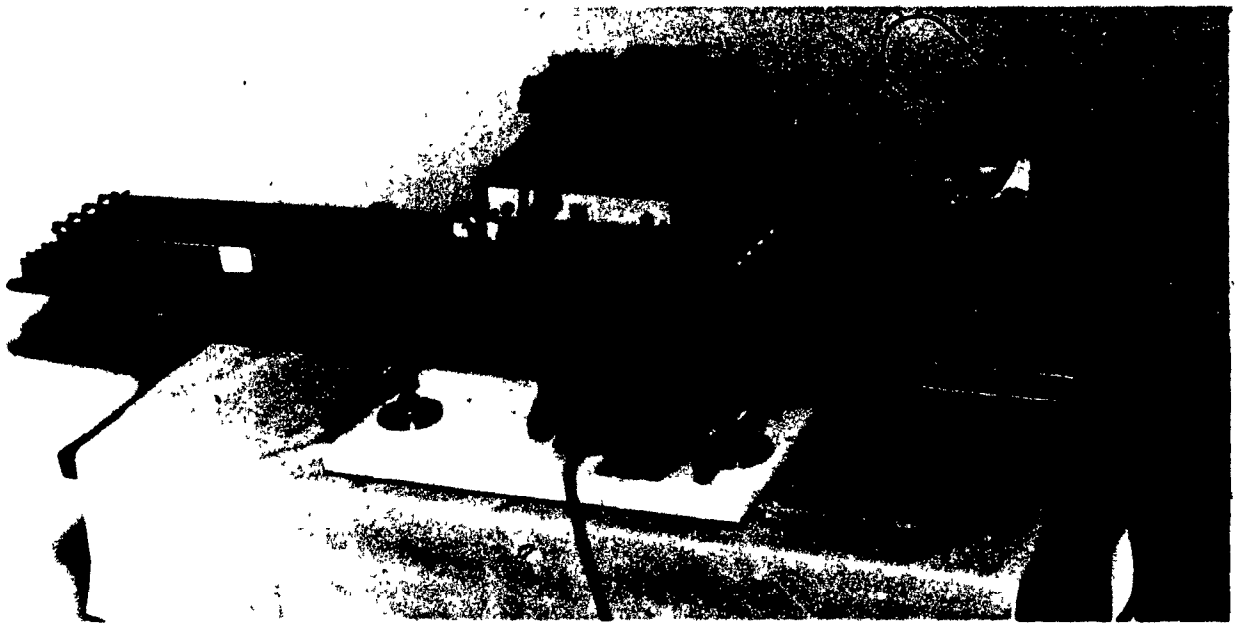

FIGURE 5: PHOTOGRAPH SHOWING THE PERISTALTIC PUMP AND THE THREE SERIAL COLLECTION RACKS USED FOR THE SAMPLING FROM PORTAL AND HEPATIC VEINS.

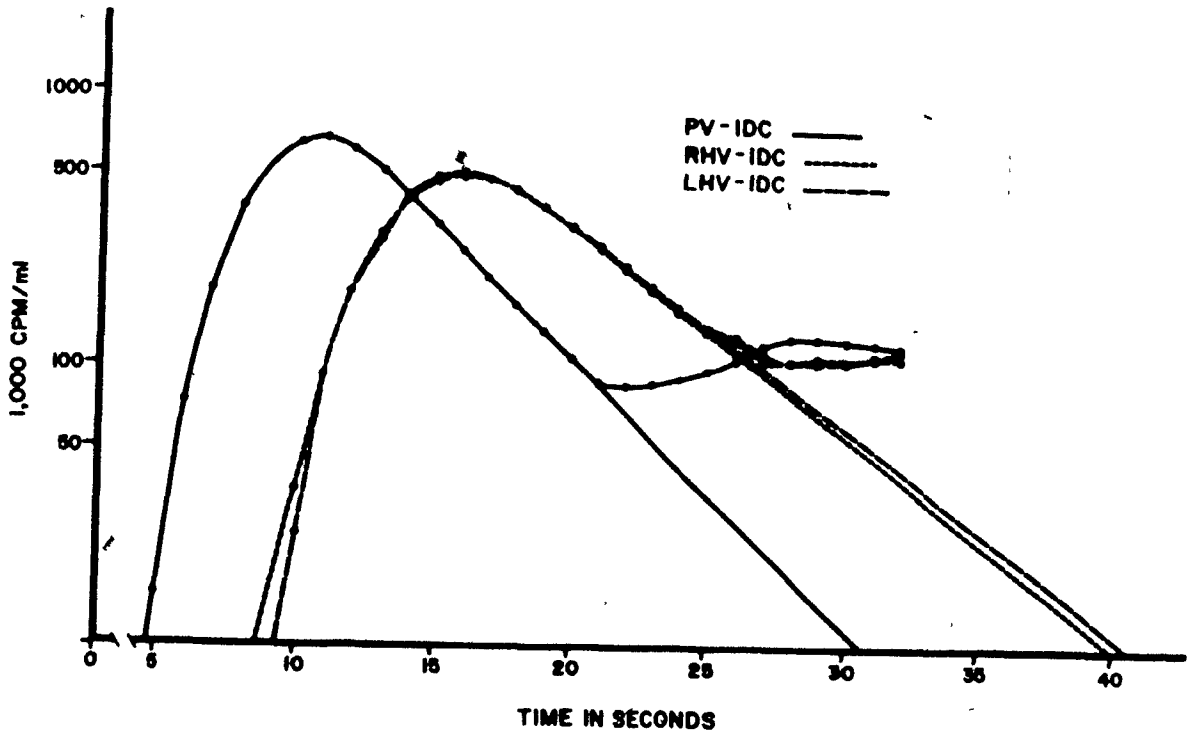

FIGURE 6: SIMULTANEOUS INDICATOR DILUTION CURVES (I.D.C.) REPLOTTED ON SEMILOGARITHMIC PAPER AFTER INJECTING Cr^{51} RED BLOOD CELLS (Cr^{51} R.B.C.) INTO THE CRANIAL MESENTERIC ARTERY.

PV-IDC: IDC OBTAINED FROM THE LEFT BRANCH OF THE PORTAL VEIN

RHV-IDC: IDC OBTAINED FROM A RIGHT HEPATIC VEIN

LHV-IDC: IDC OBTAINED FROM A LEFT HEPATIC VEIN

Flows were calculated using the formula:

$$F\ (ml/min.) = \frac{60 \times Q}{\Sigma C}$$

Q = quantity of Cr^{51} R.B.C. injected (c.p.m.)

ΣC = sum of the Cr^{51} R.B.C. concentration (c.p.m./ml/sec.)

The animals were divided into 4 groups (table 1): one group of 19 dogs (group C) with normal double supply to the liver by the hepatic artery and the portal vein; one group of 5 dogs (group D) with the hepatic artery ligated along the lesser curvature of the stomach so that only portal blood could enter the liver; one group of 5 dogs (group E) in which the effects of the radiopaque material, injected while positioning the various catheters, probes and ligatures, were studied; and finally one group of 5 dogs (group F) in which the effects of the surgical manipulation of hepatic artery and portal vein were studied.

In group C, 40 experiments and in group D, 9 experiments were recorded with simultaneous determination of flows using I.D.C. and flowmeters. In the 27 experiments of group C and 7 of group D, total hepatic blood flow could be estimated by averaging the total hepatic blood flows measured from one right and one left hepatic veins. This was impossible in 13 experiments of group C and 2 of group D where only a left hepatic vein could be catheterized. Each experiment was performed only when flows recorded by flowmeters were stable at least for 5 minutes; when several experiments were made on the same dog, at least 15 minutes separated each successive procedure.

In group E, all catheters were positioned under fluoroscopic visualization but without using radiopaque material. The arterial catheter was left into the abdominal aorta, the jugular catheters into the vena cava

	ELECTROMAGNETIC FLOWMETERS	Cr^{51} R.B.C DILUTION CURVES
GROUP C (19 normals)	Portal blood flow and hepatic arterial blood flow (40)	Portal blood flow (40) and total hepatic blood flow a) mean from R.H.V and L H V (27) b) from L.H.V (13)
GROUP D (5 ligated hepatic arteries)	Portal blood flow (9)	Portal blood flow (9) and total hepatic blood flow a) mean from R H V. and L H V. (7) b) from L.H V (2)
GROUP E (5 normals) before and after radio-peque material loading	Portal blood flow and hepatic arterial blood flow	Cardiac output.
GROUP F (5 normals) before positioning probes		Portal blood flow and total hepatic blood flow from L.H.V. (8).
after positioning probes	Portal blood flow and hepatic arterial blood flow (9)	Portal blood flow and total hepatic blood flow from L.H.V. (9)

R.H.V. right hepatic vein. L.H.V left hepatic vein.

TABLE 1 EXPERIMENTAL DESIGN TO VALIDATE THE USE OF INDICATOR DILUTION CURVES IN THE ESTIMATION OF TOTAL HEPATIC AND PORTAL BLOOD FLOWS. (THE VALUES IN PARENTHESES REFER TO THE NUMBER OF EXPERIMENTS WITH EACH METHOD)

and the pancreaticoduodenal vein into the portal vein. Hepatic arterial and portal venous blood flows were measured using electromagnetic flowmeters and cardiac output using I.D.C. from the aorta after injection of the indicator into the inferior vena cava. Hepatic arterial blood flow, portal venous blood flow and cardiac output were measured simultaneously during the control state and during two successive periods of 30 minutes following injection of 50 ml of radiopaque material (Renographin 60, Squibb) through various catheters.

In group F, catheters were positioned into a left hepatic vein, the left branch of the portal vein and the cranial mesenteric artery under fluoroscopic visualization but without using radiopaque material. In all dogs, one or two successive measurements of portal and total hepatic blood flows were performed using I.D.C. before positioning of the probes on hepatic artery and portal vein (8 experiments). Then, 15 minutes after positioning of the probes, one or two successive measurements of flows were recorded simultaneously using I.D.C. and flowmeters (9 experiments).

c) Evaluation of Indocyanine Green as indicator for the estimation of portal and total hepatic blood flows.

These experiments were performed to evaluate the Indocyanine Green used to control the position of the catheters by direct recording of curves on photographic paper.

1) Comparison between Indocyanine Green-I.D.C. and electromagnetic flowmeters.

Twenty nine (29) experiments were performed in 9 dogs (group G) prepared as described in group C. Portal and total hepatic blood flows were measured simultaneously using electromagnetic flowmeters

and Indocyanine Green-I.D.C. (as described in paragraph Bb). In all dogs, using I.D.C., total hepatic blood flow could be estimated by averaging the total hepatic blood flows measured simultaneously from one right and one left hepatic veins.

ii) Comparison between Indocyanine Green and Cr^{51} R.B.C.-indicator dilution curves.

Thirty two (32) experiments were performed in 12 dogs (group H) to compare simultaneous I.D.C. obtained after injections of a mixture of Indocyanine Green and Cr^{51} R.B.C. The Indocyanine Green concentration of the mixture was calculated from the amount of dye dissolved in a known volume of saline and added to a known volume of Cr^{51} R.B.C. suspension. Cr^{51} R.B.C. concentration was estimated from counted standards of the mixture (0.4 ml). A known amount of the mixture was injected into the cranial mesenteric artery and blood samples were withdrawn using a peristaltic pump (30 ml/min.) through the cuvette-densitometer and collected into two serial collection racks. Portal and total hepatic blood flows were calculated, as described in paragraph B_1, from I.D.C. obtained simultaneously using Indocyanine Green and Cr^{51} R.B.C. from the left branch of the portal vein and a left hepatic vein. In 6 experiments (3 dogs), cardiac output was calculated using I.D.C. obtained from the abdominal aorta after injection into the inferior vena cava.

d) Estimation of the sinusoidal fraction of portal blood flow.

i) Background.

Injected Cr^{51} R.B.C. are not metabolized or excreted by the liver and they are almost completely recovered in the hepatic veins (38). They remain in the vascular space and therefore may be used as a vascular reference substance (38).

I^{125} albumin microaggregates (I^{125} A.M.A.), a colloid almost completely phagocytized during one passage through the hepatic reticuloendothelial system (R.E.S.) (59) can be added to the Cr^{51} R.B.C. suspension injected into the cranial mesenteric artery. Theoretically this indicator (1 to 5 μ) should flow freely through the splanchnic capillary system. Portal I.D.C. could then be obtained and used to measure portal blood flow. Since this indicator is removed by the hepatic R.E.S., the hepatic I.D.C. could not be utilized to evaluate the total hepatic blood flow. However, the hepatic extraction efficiency of I^{125} A.M.A. flowing through the portal vein could be measured by comparing hepatic and portal curves obtained simultaneously with both indicators and expressed as per cent of portal blood flow.

In the following experiments, the amount of injected colloid (less than 1 mg) is well below the reported critical dose under which all particles entering the sinusoids should be totally phagocytized by the hepatic R.E.S. during one passage (28). If some I^{125} A.M.A. are not phagocytized, the I.D.C. obtained from hepatic veins could be related to a fraction of portal blood flow bypassing the sinusoidal Kupffer cells because of intrahepatic porto-hepatic shunts (27). Therefore the hepatic extraction efficiency could be utilized in the estimation of a sinusoidal fraction of portal blood flow in dogs.

1) Methods.

Fourty four (44) experiments were performed in 15 dogs. In all dogs, the cranial mesenteric artery, the left branch of the portal vein, a left hepatic vein and, in 5 dogs, a right hepatic vein were catheterized under fluoroscopic visualization using radiopaque material, as previously described in paragraph B. All catheters were perfused with saline (1 to 2 ml/min.) and heparin (10,000 u/hr) was administered intravenously after positioning of the catheters.

Portal blood flows were calculated using I.D.C. obtained from the left branch of the portal vein and total hepatic blood flows were calculated by simultaneous I.D.C. from hepatic vein(s) after a single injection of the indicator mixture into the cranial mesenteric artery (fig. 7). The indicator mixture contained Cr^{51} R.B.C. and I^{125} A.M.A. and the volume injected in each experiment was 2.7 ml. using a dye dilution glass tube. Packed Cr^{51} R.B.C. were prepared as described in paragraph B. I^{125} A.M.A. were prepared according to Kitani and Taplin (60): 0.1% I^{125} labelled albumin at pH 5.2 was heated at 100°C for 3½ min. with shaking which produced aggregates of 10 to 15 microns. After cooling at the room temperature, the suspension was ultrasonicated for 5 min. to reduce aggregate size to 1 to 5 microns. More than 90% of the radioactivity was removed from the suspension by centrifugation at 3,000 r.p.m. for 10 min. Particles of 1 to 5 microns were readily visible microscopically and their size distribution was controlled for each preparation using an hematocytometer (fig. 8). Blood was reconstitued by adding to packed Cr^{51} R.B.C., 5 ml of microaggregate suspension and isotonic saline so that each single injection contained less than 1.0 mg of I^{125} albumin (usually 0.88 mg).

After instantaneous injections of the known volume of the mixture solution into the cranial mesenteric artery, the catheter was flushed with 10 ml of blood or saline. Samples were collected using a peristaltic pump (30 ml/min.) into serial collection racks running at a speed of 1 tube per second. Known volume (0.4 ml) was withdrawn from the injection mixture (used as standards) and from each tube were counted separately on an automated gamma counter (Nuclear Chicago model 300) in two channels of appropriate energy (Cr^{51} and I^{125}). The activity due to each substance was corrected using appropriate standards. In order to

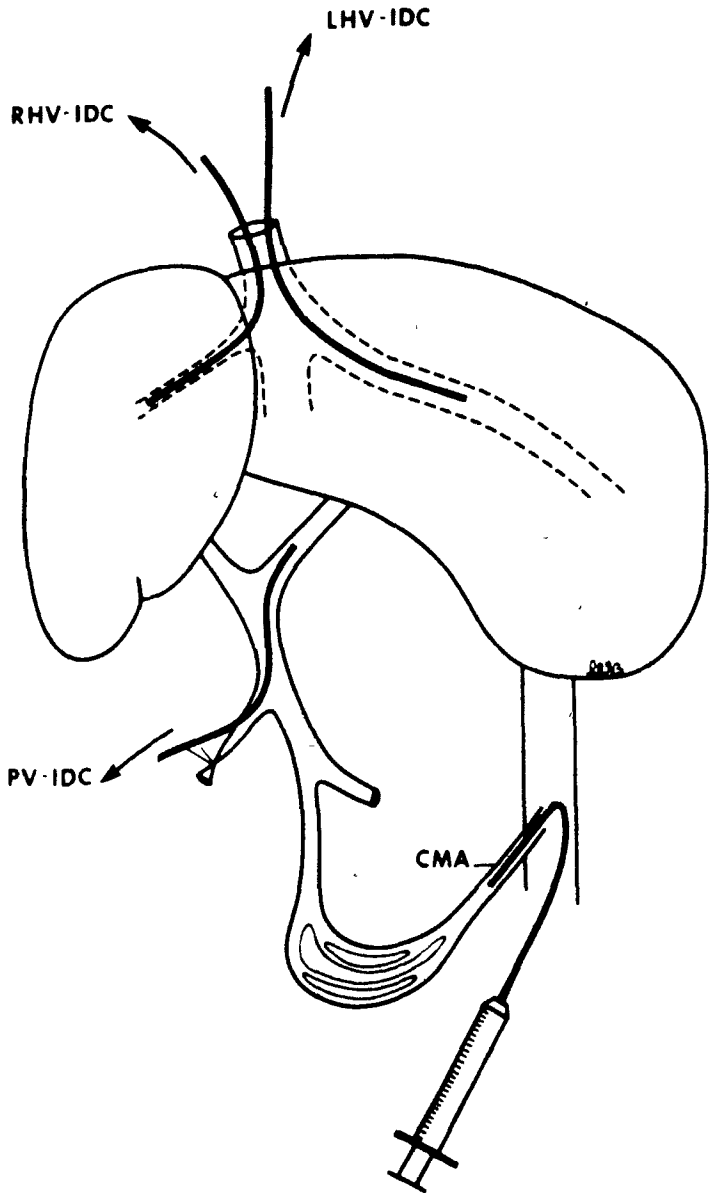

FIGURE 7: SCHEMATIC DIAGRAM ILLUSTRATING THE POSITION OF THE INJECTING AND SAMPLING CATHETERS USING I^{125} ALBUMIN MICROAGGREGATES (I^{125} A.M.A.).

