Hepatic Vascular Response Following Colloid Injection[1] (36281)

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The importance of pathophysiological alterations of the splanchnic circulation and hepatic vascular bed as a critical factor in the pathogenesis and progression of shock has been previously emphasized (1, 2). In this regard, impairment of hepatic blood flow and hepatic function has been related to decreased resistance to traumatic (3), hemorrhagic (3, 4), and endotoxin shock (5). Closely associated with the relationship between circulatory failure and hepatic function is the clearance and metabolic activity of the hepatic Kupffer cells (6). These cells represent a major segment of the reticuloendothelial system (RES) and are a key systemic host defense mechanism involved in the vascular clearance of toxic colloidal and particulate matter. Indeed, Palmerio and Fine (7) have presented data to support the concept that the maintenance of vascular integrity with reference to shock induction is closely dependent on the phagocytic and metabolic potential of the RES.

While many biochemical and physiologic factors participate in the regulation of RE function (8), the importance of hepatic blood flow as a major determinant of intravascular phagocytic activity by the RES is well documented (9, 10). A common technique utilized to alter the functional activity of the macrophage system is the intravenous injection of foreign colloids which are cleared by the RES. In this regard, colloid injection, which has been shown to alter the functional activity of the RES (8) also increases host susceptibility to traumatic and hemorrhagic shock (11). Although the mechanism mediating this response has not been defined, it has been suggested that this is due to a decrease in phagocytic and detoxifying host defense activity of RE cells, especially the Kupffer cells of the liver (12).

Since colloid-induced RE depression will increase susceptibility to shock (12) and since hepatic circulatory stability is a major determinant of shock resistance, as well as RE function, it is conceivable that decreased RE function and resistance to shock following colloid injection is due to a colloid-induced disturbance of the hepatic circulation. In the present study, the effect of colloid clearance by the liver on the stability of hepatic blood flow was evaluated in both an isolated perfused liver preparation and in the intact animal.

Materials and Methods. The method of perfusion of the isolated rat liver was similar to that described by Miller *et al.* (13). Male Holtzman rats (Holtzman Co., Madison, WI), weighing 200–400 g, were utilized as both liver and blood donors. Liver donors were anesthetized with sodium pentobarbital (30 mg/kg) and injected intravenously with 100 USP units of heparin. Cannulas were inserted into the portal vein for inflow, the thoracic vena cava for outflow, and the bile duct for bile collection. The liver, which was continuously perfused with warm, heparinized sterile saline, was rapidly extirpated and transferred to a thermo-regulated liver perfusion apparatus (Metalloglas, Inc., Boston, MA) maintained at 37°. Perfusion was maintained with a hydrostatic pressure head of 14–20 cm of water, and oxygenation was accomplished with a humidified gas mixture of 95% O_2 and 5% CO_2. Blood flow through the isolated perfused liver was monitored with the use of a calibrated outflow chamber lo-

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cated between the caval outflow and blood reservoir, and bile flow was measured by a calibrated collection chamber. The perfusion medium consisted of fresh, heparinized whole rat blood diluted 1:4 with Krebs–Ringer phosphate buffer (pH 7.4) in a final volume of 100 ml. The basic perfusion medium was supplemented with 3000 USP units of heparin and 800 mg of dextrose. Constant blood flow, bile production, and a stable perfusate hematocrit were used as criteria for preparation stability.

Hepatic sinusoidal blood flow *in vivo* was determined by an isotopic tracer technique utilizing the fractional clearance rate constant (k), as previously described by Dobson and Jones (14). In this method, the tracer utilized was an ^{131}I colloid (sp act 0.02 μCi/mg). It was injected at a dose of 5 mg/100 g, which is below the "critical colloid dose" (9) and its clearance was assessed by radioassay of timed 0.1 ml aliquots of whole blood. At this dose liver blood flow is the rate-limiting factor in the clearance of the tracer colloid and not RE phagocytic activity. The fractional clearance rate (k) was calculated from the expression:

$$k = \frac{0.693}{t/2},$$

where $t/2$ is the half-time (min) for disappearance of the colloid from the blood and k represents the fraction of the blood volume cleared per minute. The fractional clearance rate (k) multiplied by the blood volume is an index of minimal hepatic sinusoidal blood flow (9, 14). Blood volumes was determined by extrapolation of the clearance curve to zero time (C_0) and applying the dilution principle as previously described (9). Hepatic sinusoid blood flow was expressed as:ml/min; ml/min/g of liver; and ml/min/100 g of body wt.

