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Hepatic Blood Flow Determined by Colloidal Gold Clearance Compared with Direct Flow Measurements[1]

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Liver blood flow is usually evaluated clinically and experimentally by indirect methods using bromsulphalein (2) or radioactive colloid suspensions (4, 5). The value of an indirect method is contingent upon the accuracy with which it monitors actual blood flow. Selkurt compared the bromsulphalein clearance method with direct flow measurements and found a good correlation to exist (16, 17). Razzak and Wagner (15) showed that hepatic blood flow measured by colloidal gold clearance compared favorably with flows measured by direct collection of hepatic venous blood. Experiments herein reported are similar to those described by Razzak and Wagner in which liver blood flow rates were determined indirectly using radioactive gold (^{198}Au) colloid and compared with direct flow measurements obtained with a hepatic venous outflow technique. In addition, the present study included measurement of the rate of particle clearance by peripheral, splanchnic and hepatic vascular beds as well as tissue distribution of this substance following a period of infusion.

METHODS

Dogs weighing 20 to 27 kg were anesthetized with sodium pentobarbital. Using a cautery, the chest was entered through the right fifth intercostal space, and the abdomen through a right subcostal incision. (Refer to Fig. I for details of the experimental set up.) Cord tapes were passed around the inferior vena cava just below the right atrium (A) and immediately above the adrenal veins (A'). A third tape (B) was passed around the superior vena cava between the right atrium and the azygos vein. Small polyethylene catheters were inserted into the portal vein, inferior vena cava and internal mammary artery for purposes of obtaining blood samples and pressure measurements. Mean systemic pressure was measured with a mercury manometer, and venous pressure by measuring the blood level in the polyethylene catheters with a meter rule. All zero levels were adjusted to the level of the right atrium.

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Inferior and superior (No. 26F) vena cava catheters were inserted through the right atrial appendage and lateral wall respectively. The inferior cava catheter was passed into the abdomen so that its tip lay at the level of the renal vein, below tape A'. Side opening C in this catheter at the right atrium afforded an intralumenal route for return of vena cava blood to the heart when tapes A and A' were tightened. The azygos vein remained patent as an additional return channel of venous blood to the heart. A third venous catheter (No. 24F) inserted through a pursestring suture was placed in the inferior vena cava just above the diaphragm between tapes A and A'. The three caval catheters were connected in a short closed loop, (Fig. I) containing side arms for pressure measurement, flow diversion to a volumetric vessel and, during cardiopulmonary bypass, diversion of venous blood to the pump-oxygenator. Oxygenated blood re-entered the arterial tree via a cannulated femoral artery.

The segment of the inferior vena cava receiving the hepatic veins was isolated by tightening the tapes at A and A'. By simultaneously clamping at D and releasing at E, the entire hepatic blood flow could be directed to a volumetric vessel for a known time period, and appropriate minute flow calculations made. During the period of hepatic vein diversion, venous return to the heart was held constant by infusing donor blood at a rate equal to hepatic vein outflow. Hepatic venous pressure was held constant by adjusting the height of the diversion tube outlet. Thus, diversion tube resistance, outlet level and decreased venous return did not affect hemodynamics during the hepatic blood flow measurement. Inferior vena cava pressure remained constant during flow measurements, indicating that the inferior vena caval return was not significantly impeded by occlusion at A and A'.

Two groups of animals were observed. In 13 experiments (perfused group), cardiac output (75 cc/kg/min) was controled by means of total cardiopulmonary bypass with a rotating disc heart-lung machine for a period of 45 minutes. These animals were also observed before and after the bypass for periods of 15 minutes. A second group of 10 animals (control group), having the same surgical preparation, was observed for a period of 45 minutes without utilization of cardiopulmonary bypass.

In all animals, arterial and venous pressures were monitored continuously and hepatic vein blood flow measured directly every five minutes. Every 15 minutes blood specimens were drawn from actively flowing blood in the portal vein, hepatic vein, brachial vein, and internal mammary artery for gold colloid assay.