FIGURE 8: MICROSCOPIC ASPECT OF A DILUTED I^{125} ALBUMIN MICROAGGREGATES PREPARATION (1/5) ON HEMATOCYTOMETER.

provide a basis for comparison between indicators, the total amount of each indicator injected was defined as 1 unit. The activity (c.p.m.) recorded in each tube and for each indicator (Cr^{51} and I^{125}) was divided by the total amount of activity injected (Cr^{51} or I^{125}) and corrected to ml. The resulting numbers were an expression of the outflow fraction of the injected mass per ml of blood and were plotted on semilogarithmic paper against time (38). Correction for the overlaping activity between indicators and for the background, expression of the fractional recovery per ml, extrapolation of the downslope to the baseline and calculation of flows were computed by a Wang calculator (model 700B).

Flows are calculated using the formula:

$$F = \frac{60 \times Q}{\Sigma C} \qquad (1)$$

where both terms are divided by Q in order to compare indicators:

$$F = \frac{60 \times \frac{Q}{Q}}{\Sigma \frac{C}{Q}} \qquad (2)$$

or

$$F \text{ (ml/min.)} = \frac{60}{\Sigma \frac{C}{Q}} \qquad (3)$$

Q = quantity of Cr^{51} R.B.C. or I^{125} A.M.A. injected (c.p.m.)

ΣC = Cr^{51} R.B.C. or I^{125} A.M.A. concentration at one second interval (c.p.m./ml/sec.)

$\Sigma \frac{C}{Q}$ = sum of the outflow fractions of injected mass (ml/sec.)

The results of a multiple indicator dilution experiment are illustrated in figure 9. The I.D.C. obtained from the portal vein were almost identical with Cr^{51} R.B.C. and I^{125} A.M.A. suggesting that almost all microaggregates injected into the cranial mesenteric artery appeared in the portal blood. However, the I.D.C. obtained from hepatic veins were

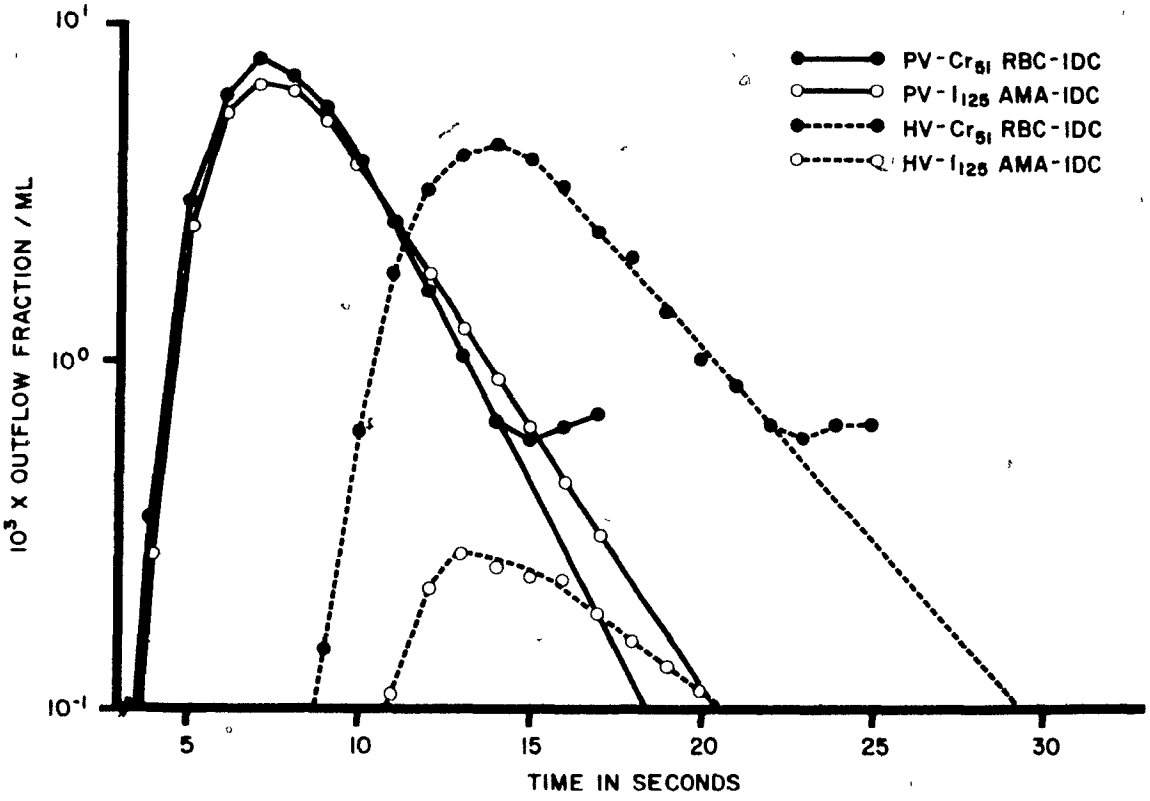

FIGURE 9: SIMULTANEOUS Cr^{51} RED BLOOD CELLS-I.D.C. (Cr^{51} RBC-IDC) AND I^{125} ALBUMIN MICROAGGREGATES-I.D.C. (I^{125} AMA-IDC) OBTAINED FROM THE LEFT BRANCH OF THE PORTAL VEIN (P.V.) AND ONE LEFT HEPATIC VEIN (H.V.) AFTER A SINGLE INJECTION INTO THE CRANIAL MESENTERIC ARTERY.

strikingly different, the I^{125} A.M.A. curve being small in relation to the reference red cell curve. These findings indicate that a large proportion of this material was irreversibly removed by the liver during a single passage.

Interpretation of data.

Theoretically, if no removal of I^{125} A.M.A. occurred during the passage through the liver, the ratio between the hepatic vein curve area (H.V.)* and the portal vein curve area (P.V.) would be the same whether using I^{125} A.M.A. (I) or Cr^{51} R.B.C. (Cr) assuming identical dilution of portal blood by the hepatic arterial blood for both indicators.

Thus, theoretically,

$$\frac{HV.I}{PV.I} \text{ (would be equal to) } \frac{HV.Cr}{PV.Cr} \qquad (1)$$

and the "theoretical HV.I" would be:

$$\text{"theoretical HV.I"} = PV.I \times \frac{HV.Cr}{PV.Cr} \qquad (2)$$

This value compared with the calculated HV.I can be used to estimate the hepatic extraction efficiency (E) of the injected microaggregates flowing through the portal vein:

$$E(\%) = 100 \times \frac{\text{theoretical} - \text{calculated}}{\text{theoretical}} \quad HV.I \qquad (3)$$

then by combining (2) and (3)

$$E(\%) = 100 \times \frac{PV.I \times \frac{HV.Cr}{PV.Cr} - HV.I}{PV.I \times \frac{HV.Cr}{PV.Cr}} \qquad (4)$$

or

$$E(\%) = 100 \times \frac{PV.I \times HV.Cr - HV.I \times PV.Cr}{PV.I \times HV.Cr} \qquad (5)$$

where each curve areas can be directly calculated from the multiple indicator dilution curves.

This hepatic extraction efficiency was expressed as per cent

* Curve area as recorded by fractional recovery against time.

of portal blood flow and was used in the estimation of the sinusoidal fraction of portal blood flow.

In 11 experiments (5 dogs), total hepatic blood flow and the sinusoidal fraction of portal blood flow could be estimated by averaging the values measured simultaneously from one right and one left hepatic veins. This was impossible in 33 experiments (10 dogs) where only a left hepatic vein could be catheterized.

In this work, correlation between pairs was tested by the Pearson r test and the mean of individual differences was tested by the Student t test for pairs (61).

2) RESULTS

A) Experimental design to validate the use of the indicator dilution method.

a) Group A.

In this group, total hepatic blood flow was estimated simultaneously from one right and one left hepatic veins using Evans Blue as indicator. In 20 dogs, 62 calculable pairs of curves were obtained after injection of the indicator into the portal vein (table 2). The difference between paired total hepatic blood flow obtained from one right and one left hepatic vein varied between 0 and 48.2% (mean: 10.6%) and no significant difference was found between paired flows (t: 1.549, $p>0.10$) (fig. 10). In 15 dogs, 54 calculable pairs of curves were obtained after injection of the indicator into the cranial mesenteric artery (table 2). The difference between paired total hepatic blood flows varied between 1 and 25.6% (mean: 6.8%) and no significant difference was found between paired flows (t: 1.539, $p>0.10$) (fig. 11).

b) Group B.

In this group, total hepatic blood flow was estimated simultaneously from one right and one left hepatic veins using Indocyanine Green as indicator. In 6 dogs, 41 calculable pairs of curves were obtained after injection of the indicator into the portal vein (table 2). The difference between paired estimated total hepatic blood flows varied between 0.8 and 35.3% (mean: 13.0%) and no significant difference was found between paired flows (t: .627, $p>0.10$) (fig. 12). In 13 dogs, 64 calculable pairs of curves were obtained after injection of the indicator into the cranial mesenteric artery (table 2). The difference between paired estimated total hepatic blood flow varied between 1.7 and 24.7%

TOTAL HEPATIC BLOOD FLOW

	n**	RIGHT HEPATIC VEIN	LEFT HEPATIC VEIN
GROUP A (Evans Blue)			
Injection into the P.V	62	1728±103 (ml/min)	1670±100 (ml/min)
Injection into the C M A	54	1804±82 (ml/min)	1859±92 (ml/min)
GROUP B (Indocyanine Green)			
Injection into the P.V	41	1605±75 (ml/min)	1574±70 (ml/min)
Injection into the C.M.A.	64	1515±72 (ml/min)	1538±68 (ml/min)

* No significant difference was found between paired values for all parameters.

** Number of paired experiments

TABLE 2 COMPARISON BETWEEN TOTAL HEPATIC BLOOD FLOWS MEASURED BY SAMPLING FROM ONE RIGHT AND ONE LEFT HEPATIC VEINS (mean±SE) AFTER INJECTION INTO THE PORTAL VEIN (P.V.) OR INTO THE CRANIAL MESENTERIC ARTERY (C.M.A.)*

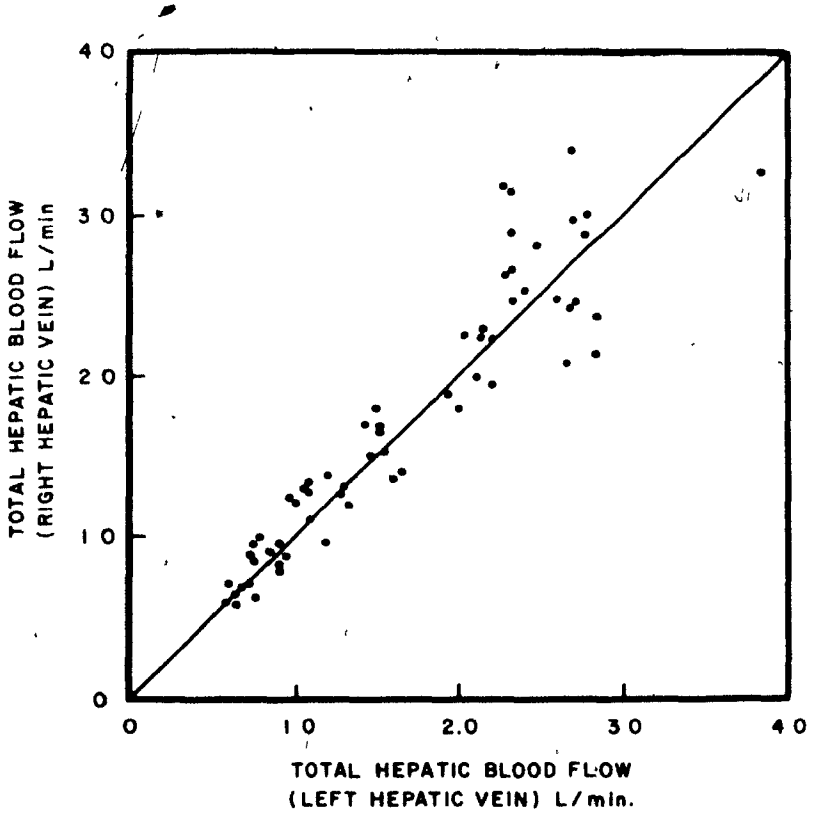

FIGURE 10: COMPARISON OF TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY FROM ONE RIGHT AND ONE LEFT HEPATIC VEINS AFTER INJECTION OF EVANS BLUE INTO THE PORTAL VEIN (GROUP A). THE SOLID LINE IS THE LINE OF IDENTITY.

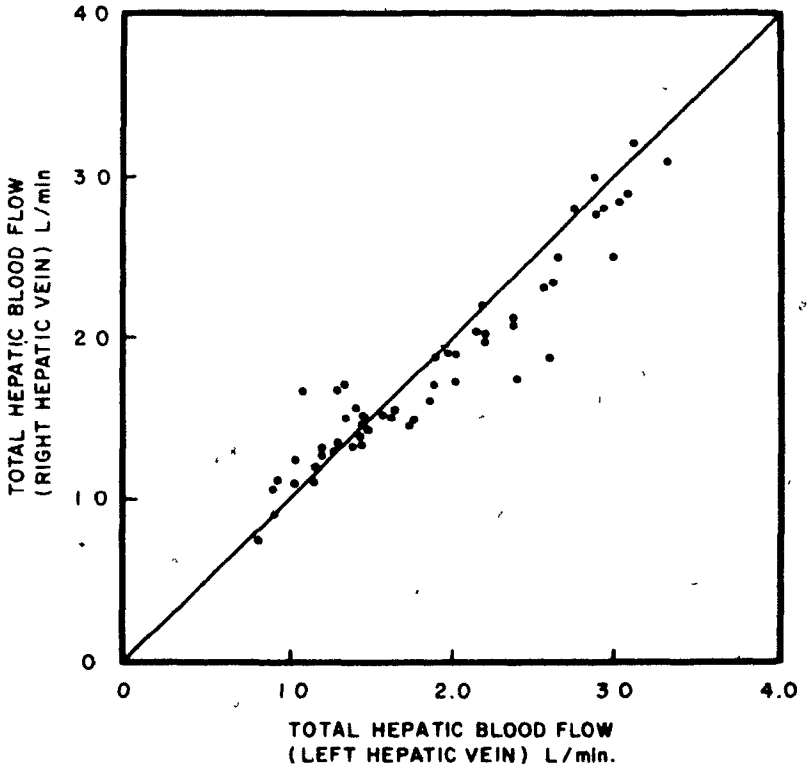

FIGURE 11: COMPARISON OF TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY FROM ONE RIGHT AND ONE LEFT HEPATIC VEINS AFTER INJECTION OF EVANS BLUE INTO THE CRANIAL MESENTERIC ARTERY (GROUP A). THE SOLID LINE IS THE LINE OF IDENTITY.

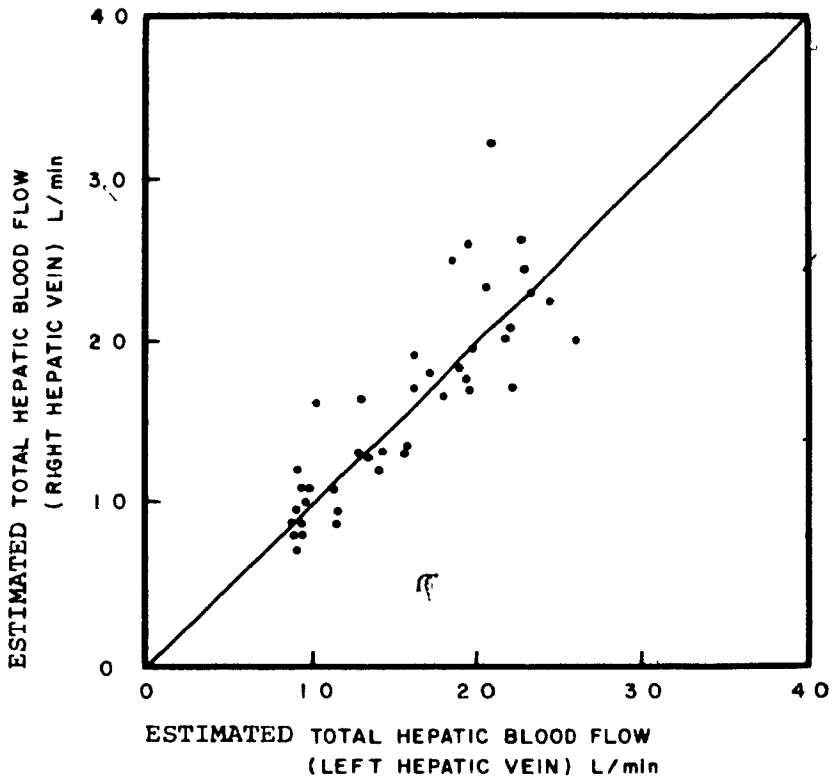

FIGURE 12: COMPARISON OF ESTIMATED TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY FROM ONE RIGHT AND ONE LEFT HEPATIC VEINS AFTER INJECTION OF INDOCYANINE GREEN INTO THE PORTAL VEIN (GROUP B). THE SOLID LINE IS THE LINE OF IDENTITY.