The particulate preparations utilized to challenge the RES were colloidal carbon (C_{11}/1431a; Gunther Wagner, Hanover, Germany) and a gelatinized ^{131}I "RE test lipid emulsion." Colloidal carbon was injected intravenously at a dose of 16 mg/100 g or added to the perfusate at doses of 8, 32, or 48 mg/liver preparation. The lipid emulsion was injected intravenously at a dose of 50 mg/100 g or added to the perfusate at doses of 100, 200, or 800 mg/liver perfusion. Both of these particulate preparations have been previously employed to evalute RE function, and their selective removal by the RES is well documented (8).

Carbon clearance by the isolated perfused liver was evaluated by spectrophotometric analysis (675 μ) of timed 1 ml perfusate samples diluted in 0.1% sodium carbonate. Clearance of the ^{131}I RE test lipid emulsion was determined by ^{131}I radioassay of timed 1 ml perfusate samples utilizing a deep well gamma counter (Auto-Gamma Nuclear-Chicago, Des Plaines, IL). The data were statistically analyzed with the t test by placement of the confidence level at 95%. Means and standard error of the mean are presented for all experimental groups.

Results. Preliminary experiments demonstrated flow stability in the isolated perfused rat liver over a 3-hr perfusion period, following an initial period during which flow increased to a stable value. Associated with constant hepatic blood flow, was a constant rate of bile production and a stable perfusate hematocrit, as previously described (15). In an attempt to determine the effect of colloid injection into the perfusate on hepatic blood flow, colloid carbon (8 mg) and the lipid emulsion (100 mg) were added to the perfusate following establishment of stable blood flow (Fig. 1). As shown, in contrast to the stable blood flow prior to colloid administration, a progressive and significant ($p < .05$) decrease in flow was observed after carbon addition, achieving a maximum depression by 30 min postcolloidal injection. This response was not colloid specific, since the phagocytic clearance of the lipid emulsion was also followed by a similar, though less intense, response (Fig. 1). Recovery of flow rate was observed with both colloids, but it was consistently faster and more complete after clearance of the lipid particles. Determination of the clearance of the particles from the perfusate by the Kupffer cells demonstrated that with these doses (8 mg carbon, 100 mg lipid) an average clearance $t/2$ of 9.4 min for carbon and 11.2 min for lipid was detectable.

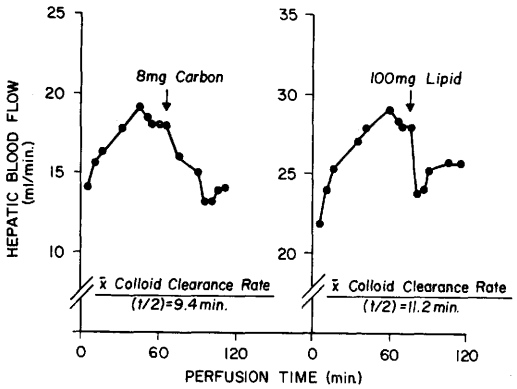

FIG. 1. Hepatic blood flow in the isolated perfused rat liver prior to and following colloid injection into the perfusate: Each graph represents composite data obtained from 3 separate perfusion experiments. The arrow (↓) indicates the point of injection of either colloidal carbon (8 mg) or the gelatinized lipid emulsion (100 mg).