This colloid was a commercial preparation of ^{198}Au having a mean particle size of 3 millimicra and a specific activity of 3 to 4 μc/mg. Constant rate intravenous infusion was begun 30 minutes before the first blood specimens were drawn in order to establish a stable level of colloid. The total dose of gold was under 10 mg, which is substantially less than the 250 mg considered capable of producing toxic effects on the reticuloendothelial system (18).

At the end of each experiment the infusion was discontinued and the gold colloid in the circulating blood allowed to clear over a period of 15 minutes.

After sacrifice of the animal, a residual blood sample was taken and the liver, spleen and lungs removed and weighed. Representative portions of each organ were prepared, weighed and assayed for ^{198}Au content, using a well-type emission counter. The gold uptake of the liver, spleen, and lungs, as well as the residual in the blood, was calculated from these data. Total gold colloid infused was calculated from the duration of the infusion period, infusion rate and infusate assay.

Colloid blood level varied widely between experiments, depending upon dog weight, activity decay of colloid and hemodynamic factors. To facilitate tabulation of data from all experiments, activity concentration of venous blood colloid was expressed as a ratio of corresponding arterial concentration. The average colloid activity concentration in the portal venous, hepatic venous, and peripheral venous blood for all dogs studied, expressed as a ratio of the corresponding arterial concentration, was computed. From these average concentrations, the clearance efficiencies of ^{198}Au (afferent-efferent concentrations/afferent concentration) of the tissues studied were calculated.

In addition to the post mortem assay of gold uptake by the tissues, a second independent estimate of uptake by the hepatic, splanchnic, and peripheral tissues was made from observed concentration gradients and blood flows. During cardiopulmonary bypass in 13 dogs, the cardiac output (perfusion pump flow) was controlled and known. The portion perfusing the liver was measured directly, and it was assumed that 75 per cent of the afferent blood supply was via the portal vein and 25 percent was via the hepatic artery.

Let:

$\underline{C}_a$ = absolute colloid concentration in arterial blood
C_h = relative concentration in hepatic venous blood (Fig. 2)
C_p = relative concentration in portal venous blood (Fig. 2)
C_b = relative concentration in peripheral venous blood (Fig. 2)
CO = cardiac output (perfusion pump flow)
$\emptyset$ = hepatic vein flow/cardiac output (determined experimentally)

Then:

$\emptyset CO$ = hepatic vein flow
$.75\emptyset CO$ = portal vein flow
$.25\emptyset CO$ = hepatic artery flow
$(1 - \emptyset)CO$ = peripheral vein flow

And:

Since uptake = concentration gradient • flow • time
(1) hepatic uptake = $(C_p - C_h)\underline{C}_a \cdot .75\emptyset \cdot CO \cdot \text{time} + (1 - C_h)\underline{C}_a \cdot .25\emptyset \cdot CO \text{ time}$
(2) splanchnic uptake = $(1 - C_p)\underline{C}_a \cdot .75\,\emptyset \cdot CO \cdot \text{time}$
(3) peripheral uptake = $(1 - C_b)\underline{C}_a\,(1 - \emptyset) \cdot CO \cdot \text{time}$

$$\text{percent of total gold dose in liver} = \frac{(1)}{(1) + (2) + (3)}$$

$$\text{percent of total gold dose in splanchnic tissue} = \frac{(1)}{(1) + (2) + (3)}$$

$$\text{percent of total gold dose in peripheral tissue} = \frac{(1)}{(1) + (2) + (3)}$$

Hepatic vein blood flow was determined indirectly using the ratio of the rate of hepatic colloid uptake to the transhepatic concentration gradient (Fick principle). The rate of hepatic uptake is the rate of colloid infusion into the animal times the percent of total dose accumulated in the liver. The transhepatic concentration gradient was taken as the difference between the portal and hepatic venous concentrations. A small error introduced by neglecting the slight difference between portal vein and hepatic artery concentration was accepted in the interest of simplicity. The indirect measurements of hepatic blood flow were compared with the corresponding direct flow measurements.