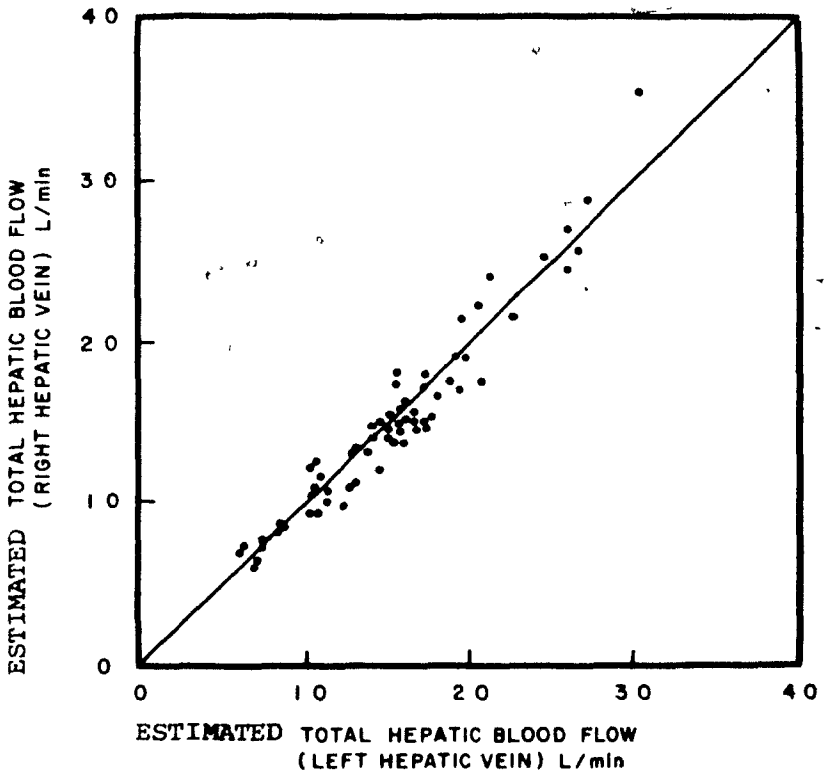

FIGURE 13: COMPARISON OF ESTIMATED TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY FROM ONE RIGHT AND ONE LEFT HEPATIC VEINS AFTER INJECTION OF INDOCYANINE GREEN IN THE CRANIAL MESENTERIC ARTERY (GROUP B). THE SOLID LINE IS THE LINE OF IDENTITY.

(mean: 7.6%) and no significant difference existed between paired flows (t: 1.230, p>0.10) (fig. 13).

In summary, no difference was found between paired estimated total hepatic blood flows obtained simultaneously from one right and one left hepatic veins using Evans Blue or Indocyanine Green whatever the injection site in the portal vein or into the cranial mesenteric artery. According to Shoemaker et al. (36) these findings validate the use of the indicator dilution method for the estimation of the total hepatic blood flows.

B) Estimation of portal and total hepatic blood flows by electromagnetic flowmeters and Cr^{51} R.B.C.-I.D.C.

a) Group C.

i) Total hepatic blood flow:

In 40 experiments (19 dogs) mean total hepatic blood flows (mean±SE) estimated simultaneously by electromagnetic flowmeters and I.D.C. were respectively 1,002±47 ml/min. and 1,070±63 ml/min. (table 3). A highly significant correlation (r: .801, p<0.001) and no significant difference (t: 1.817, 0.10>p>0.05) were found between paired flows (fig. 14). In the 27 experiments (14 dogs) where I.D.C. could be obtained from one right and one left hepatic veins, the mean total hepatic blood flow (mean±SE) were 1,096±69 ml/min. (right hepatic vein) and 1,098±69 ml/min. (left hepatic vein). No difference (t: .047, p>0.10) was found between paired flows (fig. 15).

ii) Portal blood flow:

In the same 40 experiments, mean portal blood flows (mean±SE) estimated simultaneously by flowmeters and I.D.C. were respectively 858±45 ml/min. and 918±55 ml/min. (table 3). A highly significant

	n**	ELECTROMAGNETIC FLOWMETERS	Cr^{51} R.B.C. DILUTION CURVES
GROUP C (19 dogs weighing 22±0.8 Kg)			
Total hepatic blood flow	40	1002±47 (ml/min)	1070±63 (ml/min)
Portal blood flow	40	858±45 (ml/min)	918±55 (ml/min)
Portal fraction of total hepatic blood flow	40	85.1±1.2 (%)	85.6±1.1 (%)
GROUP D (5 dogs weighing 17±0.2 Kg)			
Total hepatic blood flow	9	588±57 (ml/min)	631±61 (ml/min)
Portal blood flow	9	588±57 (ml/min)	630±54 (ml/min)

* No significant difference was found between paired values for all parameters.

** Number of paired experiments.

TABLE 3: FLOWS AND FRACTIONAL LIVER BLOOD FLOWS OBTAINED BY TWO DIFFERENT METHODS (mean±SE)*

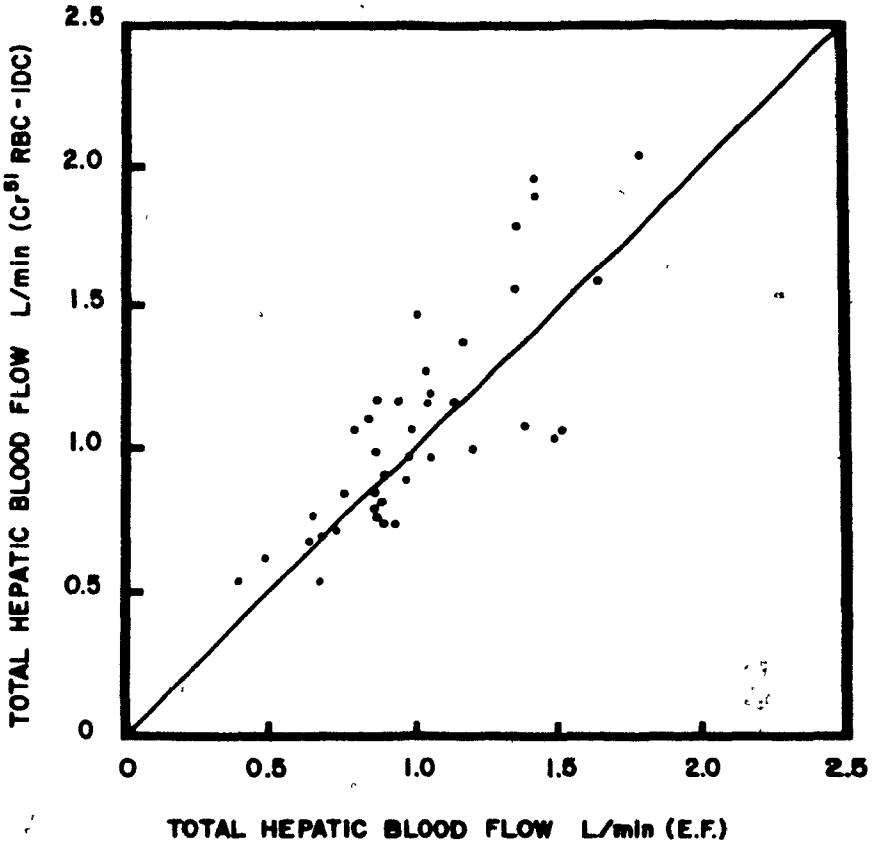

FIGURE 14: COMPARISON OF TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY BY ELECTROMAGNETIC FLOWMETERS (E.F.) AND Cr^{51} RED BLOOD CELLS INDICATOR DILUTION CURVES. (Cr^{51} R.B.C.-I.D.C.)(GROUP C). THE SOLID LINE IS THE LINE OF IDENTITY.

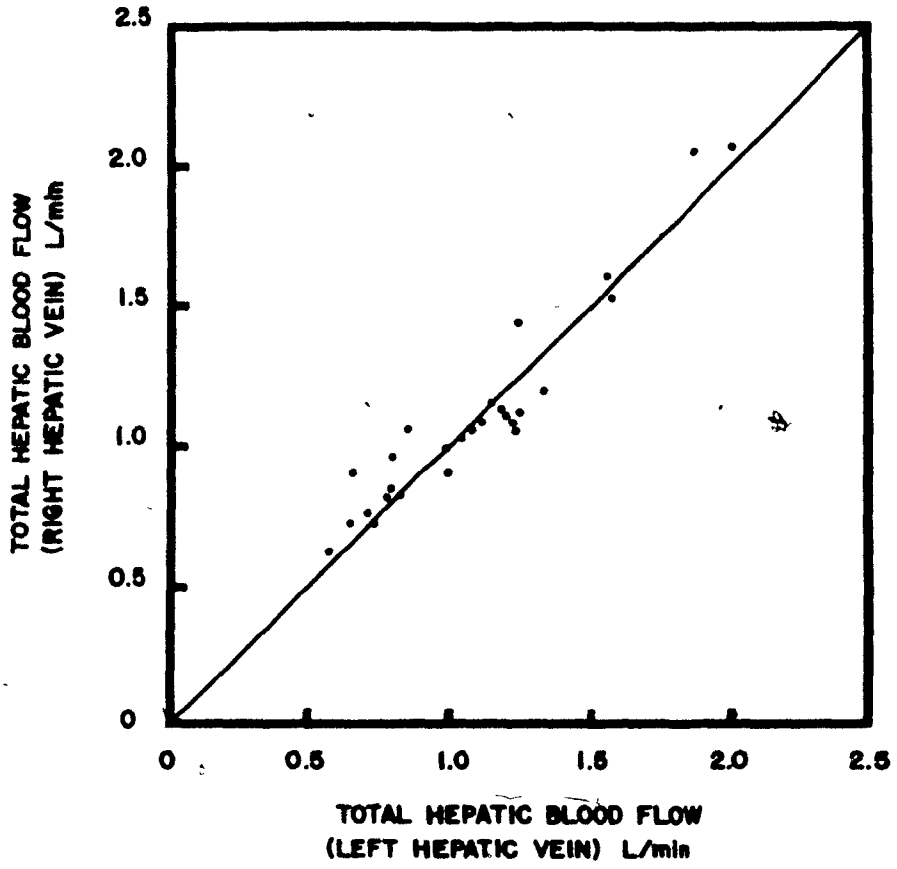

FIGURE 15: COMPARISON OF TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY FROM ONE RIGHT AND ONE LEFT HEPATIC VEINS AFTER INJECTION OF Cr^{51} RED BLOOD CELLS (Cr^{51} R.B.C.) INTO THE CRANIAL MESENTERIC ARTERY (GROUP C). THE SOLID LINE IS THE LINE OF IDENTITY.

correlation (r: .812, $p<0.001$) and no significant difference (t: 1.874, $0.10>p>0.05$) were found between paired flows (fig. 16).

iii) Portal fraction of total hepatic blood flow:

The ratio of the portal over total hepatic blood flow was calculated using simultaneous measurements by flowmeters and I.D.C. When considering this ratio, values for the 40 experiments varied between 63.0 and 96.7 (mean: 85.2%) using flowmeters and between 69.2 and 96.8% (mean 85.6%) using I.D.C. (table 3). A highly significant correlation (r: .776, $p<0.001$) and no difference (t: .554, $p>0.10$) were found between paired flows (fig. 17).

b) Group D (ligated hepatic artery).

In these dogs, only portal blood flow enters the liver so that portal blood flow equals total hepatic blood flow. Indeed, in 9 experiments (5 dogs), mean portal blood flow (estimated by flowmeters and I.D.C.) and mean total hepatic blood flow (estimated by I.D.C.) were nearly identical (table 3). No difference existed between paired flows when comparing: 1) portal blood flow (flowmeters) and total hepatic blood flow (I.D.C.) (t: 1.419, $p>0.10$); 2) portal blood flow (flowmeters) and portal blood flow (I.D.C.) (t: 1.522, $p>0.10$); 3) and finally, total hepatic blood flow (I.D.C.) and portal blood flow (I.D.C.) (t: .080, $p>0.10$).

In the 7 experiments (4 dogs) where I.D.C. could be obtained from one right and one left hepatic veins, there was, as in normals, no difference between paired total hepatic blood flow (t: .560, $p>0.10$). Mean total hepatic blood flows (mean±SE) were 657±40 ml/min. (right hepatic vein) and 667±44 ml/min. (left hepatic vein).

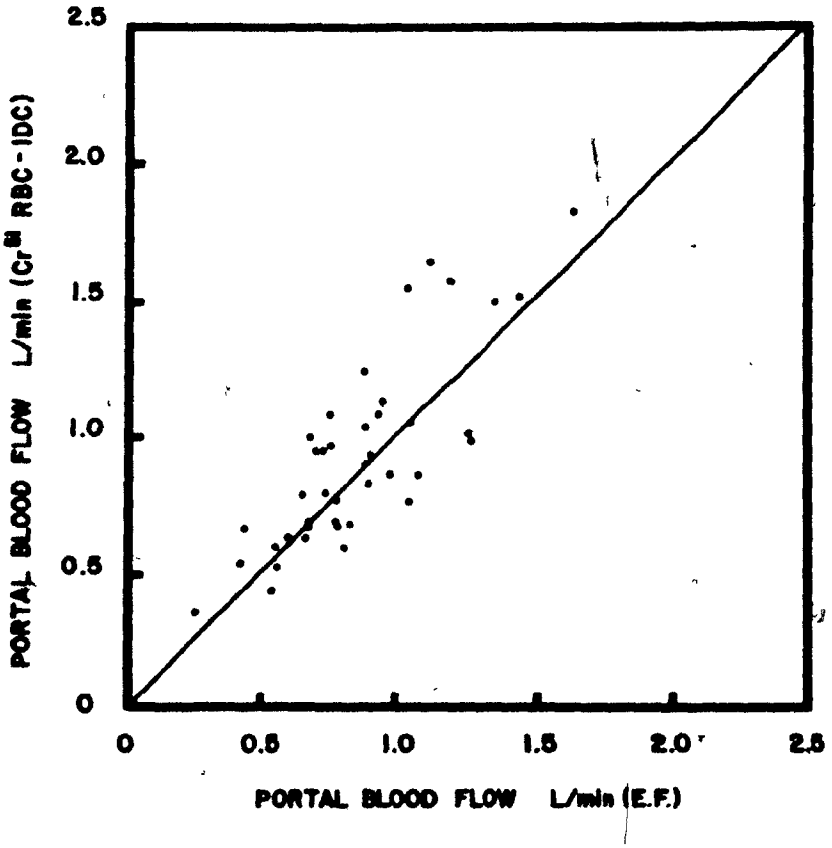

FIGURE 16: COMPARISON OF PORTAL BLOOD FLOWS OBTAINED SIMULTANEOUSLY BY ELECTROMAGNETIC FLOWMETERS (E.F.) AND Cr^{51} RED BLOOD CELLS INDICATOR DILUTION CURVES (Cr^{51} R.B.C.-I.D.C.) (GROUP C). THE SOLID LINE IS THE LINE OF IDENTITY.

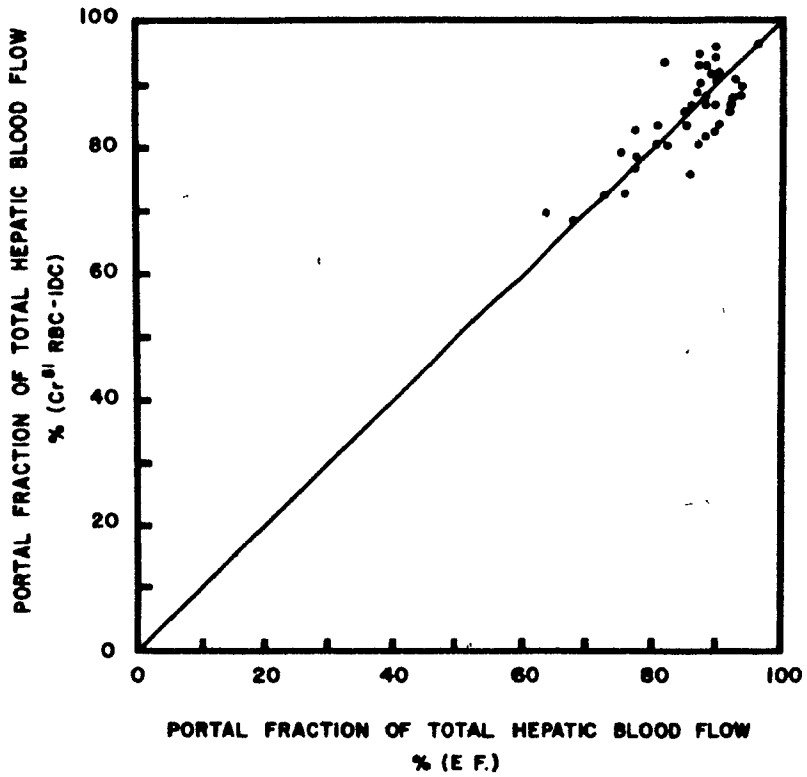

FIGURE 17: COMPARISON OF THE PORTAL FRACTION OF TOTAL HEPATIC BLOOD FLOW OBTAINED SIMULTANEOUSLY BY ELECTROMAGNETIC FLOWMETERS (E.F.) AND Cr^{51} RED BLOOD CELLS INDICATOR DILUTION CURVES (Cr^{51} R.B.C.-I.D.C.) (GROUP C). THE SOLID LINE IS THE LINE OF IDENTITY.

c) Group E. (effects of radiopaque material loading)

In these dogs, the effects of 50 ml of radiopaque material on liver circulation and cardiac output were studied by comparing flows recorded during the control state and during the first 30 minutes following loading. The mean values were calculated using at least three determinations in each period. Cardiac output, portal and total hepatic blood flows increased significantly as did the portal fraction of total hepatic blood flow. However, no major changes were observed in hepatic arterial blood flow (table 4). In all dogs, flows returned progressively to their preloading values between the 30th and the 60th minutes.

d) Group F. (effects of surgical manipulations of hepatic vessels)

In 5 dogs, the effects of surgical manipulations of hepatic artery and portal vein were studied by comparing the portal fractions of total hepatic blood flow obtained using Cr^{51} R.B.C.-I.D.C. before and after positioning probes. Comparison between absolute flows were not possible: difficulties while cannulating the cranial mesenteric artery without radiopaque material resulted in variable loss of indicator from experiment to experiment and overestimation of flows. However, the same amount of indicator was analysed from portal and hepatic veins: no significant difference (t: .199, $p<0.10$) was found between paired portal fractions of total hepatic blood flow estimated simultaneously by flowmeters and Cr^{51} R.B.C.-I.D.C. after positioning probes (table 5).