The decrease in hepatic blood following colloid injection was both dose dependent and a function of the precolloid injection level of stable blood flow. Presented in Fig. 2 is the relationship between colloid dose and percentage flow depression. As shown, increasing the colloid dose of both colloids resulted in a significant ($p < .05$) increment in the vascular response observed. Comparable to the data in Fig. 1, the percentage decrease in blood flow after carbon clearance was greater than that after clearance of the lipid.

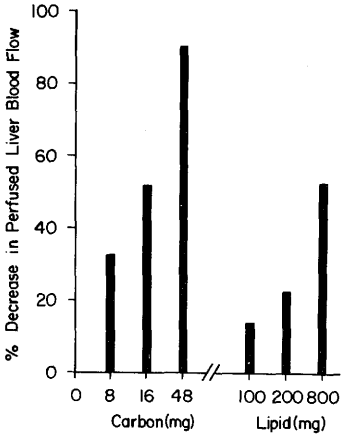

FIG. 2. Relationship between administered colloid dose and the intensity of the vascular response: Blood flow is expressed as average percentage (%) decrease in blood flow during the period of maximum vascular response, compared to the stable flow determined prior to colloid injection. Dose indicated represents mg/perfusate. Each bar represents the response as determined from an average of 3 separate liver perfusions.

The relationship of the stable control level of blood flow to the vascular response following colloid injection is presented in Fig. 3. In

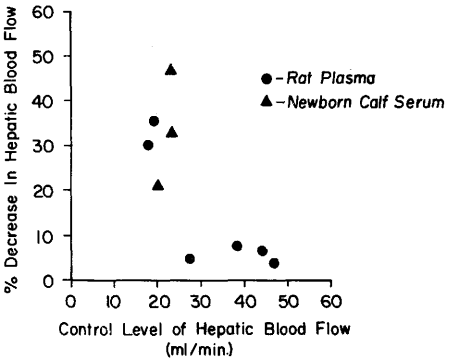

FIG. 3. Vascular response in the isolated perfused rat liver 30 min following colloid administration (8 mg carbon) as a function of the stable control flow established prior to colloid injection: Both heparinized rat plasma (●); and heparinized newborn calf serum (▲) were used in the perfusate. Each point represents an individual experiment. Vascular responses above a stable flow of 26 ml/min were not significantly ($p < .05$) different from precolloid control level.

these experiments, a constant 8 mg dose of colloidal carbon was utilized; and the stable flow was attained and maintained prior to colloid injection. Both rat plasma and newborn calf serum were utilized in an attempt to delineate the perfusate specificity of this response. At stable flow rates above 26 ml/min, a minimal vascular response was observed following colloid phagocytosis. In contrast, when the stable perfusion rate was maintained in the physiologic range (Table I) for the rat (9), there was a consistent and significant ($p < .05$) decrease in blood flow during the 20–40 min postcolloid injection period. No apparent differences existed when either rat plasma or newborn calf serum supplemented with rat blood cells was used in the perfusion medium.

TABLE I. Hepatic Sinusoidal Blood Flow Following Intravenous Colloid Administration.

Colloidal[a] preparation	Experimental parameter	Liver blood flow[b] (mean ± SE)			
		Control	(min): 30	45[c]	90
Colloidal carbon	(ml/min)	26.61 ± 1.58	18.00 ± 2.68	4.90 ± 0.56	12.00 ± 2.16
	(ml/min/g)	2.14 ± 0.12	1.74 ± 0.23	0.37 ± 0.04	0.99 ± 0.19
	(ml/min/100 g)	6.80 ± 0.80	6.29 ± 0.98	1.41 ± 0.16	3.43 ± 0.62
Lipid emulsion	(ml/min)	18.11 ± 1.77	20.13 ± 2.74	11.53 ± 2.26	14.02 ± 1.23
	(ml/min/g)	1.72 ± 0.15	2.10 ± 0.25	1.01 ± 0.15	1.56 ± 0.13
	(ml/min/100 g)	6.20 ± 0.65	7.24 ± 0.94	3.60 ± 0.61	5.86 ± 0.49

[a] Colloidal carbon was injected at a dose of 16 mg/100 g of body weight. The gelatinized lipid emulsion was injected at a dose of 50 mg/100 g of body weight.