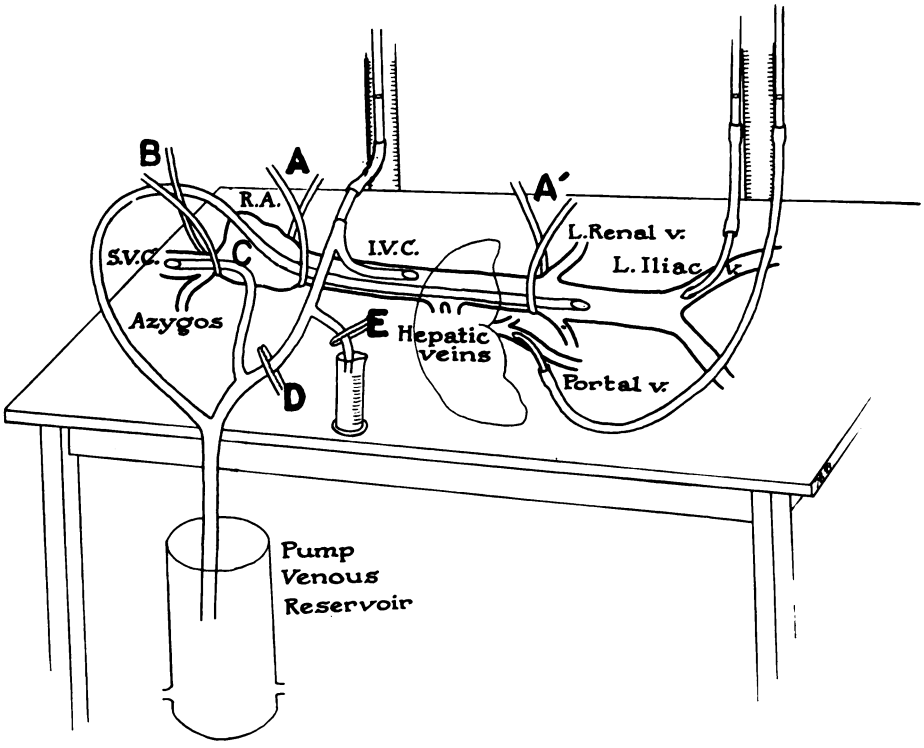

FIGURE I SCHEMATIC DIAGRAM OF PREPARATION

Fig. 1. Schematic diagram representing placement of catheters for venous flow diversion and pressure measurement.

RESULTS

Figure 2 shows gold colloid concentration expressed as a per cent of the arterial level in the peripheral vein (b), portal vein (c), and hepatic vein (d) in 57 sets of measurements in 10 control dogs and 49 sets of measurements in 13 perfused animals. These data show a concentration drop from 100 per cent in the arterial blood to 93 per cent in the portal vein blood and a further decline to 25 per cent in the hepatic vein blood. Peripheral vein concentration was equivalent to portal vein values. There was no significant difference between perfused and control groups of animals.

Figure 3 shows the clearance efficiency in the tissues studied expressed as the percent of afferent gold colloid concentration removed. The hepatic clearance efficiency of approximately 72 per cent was substantially larger than the 7 per cent clearance efficiency of the splanchnic and peripheral capillary beds.

The tissue accumulation of colloid is shown in Fig. 4. Assay of post mortem tissue showed that approximately 71 per cent of the infused ^{198}Au colloid was present in the liver. Spleen and lung tissue accounted for another 3 and 2 per cent respectively. Residual colloid in the post mortem blood was present in only trace amounts. Approximately 25 percent of the infused gold was not accounted for in the tissues assayed.

The tissue accumulations calculated from clearance efficiency and flow rates (Fig. 4) indicate that the hepatic, splanchnic, and peripheral capillary beds absorbed respectively 72.3, 6.2, and 21.5 per cent of the infused colloid in the control group and 73.3, 4.4 and 22.2 per cent in the perfused animals.