In all dogs, the portal fraction varied between 84.8 and 100% (mean: 91.2%) using Cr^{51} R.B.C.-I.D.C. before positioning probes and after this surgical manipulation, between 76.8 and 88.6% (mean: 82.2%) using Cr^{51} R.B.C.-I.D.C. and between 77 and 85% (mean: 81.8%) using flowmeters (table 5). In each dog, the portal fraction decreased significantly (t: 4.248, $p<0.025$) when comparing paired values obtained using Cr^{51} R.B.C.-I.D.C. before and after positioning probes (mean: 9.9%) (table 5).

	ELECTROMAGNETIC FLOWMETERS				INDICATOR DILUTION CURVES
	H.A.B.F. ml/min	P.B.F. ml/min	T.H.B.F. ml/min	P.F. %	C.O. ml/min
a) Controls	182±36	725±62	907±90	80.4±2.4	3,900
b) Radiopaque material loading	193±32	1157±98	1350±128	86.2±1.3	6,890
Difference between a and b	+6.0%	+59.6%	+48.8%	+7.2%	+76.9%

H.A.B.F.: hepatic arterial blood flow. P.B.F.: portal blood flow. T.H.B.F.: total hepatic blood flow
P.F.: portal fraction of hepatic blood flow. C.O.: cardiac output.
(5 dogs weighing 19.1±0.1 Kg.)

TABLE 4: EFFECTS OF 50 ml. OF RADIOPAQUE MATERIAL ON LIVER CIRCULATION AND CARDIAC OUTPUT IN FIVE DOGS (GROUP E) (mean±SE).

	P.B.F. (ml/min)		T.H.B.F. (ml/min)		P.F. (%)	
	Cr^{51} R.B.C.-I.D.C.	E.F.	Cr^{51} R.B.C.-I.D.C.	E.F.	Cr^{51} R.B.C.-I.D.C.	E.F.
Before positioning probes	1412±173		1537±161		91.2±2.2	
b) After positioning probes	1091±161	779±53	1326±123	950±61	82.2±1.0	81.8±1.2
Difference between a and b	-22.7%		-13.7%		-9.9%	

P.B.F.: portal blood flow. T.H.B.F.: total hepatic blood flow. P.F.: portal fraction of total hepatic blood flow. Cr^{51} R.B.C.-I.D.C.: Cr^{51} red blood cells-indicator dilution curves. E.F.: electromagnetic flowmeters.

TABLE 5: EFFECTS OF THE SURGICAL MANIPULATIONS OF HEPATIC ARTERY AND PORTAL VEIN WHILE POSITIONING PROBES ON LIVER CIRCULATION IN 5 DOGS (mean±SE). (GROUP F)

C) Evaluation of Indocyanine Green as indicator for the estimation of portal and total hepatic blood flows.

a) In group G, flows were estimated simultaneously using Indocyanine Green dilution curves and electromagnetic flowmeters.

i) Total hepatic blood flow:

In 29 experiments (9 dogs) mean total hepatic blood flow (mean±SE) estimated simultaneously by electromagnetic flowmeters and I.D.C. were respectively 1,094±38 ml/min. and 1,129±48 ml/min. (table 6). A significant correlation (r: .716, $p<0.001$) and no significant difference (t: 1.035, $p>0.10$) were found between paired flows (figure 18). In all experiments I.D.C. could be obtained simultaneously from one right and one left hepatic veins. The mean estimated total hepatic blood flows (mean±SE) were 1,119±50 ml/min. (right hepatic vein) and 1,121±45 ml/min. (left hepatic vein). No significant difference was found between paired flows (t: .121, $p>0.10$) (fig. 19).

ii) Portal blood flow:

In the same 29 experiments, mean portal blood flows (mean±SE) estimated simultaneously by flowmeters and I.D.C. were respectively 907±41 ml/min. and 1,024±44 ml/min. (table 6). A significant correlation (r: .849, $p<0.001$) was found between paired flows. However, a significant difference was found between paired flows (t: 4.922, $p<0.001$) (fig. 20).

iii) Finally, the portal fraction of total hepatic blood flow was calculated from the ratio of the portal over total hepatic blood flow. For both flows, simultaneous measurements were made using flowmeters and I.D.C. When considering this ratio, values for the 29 experiments varied between 69.0 and 95.0% (mean: 81.9%) using flowmeters and between 69.0 and 110.0% (mean: 91.9%) using I.D.C. (table 6). A significant difference

	n*	ELECTROMAGNETIC FLOWMETERS	I.C.G. DILUTION CURVES
Total hepatic blood flow	29	1094±38 (ml/min)	1129±48 (ml/min)
Portal blood flow	29	907±41 (ml/min)	1024±44 (ml/min)
Portal fraction of total hepatic blood flow	29	81.9±2.2 (%)	91.9±1.4 (%)

* Number of paired experiments

TABLE 6: FLOWS AND FRACTIONAL LIVER BLOOD FLOW OBTAINED SIMULTANEOUSLY BY ELECTROMAGNETIC FLOWMETERS AND INDOCYANINE GREEN (I.C.G.) DILUTION CURVES (GROUP G) (mean±SE).

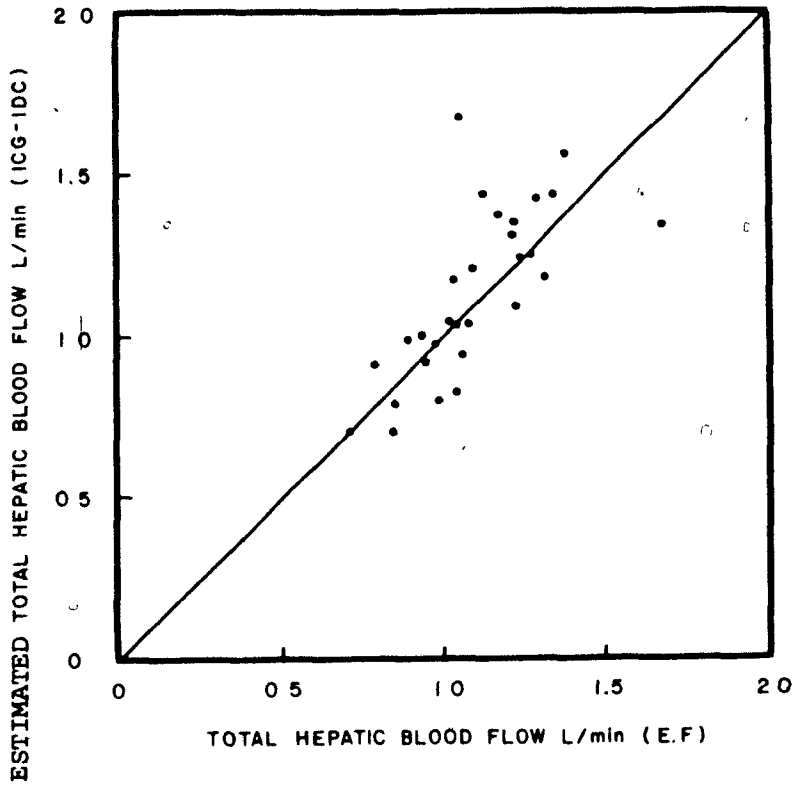

FIGURE 18: COMPARISON OF TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY BY ELECTROMAGNETIC FLOWMETERS (E.F.) AND INDOCYANINE GREEN INDICATOR DILUTION CURVES (I.C.G.-I.D.C.) (GROUP G). THE SOLID LINE IS THE LINE OF IDENTITY.

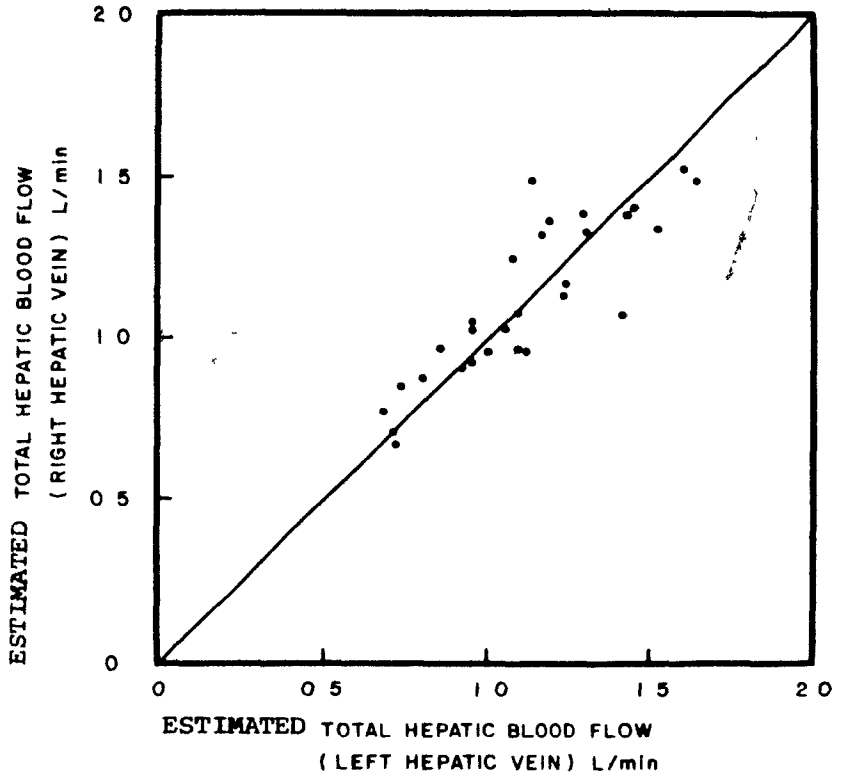

FIGURE 19: COMPARISON OF ESTIMATED TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY FROM ONE RIGHT AND ONE LEFT HEPATIC VEIN AFTER INJECTION OF INDOCYANINE GREEN INTO THE CRANIAL MESENTERIC ARTERY (GROUP G). THE SOLID LINE IS THE LINE OF IDENTITY.

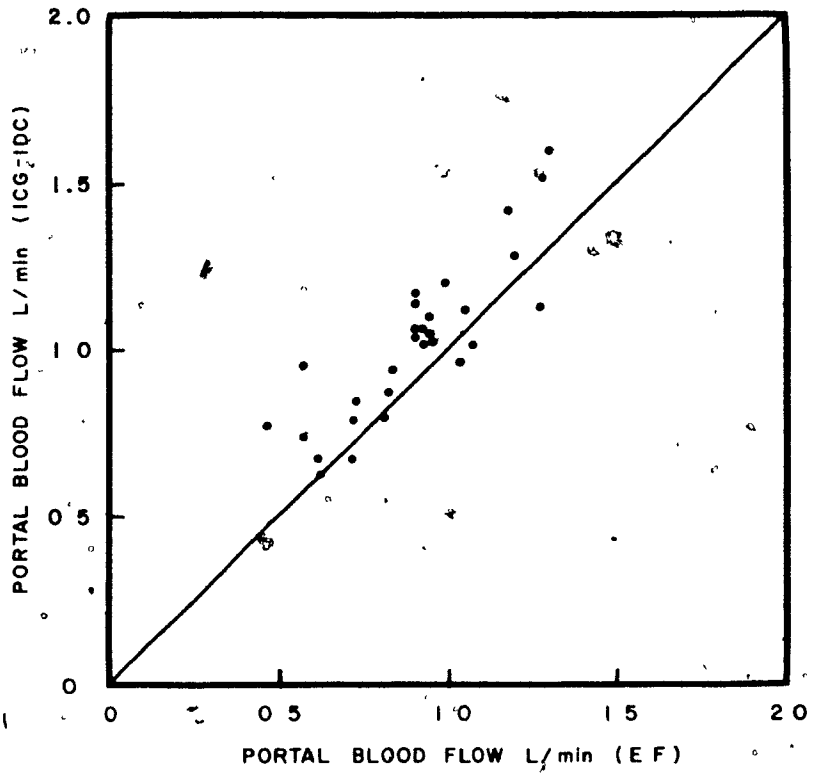

FIGURE 20: COMPARISON OF PORTAL BLOOD FLOWS OBTAINED SIMULTANEOUSLY BY ELECTROMAGNETIC FLOWMETERS (E.F.) AND INDOCYANINE GREEN INDICATOR DILUTION CURVES (I.C.G.-I.D.C.) (GROUP G). THE SOLID LINE IS THE LINE OF IDENTITY.

(r: 4.259, $p<0.001$) and no correlation (r: .296, $p>0.10$) were found between paired portal fractions of total hepatic blood flow.

b) In group H, flows were estimated simultaneously using Indocyanine Green and Cr^{51} R.B.C. dilution curves.

i) Total hepatic blood flow:

Mean total hepatic blood flows (mean±SE) were 1,334±64 ml/min. using Indocyanine Green and 1,225±70 ml/min. using Cr^{51} R.B.C. (table 7). A significant correlation (r: .734, $p<0.001$) was found between paired flows. However, a significant difference (t: 2.216, $0.05>p>0.025$) existed between paired total hepatic blood flows (fig. 21).

ii) Portal blood flow:

Mean portal blood flows (mean±SE) were 1230±52 ml/min. using Indocyanine Green and 1,034±61 ml/min. using Cr^{51} R.B.C. (table 7). A significant correlation (r: .776, $p<0.001$) was found between paired flows but a highly significant difference (t: 5.038, $p<0.001$) existed between paired portal blood flows (fig. 22).

iii) Portal fraction of total hepatic blood flow:

The portal fraction of total hepatic blood flow varied between 61.9 and 134.9% (mean: 94.47%) using Indocyanine Green and between 61.7 and 95.5% (mean: 84.6%) using Cr^{51} R.B.C. (table 7). A correlation (r: .469, $p<0.01$) was found between paired values but again a significant difference (t: 3.833, $p<0.001$) existed between paired portal fractions of total hepatic blood flow.

iiii) Cardiac output:

In 3 dogs of this group, 6 measurements of cardiac output were performed using simultaneously Indocyanine Green and Cr^{51} R.B.C.

	n*	I.C.G.-DILUTION CURVES	Cr^{51} R.B.C. DILUTION CURVES
Total hepatic blood flow	32	1334±75 (ml/min)	1225±70 (ml/min)
Portal blood flow	32	1230±52 (ml/min)	1034±61 (ml/min)
Portal fraction of total hepatic blood flow	32	94.4±2.9 (%)	84.6±1.4 (%)

* Number of paired experiments

TABLE 7: FLOWS AND FRACTIONAL LIVER BLOOD FLOW OBTAINED SIMULTANEOUSLY BY INDOCYANINE GREEN (I.C.G.) DILUTION CURVES AND Cr^{51} RED BLOOD CELLS (Cr^{51} R.B.C.) DILUTION CURVES (GROUP H) (mean±SE).

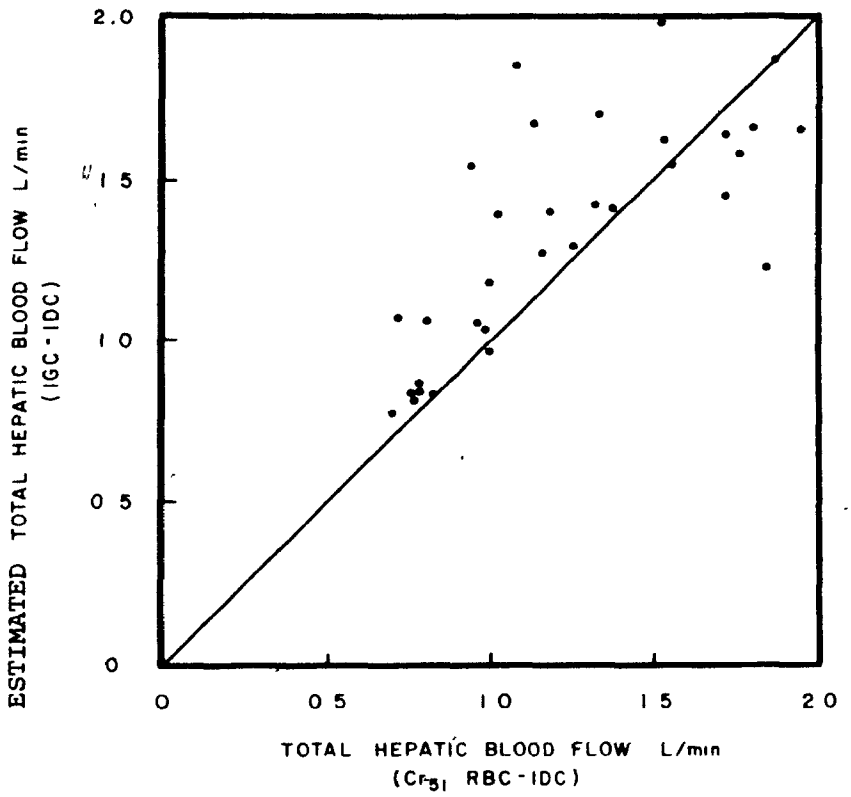

FIGURE 21: COMPARISON OF TOTAL HEPATIC BLOOD FLOWS OBTAINED SIMULTANEOUSLY BY INDOCYANINE GREEN INDICATOR DILUTION CURVES (I.C.G.-I.D.C.) AND Cr^{51} RED BLOOD CELLS INDICATOR DILUTION CURVES (Cr^{51} R.B.C.-I.D.C.) (GROUP H). THE SOLID LINE IS THE LINE OF IDENTITY.