Table Ia lists 29 hepatic vein minute blood flows determined concurrently by the indirect and direct methods in the control group of animals. It is apparent that the indirect colloid gold method approximates quite closely and consistently the corresponding hepatic flow measured directly. In Table Ib, the results of 51 flow measurements in experiments employing controlled cardiac output (perfused group) are listed, and a similar correlation between the two methods is demonstrated. In addition, it can be noted that significant changes in hepatic vein flow are reflected promptly in the indirect determination.

A statistical analysis comparing the two methods is presented in Table II. On the average, the ratio of indirect to direct flow measurements varies less than eight per cent from a perfect correlation of 1:1. This is less than the standard deviation computed for the 80 sets of flow determinations reported in this study.

DISCUSSION

Previously reported methods for the direct measurement of hepatic vein flow have involved extensive surgical preparations with major alterations in the vascular supply of the abdominal viscera. Macleod and Pierce (13) isolated hepatic outflow by ligation of the vena cava above and below the hepatic veins and the aorta below the superior mesenteric artery. Blalock and Mason (1) isolated hepatic outflow by occluding the vena cava above and below the hepatic vein ostea with a double ballooned cannula inserted from the jugular vein via the

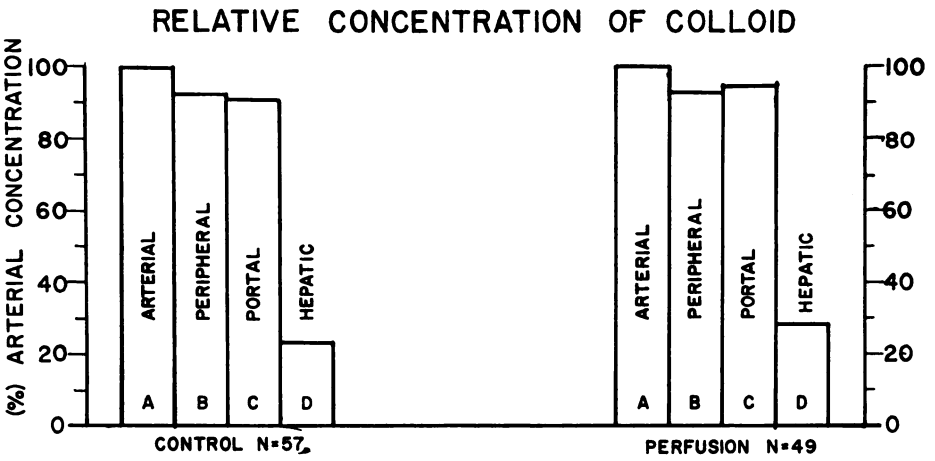

Fig. 2. Relative concentration of gold colloid expressed as per cent of arterial concentration in peripheral, portal and hepatic venous blood.

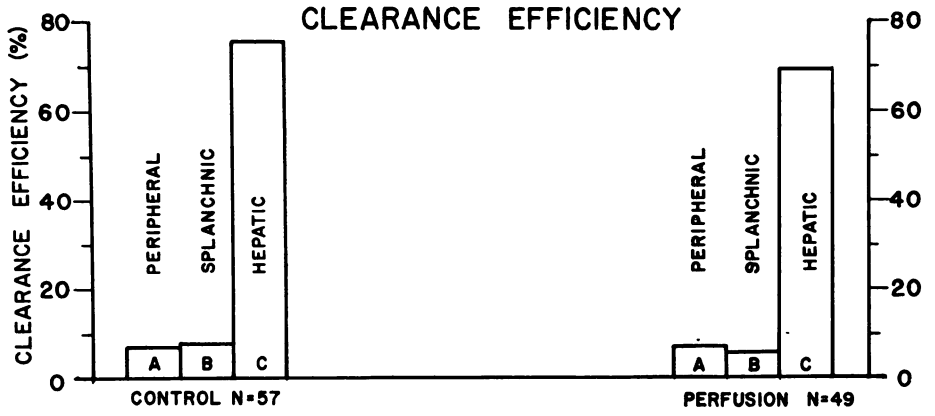

Fig. 3. Clearance efficiency of peripheral, splanchnic and hepatic tissues expressed as percentage of afferent gold colloid removed in one pass.

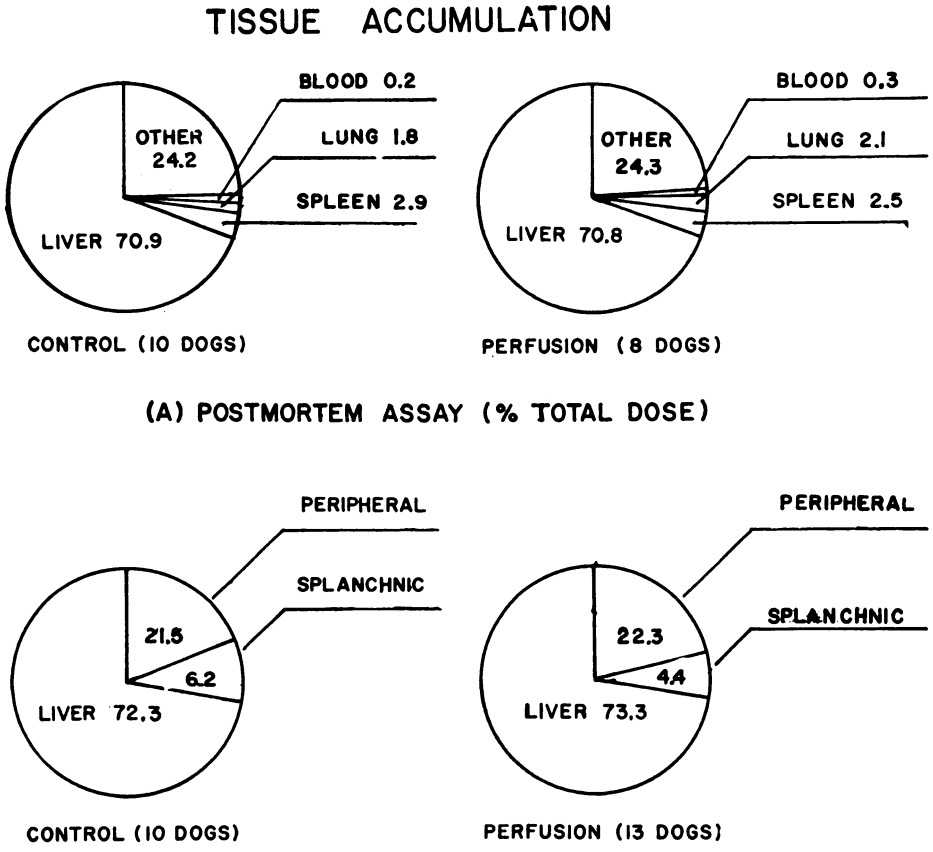

Fig. 4. Accumulation of infused gold colloid in various tissues determined by a) assay of post mortem tissue specimens and b) computation from measured flow rates and concentration gradients, expressed as per cent of total dose.

TABLE IA

COMPARISON OF HEPATIC FLOWS (CC/MIN) MEASURED CONCURRENTLY BY INDIRECT AND DIRECT METHOD IN 10 DOGS—CONTROL GROUP

	Experimental Conditions		
Expt No.	*Early*	*Middle*	*Late*
	Indir:Dir	*Indir:Dir*	*Indir:Dir*
242	275:265	234:250	262:234
245	204:262	224:246	311:294
246	279:252	278:244	231:268
250	384:372	284:331	236:262
253	258:302	269:264	152:182
254	209:288	295:264	258:240
257	275:294	261:296	228:252
260	173:208	223:273	—
261	341:324	302:330	283:306
265	401:387	340:321	381:317
Mean	280:295	271:282	260:262