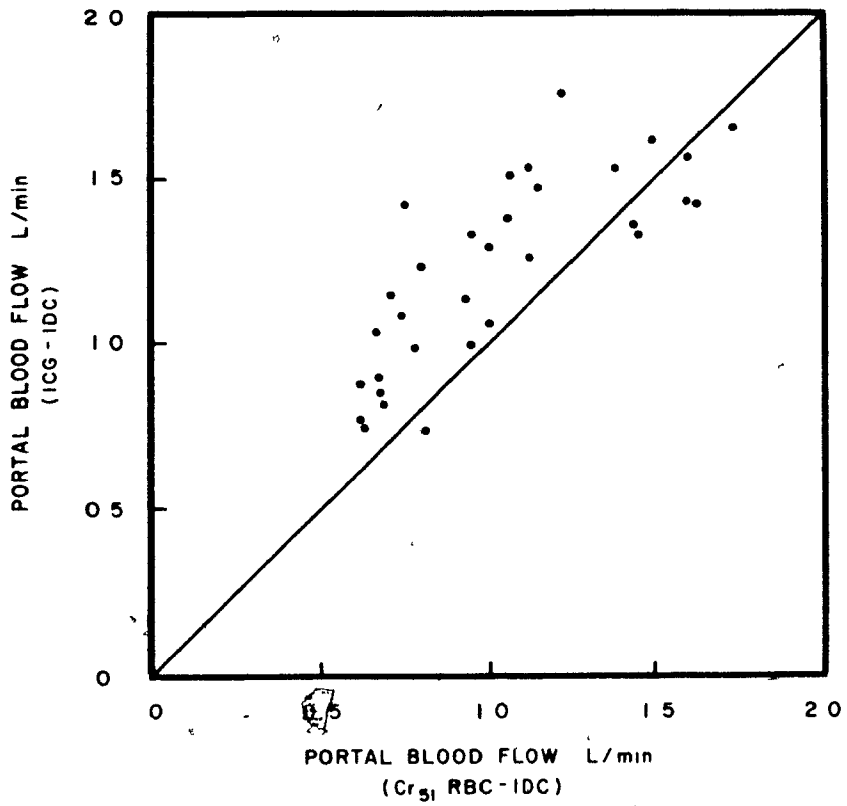

FIGURE 22: COMPARISON OF PORTAL BLOOD FLOWS OBTAINED SIMULTANEOUSLY BY INDOCYANINE GREEN INDICATOR DILUTION CURVES (I.C.G.-I.D.C.) AND Cr^{51} RED BLOOD CELLS INDICATOR DILUTION CURVES (Cr^{51} R.B.C.-I.D.C.) (GROUP H). THE SOLID LINE IS THE LINE OF IDENTITY.

The difference between paired values varied between 15.0 and 26.0% (mean: 20.8%) with, in all cases, a higher estimation of cardiac output when using Indocyanine Green.

D) Estimation of the sinusoidal fraction of portal blood flow.

a) Portal blood flow:

In the 44 experiments, portal blood flow was estimated using Cr^{51} R.B.C. dilution curves (table 8). When compared to Cr^{51} R.B.C., used as a vascular reference substance, the portal recovery of injected I^{125} A.M.A. was 90.0±0.7% (mean±SE). The loss of indicator resulted in overestimation of portal blood flow using I^{125} A.M.A. in all cases (table 8 and figure 23). A significant difference was found between paired flows ($p<0.001$) and a highly significant correlation existed between paired portal blood flows (r: 0.981, $p<0.001$).

b) Total hepatic blood flows:

In all experiments, total hepatic blood flow was estimated using Cr^{51} R.B.C. dilution curves (table 8). In 11 experiments (5 dogs), flows were estimated simultaneously from one right and one left hepatic veins and the difference between paired flows varied between 0 and 17% (mean: 5%). No significant difference ($p>0.10$) was found between paired total hepatic blood flows demonstrating adequate mixing in the portal vein and within the intrahepatic circulation, as previously reported.

c) Portal fraction of total hepatic blood flow:

The portal fraction calculated by the ratio of the portal over the total hepatic blood flows varied between 46.0 and 100% (mean: 79.5%) (table 8).

d) Sinusoidal fraction of portal blood flow:

The sinusoidal fraction varied between 68.0 and 100% of

	Cr^{51} R.B.C. DILUTION CURVES	I^{125} A.M.A. DILUTION CURVES
Total hepatic blood flow	1423±51 (ml/min)	
Portal blood flow	1120±48 (ml/min)	1248±55 (ml/min)
Portal fraction of total hepatic blood flow	79.5±2.0 (%)	
Sinusoidal fraction of portal blood flow		92.3±1.0 (%)

TABLE 8: RESULTS OBTAINED AFTER A SINGLE INJECTION OF Cr^{51} RED BLOOD CELLS (Cr^{51} R.B.C.) AND I^{125} ALBUMIN MICROAGGREGATES (I^{125} A.M.A.) INTO THE CRANIAL MESENTERIC ARTERY IN 44 EXPERIMENTS (mean±SE).

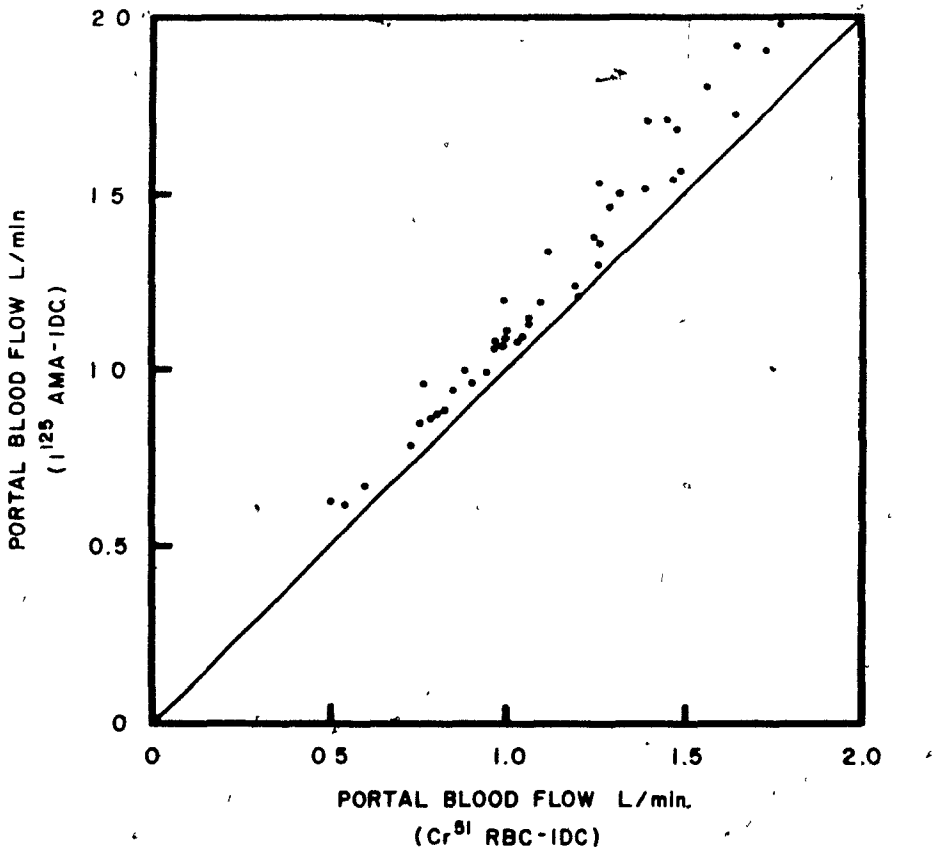

FIGURE 23: COMPARISON OF PORTAL BLOOD FLOWS OBTAINED SIMULTANEOUSLY BY Cr^{51} RED BLOOD CELLS INDICATOR DILUTION CURVES (Cr^{51} R.B.C.-I.D.C.) AND I^{125} ALBUMIN MICROAGGREGATES (I^{125} A.M.A.-I.D.C.) (GROUP I). THE SOLID LINE IS THE LINE OF IDENTITY.

portal blood flow (mean: 92.3%) and was over 90% in 36 cases (table 8). In 11 experiments (5 dogs), values were calculated simultaneously from one right and one left hepatic veins and the difference between paired values varied between 0.5 and 14.0% (mean: 4.4%). No significant difference ($p>0.10$) was found between paired sinusoidal fractions of portal blood flow.

3) DISCUSSION

In the first two groups of animals (A and B), experiments were performed to validate the use of the indicator dilution method for the estimation of total hepatic blood flow.

Nearly identical values were repeatedly obtained from paired estimated total hepatic blood flows estimated from two different hepatic veins after injection of the indicator into the portal vein or the cranial mesenteric artery (table 2 and figures 10-13). According to Shoemaker et al (36), these findings strongly suggest that the indicator was uniformly mixed with blood within the intrahepatic circulation, and validate the sampling from one hepatic vein for the total hepatic blood flow estimation. Similar findings were obtained by Shoemaker et al (36) in chronic experimental models after injecting Cr^{51} red blood cells into the portal vein.

Moreover, these data could only signify that adequate mixing of the indicator was already achieved in the portal vein (at least at its bifurcation) as well as within the intrahepatic circulation after injection of the indicator into the cranial mesenteric artery. Then, indicator dilution curves (I.D.C.) obtained from one portal vein branch could be used for the portal blood flow estimation.

However, certain experimental conditions must be met for an accurate measurement of both portal blood flow and total hepatic blood flow using the indicator dilution method (33,36,62):

1) the indicator must be conserved, i.e., it must not be metabolized or excreted by the liver and it must remain in the vascular space.

2) the same amount of injected indicator must flow through the portal vein and the liver, i.e., there must be no loss of indicator through extrahepatic collaterals.

3) the indicator must be completely mixed with the blood studied at the sampling sites in the portal vein and in the hepatic veins.

4) sampling must be representative of mixed portal and hepatic vein blood at the sampling sites.

5) the intrahepatic circulation must be in a steady state.

In group C and D, the selective measurement of total hepatic and portal blood flows were performed using I.D.C. obtained simultaneously from hepatic and portal veins after injection of Cr^{51} red blood cells (Cr^{51} R.B.C.) into the cranial mesenteric artery. Results were compared with those obtained simultaneously with a direct method of measurement using non cannulating electromagnetic flowmeters. Our data show nearly identical values for paired total hepatic blood flows using flowmeters and I.D.C. (table 3 and fig. 14). These findings demonstrate that our method fulfilled all the above requirements for such an experiment. Here again, adequate mixing of the indicator was demonstrated in the portal vein as well as within the intrahepatic circulation: 1) lack of difference between flow values obtained simultaneously from two hepatic veins as observed in groups A and B; and, 2) identical values for total hepatic and portal blood flows found in the ligated hepatic artery model (group D). These also show the reliability of portal vein sampling for the portal blood flow estimation as verified by the nearly identical flows using flowmeters and I.D.C. (table 3 and fig. 16).

In this experimental work, Evans Blue was first selected because it is bound to albumin and excreted only in minute amount by the liver of dogs (63). Goresky found that, after injection into the portal vein, the recovery of Evans Blud (T 1824) was virtually complete in the outflow (38). In our laboratory, Evans Blue was abandonned because of difficulties in densitometry, mainly in the calibration in venous blood: Evans Blue preferentially absorbs light at 640 mμ and transmits practically all of the incident light at 800 mμ as does reduced hemoglobin. The photocell cannot readily discriminate between the dye and reduced hemoglobin (64).

Indocyanine Green, then used, can be readily utilized (absorption peak: 800 mμ) but is excreted by the liver. In two groups of animals (G and H), experiments were performed to study the behavior of Indocyanine Green during one passage through the splanchnic bed and the liver when flows recorded were compared with those obtained simultaneously using electromagnetic flowmeters or Cr^{51} R.B.C. dilution curves. In both groups the mean portal blood flow was significantly higher when using Indocyanine Green (figures 20 and 22). This finding suggests loss of indicator in the mesenteric circulation and invalidates the use of this indicator for the measurement of the portal blood flow as previously reported (Comparison of measured and Indocyanine Green blood flows in various organs and systems, D.E. Donald and T. Yipintsoi, Mayo Clinic Proceedings, 48: 492, 1973).

When considering total hepatic blood flow, overestimation could be expected because of the loss of indicator in mesenteric bed and also from uptake of the Indocyanine Green by the liver. Both in the steady state infusion and during disappearance of a load of dye from the plasma, the hepatic venous concentration of Indocyanine Green is lower than in blood

taken simultaneously from the portal vein and the hepatic artery (24). Such overestimation of total hepatic blood flow was not found in both groups of animals (figures 18 and 21) suggesting at least no significant uptake of the dye during one passage through the liver.

Such unexpected findings could be explained by the metabolism of the Indocyanine Green within the liver: extravascular diffusion in the hepatic sinusoids (bound to albumin), transport through the hepatocyte membrane and excretion in the bile canaliculi. This last phenomena is the rate limiting step in the hepatic clearance from the plasma of organic anions such as Indocyanine Green. Therefore, in dogs with low extraction ratio of Indocyanine Green (24), excess of dye non transported across the canaliculi membrane could diffuse back into the sinusoid through the hepatocyte membrane and modify the downslope of the I.D.C. resulting in an overestimation of the curve area. This occurs uniformly through different partsof the liver since no difference was found between paired flows recorded from two main hepatic veins in group G (figure 19) as in group B (figures 12 and 13). Similar findings were reported by Goresky, Bach and Nadeau (J. Clin. Invest., 52: 991, 1973) using galactose.

Therefore, in the present experiments, labelled red blood cells were selected because of their lack of metabolic interaction with the liver cells and of their almost complete recovery in the hepatic veins (38). Only severely damaged red cells are sequested by the liver (66). Indocyanine Green was only used to control the position of the catheters by direct recording of curves on photographic paper before using Cr^{51} R.B.C. With Cr^{51} R.B.C., mean flow values were slightly higher than those obtained with flowmeters; this non significant difference (7%) is almost identical for the estimation of either total hepatic or portal blood flows (table 3). Overestimation of flows could result from an underestimation of the amount of indicator injected, but careful calculation of the indicator collected in vitro as well as of the residual activity within the injecting system could

not verify this possibility.

However, in some cases, we experienced loss of indicator within the aorta because of difficulty while cannulating the cranial mesenteric artery. Loss of indicator was also encountered when the hepatic artery originated from the cranial mesenteric artery, as verified by an early peak of the indicator in the hepatic veins, before the major curve. These conditions would result in an overestimation of flows. Whatever the reason, in our experimental model, the same amount of indicator has been analysed from the left branch of the portal vein as well as from one or two main hepatic veins. This was substantiated by our finding that no difference existed between the paired ratio of portal over total hepatic blood flows (portal fraction of total hepatic blood flow) simultaneously estimated by flowmeters and I.D.C. (table 3).

In this study, the reported values of flows and portal fractions of total hepatic blood flows are somewhat higher than those reported by others (11,16,67). This could be explained by the fact that repeated injections of radiopaque material (about 50 ml of Renographin 60) were necessary to visualize the position of various probes and catheters. Specifically, before each experiment, the position of the right hepatic vein catheter had to be verified to prevent contamination by caval blood or intrahepatic obstruction because of an usually narrow and/or short vein. In group E, injections of 50 ml of radiopaque material increased significantly the cardiac output, the portal and total hepatic blood flows without major changes in hepatic arterial flows, as previously reported (68,69). These changes were observed for 30 minutes and flows returned progressively to preloading values within one hour. In some experiments, repeated injections of small amount of radiopaque material is the probable explanation for maintained high flow values. However, for further studies in dogs,

only the usually large left hepatic vein could be cannulated since sampling from this vein has been shown to be representative of mixed hepatic vein blood under the conditions described.

In group F, experiments were performed to evaluate the effects of the surgical dissection of hepatic artery and portal vein while positioning probes, on liver circulation. In all cases, a significant decrease of the portal fraction of total hepatic blood flows (10%) was observed after positioning probes (table 5). An increased hepatic arterial flow or a decreased portal blood flow could not be advocated since comparison between absolute flows was impossible (variable loss of indicator within the aorta from experiment to experiment). However, if confirmed, these data suggest that the direct method of measurement using electromagnetic flowmeters is not reliable for the study of normal physiology of the liver circulation in dogs. These findings also explain some high portal fractions of total hepatic blood flow (100%) obtained in group I while using only the indicator dilution method even without radiopaque material injections.

In summary, these data demonstrated the usefulness of the Stewart Hamilton method for the simultaneous estimation of total hepatic blood flow, portal blood flow and the portal fraction of total hepatic blood flow, especially with the use of Cr^{51} R.B.C. in normal dogs.

This experimental model, using a multiple indicator dilution method, can be applied to study the behavior of one substance almost completely removed by the hepatic reticuloendothelial system (R.E.S.) when compared to Cr^{51} R.B.C., used as a vascular reference substance (70).

Colloidal particles of proper size, which do not cross the capillary wall, are almost exclusively phagocytized by the reticuloendothelial system (R.E.S.) which is mainly located in the hepatic sinusoids (Kupffer cells) (71). Chromium phosphate (Cr^{51}), colloidal gold (Au^{198}), colloidal sulfure (Tc^{99m}) and heat denatured human serum albumin (I^{131}) have been the most widely used colloids for the evaluation of the phagocytic function of the R.E.S. The blood clearance of such particles, injected intravenously, has been used to estimate the blood flow through the liver (20,21,22) and intrahepatic shunted blood flow (25,26).