TABLE IB

COMPARISON OF HEPATIC FLOWS (CC/MIN) MEASURED CONCURRENTLY BY INDIRECT AND DIRECT METHOD IN 13 DOGS—PERFUSED GROUP

	Experimental Conditions			
Expt No.	*Before Perfusion*	*Early Perfusion*	*Mid Perfusion*	*Late Perfusion*
	Indir:Dir	*Indir:Dir*	*Indir:Dir*	*Indir:Dir*
243	247:224	415:510	676:690	635:690
244	243:222	413:491	382:519	430:562
248	208:240	269:357	424:471	461:476
249	402:386	450:480	238:304	190:206
251	384:405	333:357	403:376	372:381
252	364:453	483:570	484:546	496:479
255	320:360	360:420	342:324	309:345
256	323:300	391:480	383:408	402:384
258	217:354	369:447	336:480	354:486
259	268:255	430:432	332:297	273:282
262	289:318	438:462	—	361:327
263	219:225	343:327	340:345	362:315
264	162:150	207:222	273:384	323:315
Mean	280:299	376:427	384:420	382:404

right atrium. Grindley, Herrick, and Mann (7) ligated the abdominal vena cava several months prior to flow measurements. The method employed in this investigation is considered to closely approximate the physiological state because the integrity of the abdominal circulation was maintained and intralumenal pressures did not vary during flow measurement.

Indirect methods, through the avoidance of surgical trauma and disruption of vascular supply, have the advantage of estimating hepatic vein flow in an optimal physiological milieu. However, certain assumptions must be made concerning the fate of the indicator substance employed. The method of Lipscomb and Crandall (11) using endogenous urea production and transhepatic urea concentration gradient has, as they point out, the disadvantage of a large inherent error due to the small differences in the concentrations measured. The work of Bradley *et al* (2) has led to the use of bromsulphalein as the most commonly employed method for clinical and experimental hepatic vein flow evaluation. This method assumes a functioning hepatic parenchyma and biliary tree (8) as well as negligible extrahepatic clearance.

The selective uptake of intravenously injected particulate material by the liver and spleen has long been known (12). The work of Jones *et al* (9) has prompted the investigation of a wide variety of radiation emitting colloids which are cleared from the blood stream principally by the hepatic reticuloendothelial system (3,5,18). This phenomenon has been employed in clinical evaluations of hepatic blood flow using gold colloid (10,14). However, these studies did not consider the extrahepatic clearance of colloid. Razzak and Wagner (15) measured hepatic blood flow employing the rate of clearance of radioactive cold colloid from the peripheral blood following a single injection of the colloid. When compared with direct flow measurements obtained concurrently with a hepatic vein diversion technique, they demonstrated a good correlation between the two methods. In their calculations, they employed hepatic clearance efficiency as a correction factor in determinating calculated hepatic blood flow.

Under the conditions employed in this study, the liver accumulated about 72 per cent of the total infused dose of colloid (Fig. 4). Thus the effective infu-

TABLE II

SUMMARY OF COMPARISON OF CONCURRENT FLOW MEASUREMENTS

Control Group			*Perfused Group*		
Mean	*Std Dev*	*N*	*Mean*	*Std Dev*	*N*
Indir:Dir			*Indir:Dir*		
271:280		29	355:387		51
0.97:1.0	0.07	29	0.92:1.0	0.08	51

sion of gold to the liver was 72 per cent of the actual infusion rate; the remaining 28 per cent of the infusion being cleared by extrahepatic tissues. Failure to consider the significant extrahepatic clearance when calculating estimated hepatic blood flow would result in overstatement of flow by a commensurate amount. Thus, in this study, the numerator of the Fick equation (flow = accumulation rate/concentration gradient) was taken as 0.72 × infusion rate to the animal. Use of this factor produced good agreement between calculated hepatic blood flow and the corresponding direct flow for 80 pairs of measurements in 23 animals as shown in Table I. The statistical analysis presented in Table II shows that the agreement approached the ideal of 1:1 and was within one standard deviation. The wider differences observed within individual pairs of measurements were most likely due to errors in direct flow measurement, colloid gold analysis, and contamination of hepatic vein samples by caval blood which, though individually small, could compound to produce the variation observed within individual pairs of determinations.