As blood clearance was demonstrated to be directly proportional to the particle size (20,72), I^{125} albumin microaggregates (I^{125} A.M.A.)were chosen in this study because their size range was 1 to 5 microns and their preparation easy and reproducible. In fact, the blood clearance of radio-albumin microaggregates (I^{131} or Tc^{99m}) has been demonstrated to be twice as high than that of radio-albumin colloids (60). Moreover, after removal by the hepatic R.E.S., almost 100% of the peak activity was found to remain in the liver during one hour (60). These findings suggest that the metabolism and thus, the release of protein free I^{131} or Tc^{99m} by the liver was low in the first hour (60). I^{125} was chosen instead of I^{131} because there was less overlaping activity with Cr^{51}, the photo-peak energies being 0.035 and 0.321 Mev respectively. Therefore, I^{125} A.M.A. fulfilled the criteria needed for such an experiment: high extraction efficiency and no immediate release by the Kupffer cells.

The particle size of this material allows it to flow freely through the splanchnic capillary system and to remain in the vascular space like the slightly larger red cells. However, after a

single passage through the splanchnic capillary system, the portal recovery of injected I^{125} A.M.A. was about 90% when compared with Cr^{51} R.B.C. used as a vascular reference substance (38). These findings could only signify that appreciable uptake of particles occurred during a single passage through the mesenteric bed and therefore invalidate the use of the I^{125} A.M.A. dilution curve for the measurement of portal blood flow. Since no R.E.S. has been found in the intestine (71), the I^{125} A.M.A. removed by the splanchnic capillaries could be due to the presence of some macroaggregates (15-20 μ) not reduced by ultrasonic agitation and larger than the capillary channels. Free I^{125} or labelled colloidal albumin, not removed from suspension by centrifugation, may also be trapped in the extra capillary space. Thus, the splanchnic capillary system behaved as a filter allowing "homogenous" I^{125} A.M.A. suspension to flow through the portal vein and to come into contact with the Kupffer cells.

Under these experimental conditions, generally more than 90% of the I^{125} A.M.A. flowing through the portal vein was removed by the hepatic R.E.S. during a single passage. This hepatic extraction efficiency was constant in the same dog even after four experiments and was nearly identical from different hepatic lobes in 5 dogs (11 experiments). Moreover, with the multiple indicator dilution method, the estimation of the hepatic extraction efficiency was reliable since the removed substance and the vascular reference substance were studied in the portal vein (before the hepatic R.E.S.) and in the hepatic vein (after the hepatic R.E.S.).

The total amount of injected albumin was less than 1.0 mg in each experiment. This dose was far below the critical R.E.S. dose reported in different species (0.19 to 0.51 mg/100 gm weight) in the evaluation of the phagocytic function of the hepatic R.E.S. (28).

Biozzi and Stiffel stated that under this critical dose, the concentration of albumin colloid particles entering the liver is so low that they are completely removed during a single passage through the hepatic R.E.S. (28). The blood clearance is therefore only dependent on the blood flowing through the hepatic sinusoids and no longer on the phagocytic activity of the R.E.S. In previous studies, the hepatic extraction efficiency, evaluated after peripheral injection of the colloid, was expressed as a fraction of the total hepatic blood flow. This hepatic extraction efficiency is generally incomplete varying from 77 to 84% in different species and reaching 94% in normal man (25,26). The incomplete extraction efficiency has been related to intrahepatic communications occurring under physiological conditions, between branches of portal and hepatic veins (27), shunting part of the colloid from Kupffer cells (25,26).

In our experimental study using the indicator dilution method the incomplete extraction efficiency may be due to the fact that a small proportion of the colloid is not removed by the R.E.S. because of lack of time for the uptake mechanism to take place during fast transit through the sinusoids. Also I^{125} unbound to A.M.A. may flow freely through the liver. However, there is no significant difference between the transit time from portal to hepatic veins in dogs with different extraction efficiency. The removal by the R.E.S. does not need an active transport mechanism necessary for the uptake of galactose by the hepatocyte membrane (Goresky, C.A., Bach and Nadeau, J. Clin. Invest., 52: 991, 1973). Finally, our results agree well with the reported values in dogs and men (25,26) using a clearance method where the rate of portal blood flow has no influence on the colloid clearance (Norman, S.J., R.E.S. J. Reticulo-endothel. Soc., 13: 47, 1973).

In the present data, using the non removed I^{125} A.M.A. as an index of the intrahepatic veno-venous shunted blood, the hepatic extraction efficiency could be used to evaluate the sinusoidal fraction of portal blood flow or a functional fraction of portal blood flow. Therefore, after a single injection of Cr^{51} R.B.C. and I^{125} A.M.A. into the cranial mesenteric artery, the total hepatic blood flow, the portal blood flow, the portal fraction of total hepatic blood flow and the sinusoidal fraction of portal blood flow could be estimated simultaneously in normal dogs.

IV) PART II: APPLICATION OF THE INDICATOR DILUTION METHOD IN AWAKE CIRRHOTIC PATIENTS

1) INTRODUCTION

In part I, the use of portal and hepatic indicator dilution curves after injection of Cr^{51} R.B.C. into the cranial mesenteric artery has been validated for the estimation of portal blood flow, total hepatic blood flow and the portal fraction of total hepatic blood flow in normal dogs. This technique was applied in 17 compensated cirrhotic patients with severe portal hypertension undergoing combined umbilicoportal, hepatic vein and superior mesenteric artery catheterization. Samples were obtained simultaneously from the portal bifurcation, one right hepatic vein and when possible, a left hepatic vein, after injecting Cr^{51} R.B.C. into the superior mesenteric artery.

Spontaneous portosystemic collaterals might result in loss of indicator flowing through the portal vein and the liver and in overestimation of flows. However, the portal fraction of total hepatic blood flow should be reliable if no extrahepatic shunts existed after the bifurcation of the portal vein: the same amount of indicator being analysed from portal and hepatic veins.

In 4 patients, I^{125} A.M.A. were added to the Cr^{51} R.B.C. suspension for the simultaneous estimation of the sinusoidal fraction of the portal blood flow.

2) MATERIAL AND METHODS

A) MATERIAL

Seventeen (17) patients with cirrhosis of the liver, thirteen males and four females, aged 36-59 years (mean: 49 years), had a hemodynamic evaluation related to their portal hypertension. Sixteen of these patients were alcoholics. Diagnosis of cirrhosis was confirmed by needle biopsy of the liver in all patients. The relative clearance of Indocyanine Green (K-I.C.G.) (18) was performed the week before the hemodynamic studies.

The surgical technique for portal catheterization has been described elsewhere (51). Under epidural anesthesia or general anesthesia, the round ligament was identified extraperitoneally and was catheterized up into the portal vein and usually into the splenic vein using fluoroscopy. In all cases, following portal catheterization, retrograde splenography and portography were obtained using a rapid film changer, by injecting 50 ml of radiopaque material (Renographin 76, Squibb) using a pressure of 60 pounds per square inch.

Hepatic vein(s) and superior mesenteric artery catheterizations were performed two days after portal catheterization, under fluoroscopic visualization, using minimal amount of radiopaque material (less than 20 ml). The patients were in a fasting state and were premedicated with meperidine (Demerol) 25-50 mg and promethazine (Phenergan) 25 mg intramuscularly, half an hour before catheterization procedure.

In all patients, a right hepatic vein was cannulated through an antecubital vein of one arm using a Cournand catheter (no. 8 or 9F). In 6 patients, a left hepatic vein was cannulated through a femoral vein by the Seldinger technique using a precurved polyethylene catheter (no. 8F). The superior mesenteric artery was then cannulated through a femoral artery

on the opposite side by the Seldinger technique using a Cordis polyethylene catheter (Cordis Corporation, Miami). In two cases where a hepatic artery originated from the superior mesenteric artery the arterial catheter was advanced well into this artery beyond the take off of the hepatic artery.

B) METHODS

a) Pressures were recorded in the hepatic veins, in the wedged (W.H.V.P.) and free (F.H.V.P.) positions and in the portal vein, free position (F.P.V.P.). Pressures were obtained using a Statham gauge transducer (model P23BB) with the patient in a supine position (zero level assumed to be 5 cm below the sternal angle). Each pressure was recorded on at least two occasions, and for each an electromagnetic mean was obtained.

b) Total hepatic blood flow:

Total hepatic blood flow was estimated using Bradley's method using a constant infusion of Indocyanine Green (19). Samples were obtained simultaneously from hepatic and portal veins at 4 minutes intervals for 20 minutes, after an equilibration period of 15 minutes.

c) Portal fraction of total hepatic blood flow:

Before performing indicator dilution curves (I.D.C.), the portal catheter was withdrawn and positionned at the bifurcation of the portal vein under fluoroscopy without using radiopaque material. Portal and total hepatic blood flows were calculated after a single injection of the indicator into the superior mesenteric artery. Portal blood flow was calculated using the I.D.C. obtained from the bifurcation of the portal vein and total hepatic blood flow was calculated by simultaneous I.D.C. obtained from hepatic vein(s) (fig. 24). Indocyanine Green was used to control the position of various catheters by direct

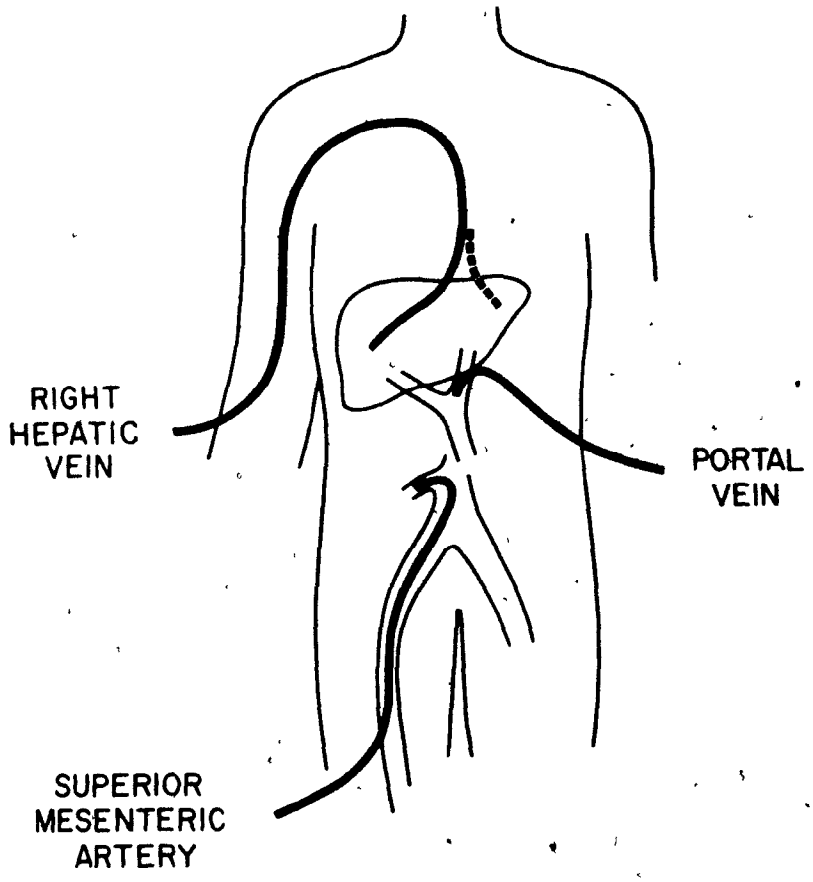

FIGURE 24: SCHEMATIC DIAGRAM ILLUSTRATING THE POSITION OF THE INJECTING AND SAMPLING CATHETERS IN MAN.

recording of curves on photographic paper. However, a Cr^{51} R.B.C. suspension (250 µc in 15 ml of autologous blood) prepared according to Wagner (57)(with three washings) was chosen as indicator because it was demonstrated to be the most reliable in dogs (part I).

After instantaneous injections of 5 ml of Cr^{51} R.B.C. into the superior mesenteric artery, the catheter was flushed with blood or saline. Known volumes (0.4 ml) of the same suspension counted on the automated gamma counter (Nuclear Chicago) were used as standards. Samples were collected using a peristaltic pump (30 ml/min) into 2 or 3 serial collection racks with heparinized tubes, running at a speed of 1 tube per second. A standard volume (0.4 ml) was withdrawn from each tube, counted separately and results were plotted on semilogarithmic paper (fig. 25,26,27). Extrapolation of the downslope to baseline, correction for background, expression of c.p.m. per ml and calculation of flows were computed by a Wang calculator, as previously described.

Twenty two (22) experiments were performed in the 17 patients. In 6 experiments (6 patients), samples were obtained simultaneously from one left and one right hepatic veins and, when calculable I.D.C. could be obtained, total hepatic blood flow was estimated by averaging flows measured from these two different hepatic veins.

d) <u>Sinusoidal fraction of portal blood flow</u>:

In 4 patients (IX, XI, XII, XIII) I^{125} A.M.A., prepared

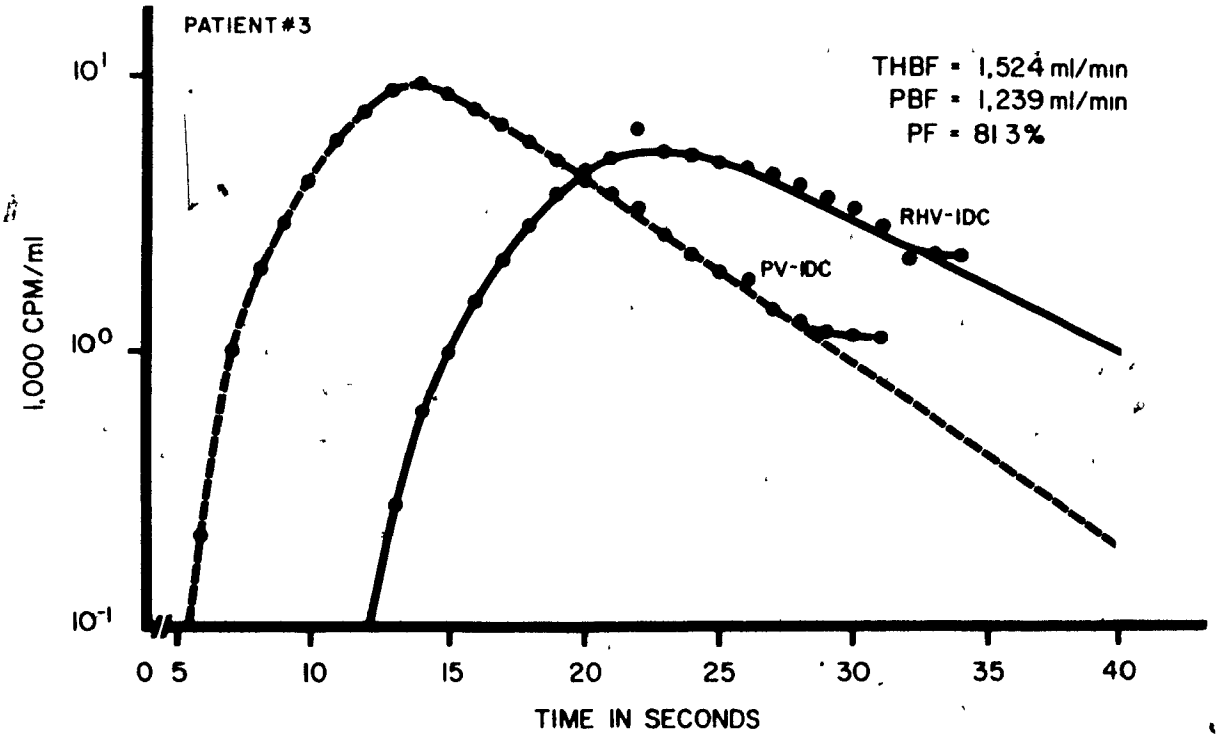

FIGURE 25: SIMULTANEOUS INDICATOR DILUTION CURVES (I.D.C.) OBTAINED FROM THE PORTAL VEIN (P.V.) AND A RIGHT HEPATIC VEIN (R.H.V.) AFTER INJECTION OF Cr^{51} R.B.C. INTO THE SUPERIOR MESENTERIC ARTERY.

THBF: TOTAL HEPATIC BLOOD FLOW

PBF : PORTAL BLOOD FLOW

PF : PORTAL FRACTION OF TOTAL HEPATIC BLOOD FLOW.

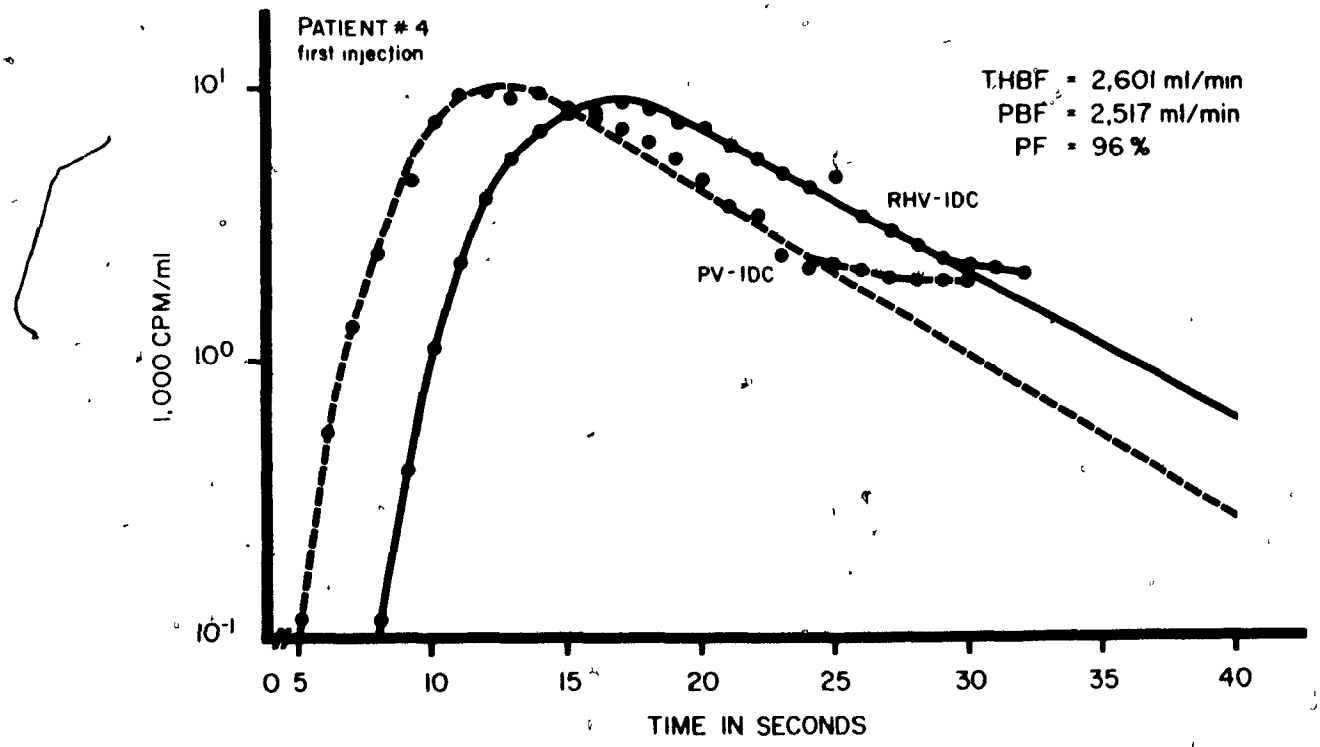

FIGURE 26: SIMULTANEOUS INDICATOR DILUTION CURVES (I.D.C.) OBTAINED FROM THE PORTAL VEIN (P.V.) AND A RIGHT HEPATIC VEIN (R.H.V.) AFTER INJECTION OF Cr^{51} R.B.C. INTO THE SUPERIOR MESENTERIC ARTERY (FIRST INJECTION).

THBF: TOTAL HEPATIC BLOOD FLOW

PBF : PORTAL BLOOD FLOW

PF : PORTAL FRACTION OF TOTAL HEPATIC BLOOD FLOW.

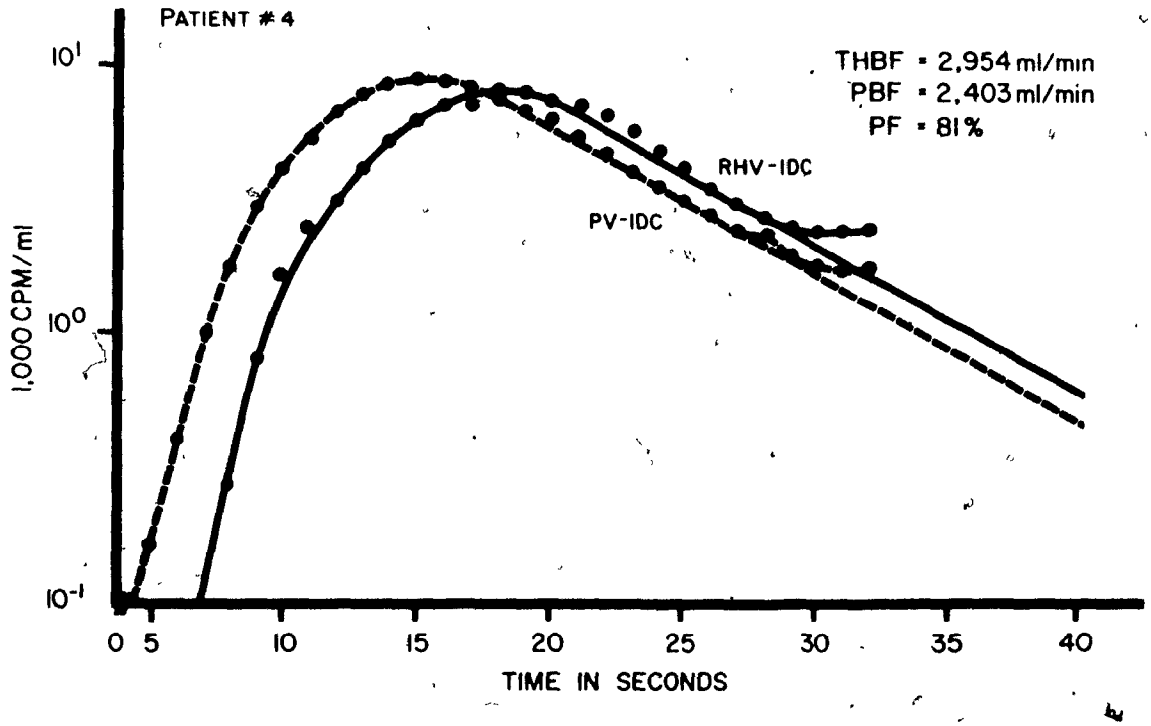

FIGURE 27: SIMULTANEOUS INDICATOR DILUTION CURVES (I.D.C.) OBTAINED FROM THE PORTAL VEIN (P.V.) AND A RIGHT HEPATIC VEIN (R.H.V.) AFTER INJECTION OF Cr^{51} R.B.C. INTO THE SUPERIOR MESENTERIC ARTERY (SECOND INJECTION).

THBF: TOTAL HEPATIC BLOOD FLOW

PBF : PORTAL BLOOD FLOW

PF : PORTAL FRACTION OF TOTAL HEPATIC BLOOD FLOW.

according to Kitani and Taplin (60), were added to the Cr^{51} R.B.C. suspension. Blood was reconstitued by adding to packed Cr^{51} R.B.C. 2.5 ml of the microaggregate preparation and isotonic saline, so that each single injection of the indicator mixture (5 ml) contained less than 1.0 mg of I^{125} albumin.

Samples were analysed as described in part I. The results of a multiple indicator dilution experiment obtained after injection of the indicator mixture into the superior mesenteric artery are illustrated in figures 28 and 29. The I.D.C. obtained from the portal veins are almost identical with Cr^{51} R.B.C. and I^{125} A.M.A., suggesting that most of the microaggregates injected into the superior mesenteric artery appeared in the portal blood. The I.D.C. obtained from the hepatic vein are different in one patient (fig. 28), the I^{125} A.M.A. curve being small in relation to the reference red cell curve, suggesting that most of the I^{125} A.M.A. were removed by the liver during one passage, as observed in dogs. However, in the other patient (fig. 29), the I.D.C. obtained from the hepatic vein are almost identical, indicating that only few amount of I^{125} A.M.A. flowing through the portal vein was removed during a single passage.

As described in part I, the hepatic R.E.S. extraction efficiency of injected microaggregates flowing through the portal vein was estimated. This hepatic extraction efficiency was dependent on portal inflow and expressed as per cent of portal blood flow. In these experiments, the amount of injected colloid (less than 1 mg) was below the reported critical dose under which all particles entering the sinusoids should be totally phagocytized by the hepatic R.E.S. during one passage, even by cirrhotic liver (28). The I^{125} A.M.A.-I.D.C. obtained from hepatic veins could be related to a fraction of portal blood flow

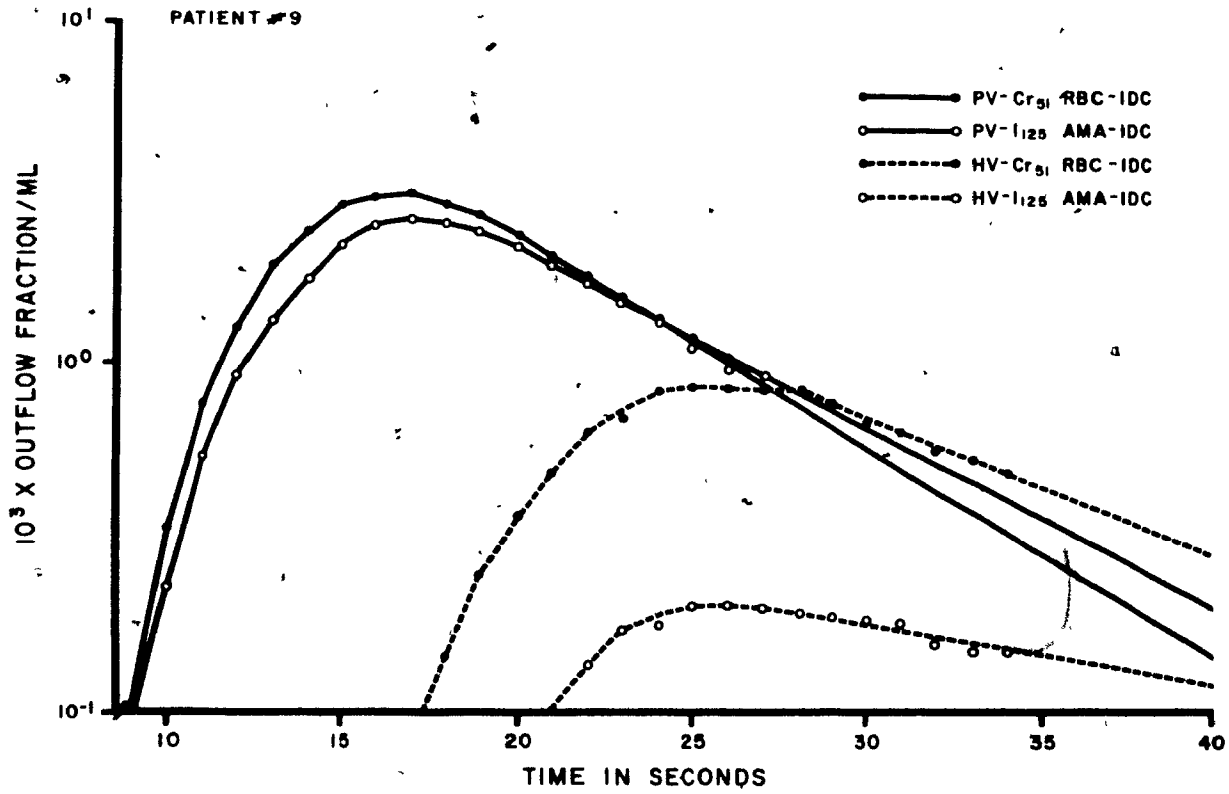

FIGURE 28: SIMULTANEOUS INDICATOR DILUTION CURVES OBTAINED FROM PORTAL VEIN (P.V.) AND A RIGHT HEPATIC VEIN (H.V.) AFTER INJECTION OF Cr^{51} RED BLOOD CELLS (Cr^{51} R.B.C.) AND I^{125} ALBUMIN MICROAGGREGATES (I^{125} A,M.A.) INTO THE SUPERIOR MESENTERIC ARTERY.

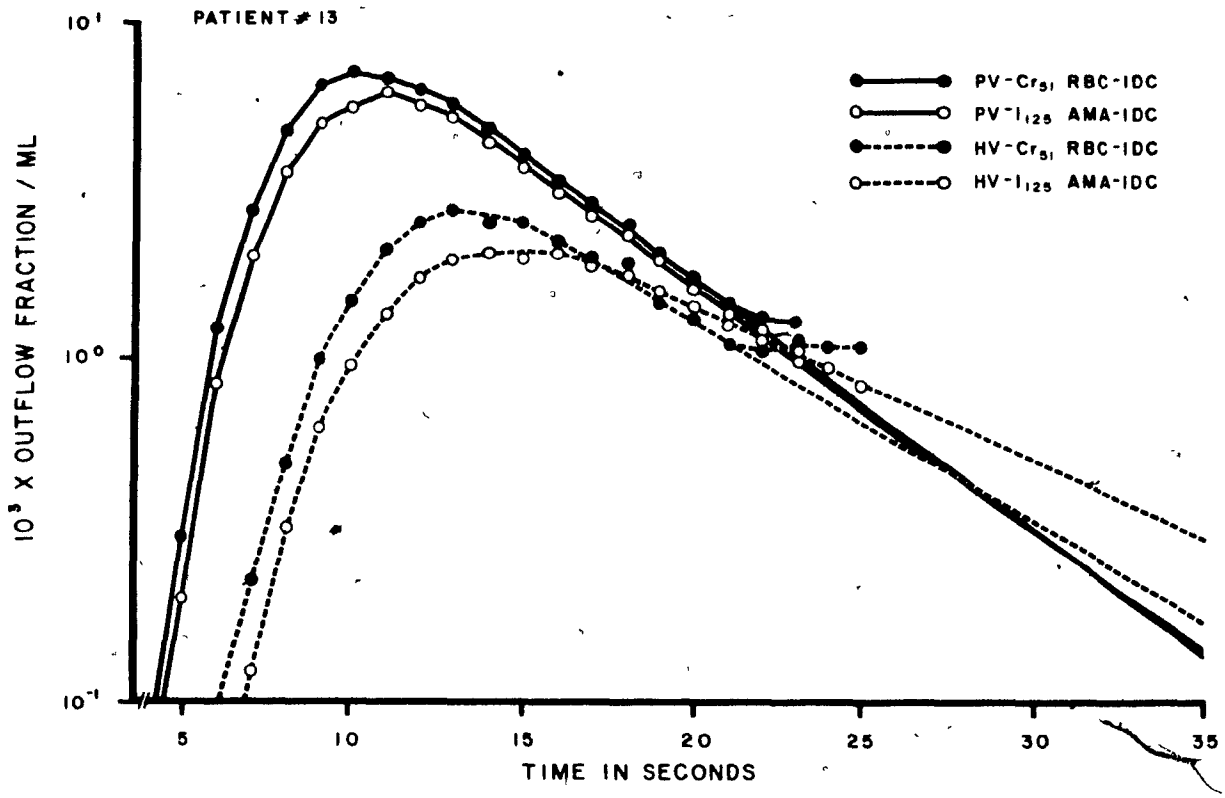

FIGURE 29: SIMULTANEOUS INDICATOR DILUTION CURVES OBTAINED FROM PORTAL VEIN (P.V.) AND A RIGHT HEPATIC VEIN (H.V.) AFTER INJECTION OF Cr^{51} RED BLOOD CELLS (Cr^{51} R.B.C.) AND I^{125} ALBUMIN MICROAGGREGATES (I^{125} A.M.A.) INTO THE SUPERIOR MESENTERIC ARTERY.

bypassing the sinusoidal Kupffer cells (25,26) because of increased intrahepatic portohepatic shunts (73). Therefore, as in normal dogs, the hepatic extraction efficiency could be utilized in the estimation of a sinusoidal fraction of portal blood flow in cirrhotic patients.

Eight (8) experiments were performed in the 4 patients. In 3 experiments (2 patients), the sinusoidal fraction of portal blood flow could be estimated by averaging the values measured simultaneously from one right and one left hepatic veins.

3) RESULTS

In 4 patients, complete hemodynamic data could not be obtained because of technical difficulties: in 2 patients, hepatic vein samples could not be obtained because of clotting obstruction of the catheter; in 1 patient, portal samples could not be obtained because the portal catheter was positioned in a narrow portal branch; and finally, in one patient, the arterial catheter was accidentally withdrawn from the superior mesenteric artery into the aorta just before the injection, as verified by fluoroscopy. These 4 patients (4 experiments) are not included in these data.

In the 13 remaining patients:

A) Relative clearance of Indocyanine Green (I-I.C.G.):

The K-I.C.G. varied between 1.42 and 13.07% (mean±SE: 6.28±1.13%) (table 9).

B) Portohepatography:

In all cases, portohepatography was obtained. In 10 cases, the coronary vein was dilated and tortuous with esophageal varices graded as 1+ to 4+ (74) but no extrahepatic shunts were demonstrated after the portal bifurcation. In 3 cases (V, VI, VIII), large spontaneous portacaval shunting was shown with reverse circulation in the portal vein (fig. 30,31), as verified by arteriographies in following days.

C) Pressures:

The mean (±SE) free hepatic venous pressure (F.H.V.P.) was 11.8±1.3 mm Hg and varied between 6.5 and 24.5 mm Hg. The mean wedged hepatic venous pressure (W.H.V.P.) was 26.8±1.6 mm Hg and varied between 17 and 38 mm Hg. The mean free portal venous pressure (F.P.V.P.)

PATIENT	AGE	SEX	K-I C G %	PORTO-HEPATIC GRADIENT* mm Hg	T.H B F ** ml/min	Cr^{51} RED BLOOD CELLS-I D C			I^{125} ALBUMIN MICROAGGREGATES-I D.C	
						T H B F ml/min	P B F ml/min	P F %	P B F ml/min	S F %
I	44	F	3 02	13	1,000	1,340	980	73 4		
II	54	M	4 77	13 5	2,690	3,380	2,820	83		
III	58	M	11 55	12	2,160	1,520	1,240	81 3		
IV	52	M	3 64	19	1,780	2,600 2,950	2,520 2,400	96 81		
V	41	F	10 19	10	800	--	---	0		
VI	56	M	10 26	11	1,070	---	---	0		
VII	58	M	13 07	8	1 720	2,100*** 2,200	1,920 1,910	91 4 87		
VIII	41	M	2 00	17	---	- -	---	0		
IX	57	M	10 19	17	1,600	4,160 4,340	1,580 1,310	38 1 30 1	1,680 1,480	79 3 86 3
X	36	M	4 46	19	1,240	2,960***	2,960	100		
XI	50	M	4 62	17	1,930	2,340*** 2,750	1,600 1,520	68 6 57 7	1,650 1,570	33 4 30 6
XII	46	M	2 52	15	6,370	5,290	5,230	98 7	5,900	13
XIII	38	F	1 42	21	3,620	1,905*** 1,904	840 711	44 37	944 886	7 5 3 8

* Porto-hepatic gradient difference between free portal venous pressure and free hepatic vein pressure

** Bradley's method using Indocyanine Green

***Mean T H B F estimated simultaneously from one right and one left hepatic veins

The difference between paired T H B F was 11% for patient VII, 7% for patient X, 12% for patient XI and 30% for patient XIII

K-I C G. fractional clearance of Indocyanine Green; T H B F total hepatic blood flow

P B F portal blood flow; P F portal fraction of total hepatic blood flow;

S F sinusoidal fraction of portal blood flow

TABLE 9 DATA OBTAINED IN 13 CIRRHOTIC PATIENTS

FIGURE 30: PORTOGRAPHY SHOWING THE SPONTANEOUS PORTO-SPLENO RENAL SHUNT WITH OPACIFICATION OF THE INFERIOR VENA CAVA.