The use of coarser colloid particles could produce the selective hepatic uptake of about 95 percent of the total dose attained with bromsulphalein (17). Dobson *et al* (4) have shown that particle size has an important effect upon the selective uptake. Their studies have shown that very coarse particles, about one micron, are taken up almost exclusively by the liver and spleen, while the finer particles commonly employed in commercial suspension are cleared by these organs to the extent of about 80 per cent. This compares with a combined liver-spleen clearance of about 75 per cent observed with the fine commercial preparation used in this study.

The clinical investigator would not be able to obtain hepatic and portal vein blood specimens as was done in these animal experiments, and alternatives must be considered. The fact that the portal and peripheral venous blood colloid concentrations were equal (Fig. 2) suggests use of the latter to estimate the former; thus, substituting a readily available specimen for a much less available one. Hepatic vein concentration can be calculated from the portal (peripheral) vein concentration using the observed clearance efficiency of the liver (Fig. 3).

A constant rate infusion of colloid was employed in this investigation in order to facilitate investigation of clearing mechanisms. The validity of using gold colloid to estimate hepatic blood flow shown in this study could be extended to a single injection technique, as demonstrated by Razzak and Wagner, since the same clearing mechanisms apply. The single injection method, which is adequately described elsewhere (4,15,16) would be much more applicable to clinical investigations because it requires only the measurement of activity decay in peripheral blood following the injection of colloid. Both approaches, however, depend upon preferential clearance of colloid by the liver. Thus, the extrahepatic clearance must be considered if hepatic flow is not to be overestimated. This is particularly important in disease states such as advanced cirrhosis, where shunting of blood past the liver sinusoids would tend to increase the extrahepatic clearance of colloid.

Though the value of the bromsulphalein technique is well established in most clinical situations, evaluation of hepatic blood flow using infused gold colloid has several potential advantages. First, the assay of specimens is relatively simple, requiring only the measurement of radioactivity in a volume of whole blood. Plasma pigments do not complicate the determination. Second, the colloid method responds more sensitively to changing flow rates than bromsulphalein since the former is cleared from the blood stream almost twice as fast as the latter (6), and thus reaches a new equilibrium sooner. Inspection of Table I shows that abrupt changes in flow rate are reflected accurately in indirect flow measurements using colloid gold. Third, since a colloid suspension is cleared by the kupfer cell and not the hepatic cell, tests using it do not depend upon a functioning liver parenchyma or biliary tree and, therefore, could be employed in the presence of active liver disease or biliary obstruction.

The difference between colloid gold and bromsulphalein clearing mechanisms suggests a potential method for differentiating reduced hepatic blood flow, biliary obstruction and parenchymal liver disease. Bromsulphalein clearance is compromised in all three situations while gold colloid clearance would be depressed only if hepatic blood flow were reduced since it does not depend upon a functioning hepatic cell and is not excreted significantly in bile.

SUMMARY

The validity of indirect hepatic blood flow measurement employing clearance of exogenous gold colloid was investigated in dogs. In one series of animals, cardiac output was controlled by extracorporeal perfusion and, in another, cardiac function was intact. In both groups of animals hepatic flow was measured directly by diverting the hepatic outflow into a volumetric flask without introducing hemodynamic changes. With a constant infusion rate of radioactive gold colloid, the activity concentration was measured with the arterial, portal venous, hepatic venous and peripheral venous blood. From these blood levels and post mortem tissue assay, clearance of colloid by the liver, and extrahepatic tissue was calculated and liver blood flows computed using the modified Fick principle.

In 80 sets of observations in 10 uncontrolled and 13 controlled cardiac output dogs, good agreement was observed between hepatic vein blood flow determined by gold colloid clearance and concurrent direct flow measurements. The indirect determinations responded quickly to changes in hepatic flow. The results of this study confirm the findings of Razzak and Wagner, who have demonstrated a good correlation between hepatic blood flow determined by a gold colloid clearance method and a direct flow measurement technique.

Extrahepatic clearance of gold colloid was significant and must be considered when evaluating hepatic vein flow with this material. This is particularly important in certain disease states where blood is shunted past the liver sinusoids. Peripheral vein colloid concentrations closely approximated portal vein concentration and, thus, could be used to approximate a sample from this inaccessable vessel.

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