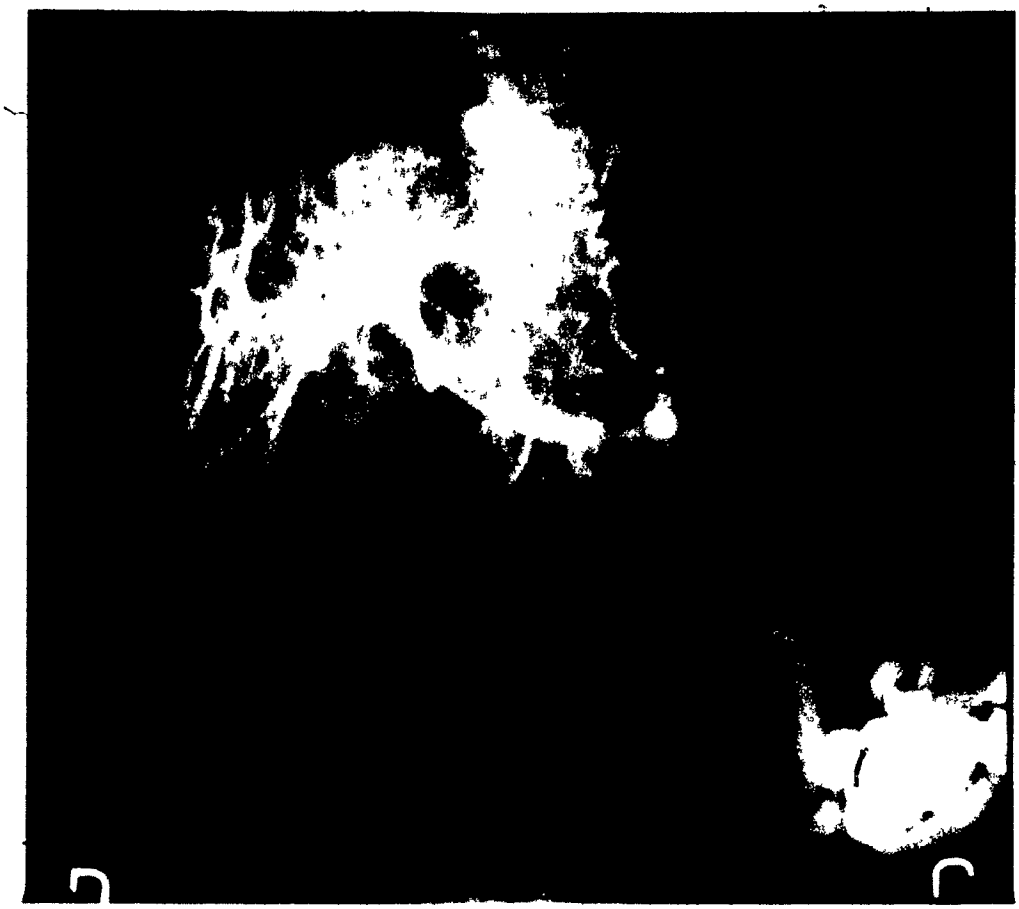

FIGURE 31: PORTOGRAPHY SHOWING THE SPONTANEOUS SPLENO-OVARO-RENAL SHUNT.

was 26.5±1.6 mm Hg and varied between 19 and 38 mm Hg. The mean portohepatic gradient (F.P.V.P. - F.H.V.P.) was 14.8±1.0 mm Hg (8 to 21 mm Hg) and was used as an index of the portal hypertension (table 9).

D) Total hepatic blood flow:

In 12 cases, the total hepatic blood flow could be estimated and varied between .800 and 6.370 L/min (mean±SE: L/min). However, in the two cases (XII and XIII) with high absolute values, the Indocyanine Green extraction was less than 5 per cent and the reliability of the constant infusion method is questionable (40). In one case (VIII), total hepatic blood flow could not be estimated because there were no differences in the I.D.C. concentrations in hepatic and portal veins.

E) Portal fraction of total hepatic blood flow:

In 10 patients (I, II, III, IV, VII, IX, X, XI, XII, XIII), 15 series of I.D.C. were calculable. The mean values (±SE) were: portal blood flow 1.97±.29 L/min and total hepatic blood flow 2.78±.28 L/min. In 4 of these patients (VII, X, XI, XIII), the mean difference between paired total hepatic blood flow estimated simultaneously from two hepatic veins was 15.1% (7 to 30%). In these 10 patients, the portal fraction of total hepatic blood flow varied from 30.1 to 100% (mean: 71.1±6.2%) (table 9).

In the other 3 patients (V, VI, VIII), where reverse circulation in the portal vein was found on portography, only delayed activity from recirculation was detected from portal and hepatic veins samples (fig. 32). In two patients (V, VI), samples were obtained simultaneously from two different hepatic veins. In these cases, the portal fraction of total hepatic blood flow was 0% (*).

(*) In patient VIII, after the injection of Cr^{51} R.B.C. into the superior

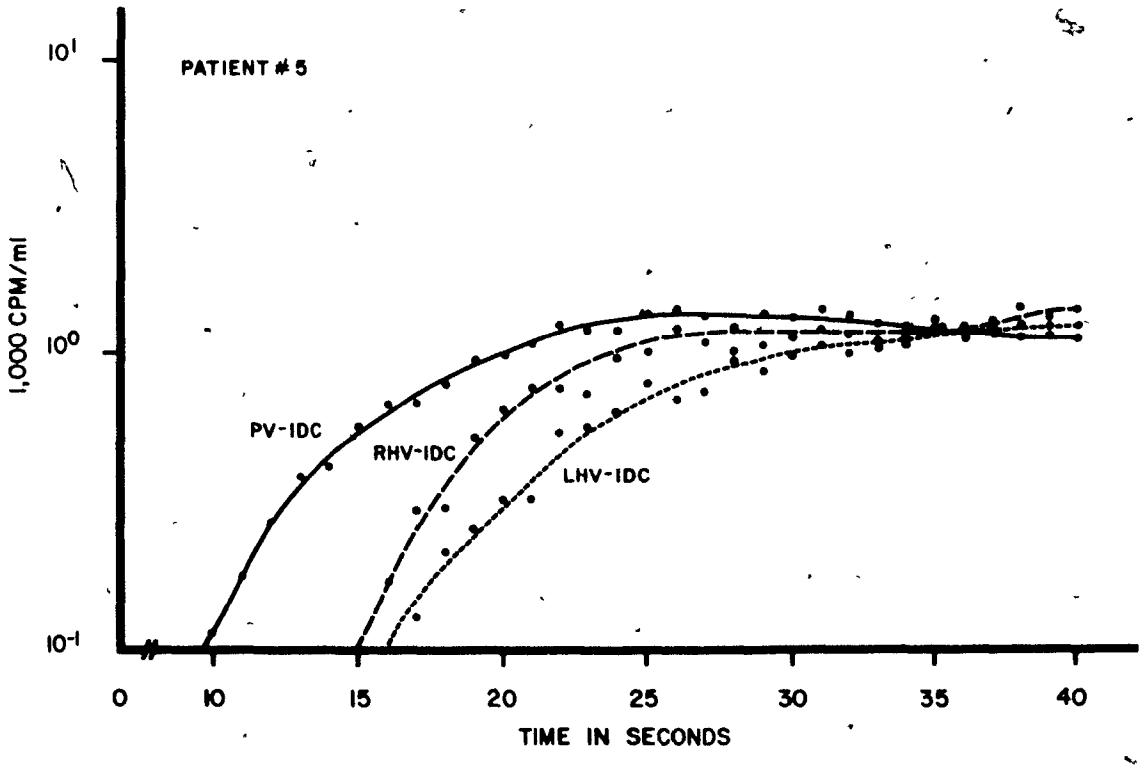

FIGURE 32: ACTIVITY RECORDED IN SAMPLES OBTAINED FROM THE PORTAL VEIN (PV-IDC), A RIGHT HEPATIC VEIN (RHV-IDC) AND A LEFT HEPATIC VEIN (LHV-IDC) AFTER INJECTION OF Cr^{51} R.B.C. INTO THE SUPERIOR MESENTERIC ARTERY IN A PATIENT WITH REVERSE CIRCULATION IN THE PORTAL VEIN.

In the thirteen patients, no correlation existed between the portal fraction of total hepatic blood flow and the K-I.C.G. (r: -.234, p 0.1) or the portohepatic gradient (r: .356, p 0.1).

F) Sinusoidal fraction of portal blood flow:

In 4 patients (IX, XI, XII, XIII), the sinusoidal fraction of portal blood flow varied between 3.8 and 86.3% (mean±SE: 36.2±12.7%) (table 9). In 3 experiments (2 patients: XI, XIII) where the sinusoidal fraction of portal blood flow could be estimated from two hepatic veins, the difference between paired values varied between 7.6, 15 and 34%. In the 4 patients, a significant correlation existed between the sinusoidal fraction of portal blood flow and the K-I.C.G. (r: .999, $p<0.001$). However, no correlation was found between the sinusoidal fraction and the portohepatic gradient (r: -.267, $p>0.10$).

(*) mesenteric artery, the arterial catheter was positioned into the hepatic artery under fluoroscopic visualization. Five (5) ml of Cr^{51} R.B.C. were injected into the hepatic artery and samples were simultaneously obtained from hepatic and portal veins. Similar I.D.C. were obtained (fig. 33) from these two sampling sites, demonstrating a complete inversion of portal flow. Total hepatic blood flow could be estimated from these I.D.C. and was 2.0 L/min using the portal I.D.C. and 2.4 L/min using the hepatic I.D.C. (difference: 17%) suggesting adequate mixing of the indicator within the hepatic circulation.

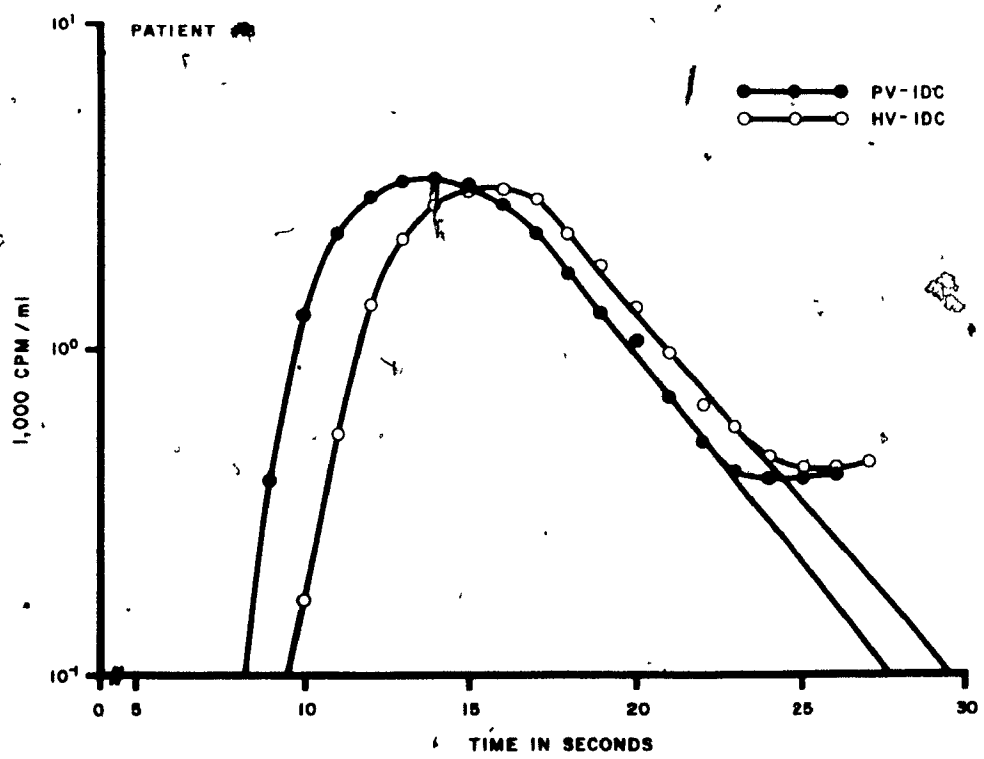

FIGURE 33: SIMULTANEOUS INDICATOR DILUTION CURVES OBTAINED FROM THE PORTAL VEIN (P.V.) AND ONE RIGHT HEPATIC VEIN (H.V.) AFTER INJECTION OF Cr^{51} RED BLOOD CELLS INTO THE HEPATIC ARTERY.

4) DISCUSSION

The use of portal and hepatic indicator dilution curves after injection of Cr^{51} R.B.C. into the cranial mesenteric artery has been validated for the estimation of portal and total hepatic blood flows in normal dogs. With the introduction of portal catheterization via the round ligament of the liver (51), a sampling site from the portal vein is now available in man. Therefore, this technique can be applied to conscious cirrhotic patients with no surgical manipulation of hepatic vessels and no circulatory impairment to the liver.

In man, as in dogs, the same experimental conditions have to be fulfilled (33,36,62). In cirrhotic patients, loss of indicator through spontaneous portosystemic collaterals resulted in overestimation of flows when compared with values obtained by the I.C.G. clearance method for the total hepatic blood flow (table 9). However, no extrahepatic shunts occurred after the portal bifurcation and therefore the same amount of indicator was analysed at the bifurcation of the portal vein and in hepatic vein(s). Thus, the portal fraction of total hepatic blood flow (the ratio of the portal blood flow over the total hepatic blood flow) should be reliable.

In normal dogs, adequate mixing of the indicator injected into the cranial mesenteric artery was demonstrated in the portal vein (at least at its bifurcation) as well as within the hepatic circulation. In man, although the phenomenon of preferential lobar distribution of portal blood has never been clearly established, it has nevertheless been popular clinical teaching to attribute localization of liver metastases and abscesses to the selective distribution

within the liver of blood flow from the area of the primary tumor or infection (75,76). In four patients, no significant difference was found between total hepatic blood flows estimated simultaneously from two different hepatic veins. This finding strongly suggest that when using the indicator dilution method, uniform mixing of the indicator was already achieved in the portal vein and the hepatic circulation in man as in the experimental model. Similar finding has been reported in normal and cirrhotic patients after injection of an I^{131} albumin into the superior mesenteric or the splenic artery (42).

If confirmed by further nearly identical total hepatic blood flows obtained from two different hepatic veins, these data would indicate that the Cr^{51} R.B.C.-I.D.C. can be used for the estimation of the portal fraction of total hepatic blood flow in cirrhotic patients. These preliminary reports also show that the portal fraction of total hepatic blood flow is not correlated with K-I.C.G. and the portohepatic gradient, parameters generally used as indices of severity in cirrhosis.

This model can be applied to study the behavior of I^{125} A.M.A., a colloid almost completely removed by the hepatic R.E.S., when compared to Cr^{51} R.B.C. as described in dogs. The hepatic extraction efficiency estimation should be reliable since the removed substance and the vascular reference substance were studied simultaneously in the portal vein (before the hepatic R.E.S.) and in the hepatic vein(s) (after the hepatic R.E.S.), whatever the loss of injected indicator through spontaneous portosystemic collaterals and the extrahepatic uptake of the indicator. Assuming that the concentration of albumin microaggregates is low enough to be completely removed during a single passage through the hepatic R.E.S., even in cirrhosis (28), the non-removed I^{125} A.M.A.

could be used as an index of intrahepatic shunted blood. Therefore, the hepatic extraction efficiency could be utilized to estimate the sinusoidal fraction of portal blood flow of a functional fraction of portal blood flow in cirrhotic patients.

Preliminary data in cirrhotic patients indicate that the estimated sinusoidal fraction of portal blood flow is nearly identical from two different hepatic lobes (2 patients) but varies greatly in different patients (table 9). Shaldon et al (26) estimated the hepatic extraction efficiency of heat denatured human serum albumin labelled with I^{131} in 6 cirrhotic patients with patent portal vein. Values varied between 64 and 90% of total hepatic blood flow (mean±SD: 75.8±11.5%) but no correction was performed for the extrahepatic uptake of the colloid which could result in overestimation of the hepatic extraction efficiency.

In the 4 patients, no correlation was found between the sinusoidal fraction of portal blood flow and the degree of the portal hypertension. However, these data show a significant correlation with the K-I.C.G. If confirmed, these findings would suggest that changes in the K-I.C.G. could be mainly secondary to vascular abnormalities of the cirrhotic liver.

In summary, after a single injection of Cr^{51} R.B.C. and I^{125} A.M.A. into the superior mesenteric artery, the portal fraction of total hepatic blood flow and the sinusoidal fraction of portal blood flow could be estimated simultaneously in awake cirrhotic patients. This is a non surgical method, with no anesthesia and dissection of hepatic vessels which can modify hepatic arterial and portal blood flows, as we demonstrated in dogs. These selective measurements are of great

importance clinically in the understanding of portal hypertension in cirrhotic patients and perhaps in the evaluation of long term results and the selection of the surgical procedure most appropriate for each individual case.

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