[b] Blood flow is expressed as: ml/min; ml/min/g of liver; and ml/min/100 g body wt. Data in each time interval represents mean ± standard error from an average of 6 experimental animals.

[c] Flow values at 45 min postcolloid injection are significantly ($p < .01$) less than control precolloid injection flow levels.

In an attempt to relate the changes observed in the isolated liver to possible *in vivo* alterations in liver blood flow following intravascular phagocytosis, hepatic blood flow was evaluated in normal animals and in animals at 30, 45, and 60 min after intravenous injection of both colloids. As presented in Table I, with colloidal carbon, a slight decrease in flow was observed by 30 min, which attained a significant ($p < .001$) maximum 82% decrease at 45 min on a ml/min basis. On a per gram basis, which is actually the more consistent physiological base, this degree of perfusion was only 17% of control levels. Significant ($p < .05$) vascular recovery was observed by 90 min postcarbon injection. With the lipid emulsion, flow was not significantly ($p < .05$) altered by 30 min, but demonstrated a significant ($p < .01$) 36% decrease at 45 min. On a per gram basis, this corresponds to a perfusion at 59% of control levels. Flow recovery was complete by 90 min postinjection. Comparable to the perfused liver, the *in vivo* data (Table I) demonstrated less of a depression and a faster rate of recovery following injection of the lipid particles compared to the vascular response following colloid carbon.

Discussion. The effect of hepatic blood flow alterations on the relative distribution of colloids and the efficiency of hepatic Kupffer cell phagocytosis has been previously investigated (9, 10). However, the cardiovascular consequences of intravenous injection and hepatic phagocytosis of foreign colloids specifically with reference to hepatic blood flow has not been well defined. The present data demonstrates that the clearance of both an inert and metabolizable colloid results in a significant decrease of hepatic blood flow *in vivo* and in the isolated perfused liver experimental model. These findings confirm and extend previous *in vitro* observations on the hepatic vascular changes following Kupffer cell phagocytosis (15). The flow changes in the isolated perfused liver appear to be a result of variations in vascular resistance, since a constant perfusion pressure was maintained throughout the experimental interval.

While the response is not colloid specific, the blood flow depression following the phagocytosis of inert colloidal carbon was more intense and prolonged than that following the clearance of the metabolizable lipid particle at doses manifesting quite comparable rates of phagocytic clearance. This may be due to the difference in particle size, surface charge, or chemical composition. Smith and Filkins (16) observed that the release of lysosomal enzymes from the liver was much greater after injection of colloidal carbon than after clearance of this lipid emulsion. Furthermore, the severe tachycardia, tachypnea, hemoconcentration, and hypotension observed by

Wiedmeier *et al.* (17) after carbon administration in the dog was not apparent after injection of comparable doses of this particulate lipid (Scovill and Saba, unpublished observations). These distinct differences may be related to the potential endotoxin contamination of carbon (8), especially in view of the ability of endotoxin to elicit a similar vascular response in the isolated perfused rat liver (18).

Whether the hepatic vascular response following colloid clearance is due to a direct effect of the colloid *per se* at the intracellular level or mediated in part by the presence of vasoactive substances in the perfusate remains to be determined. The flow drop is not a consequence of the mere presence of the particles in the liver vasculature, since the response is not demonstrable when the perfusate is devoid of plasma or serum (15). Furthermore, the *in vivo* response does not occur rapidly after colloid injection at a time during the highest blood concentration of colloid. The lack of a response in the absence of plasma or serum correlates well with the role of opsonic protein as a factor regulating Kupffer cell phagocytosis (8). In this regard, the event appears to be postphagocytic in nature and dependent on the active uptake of colloid by the Kupffer cells. Recently, endotoxin-induced vascular resistance by the isolated perfused liver has been shown to be dependent on the presence of both plasma and buffy coat cells (19). Conceivably, leukocytes may be involved in the vascular response: but this remains to be determined.

Zweifach (12) has reported that normal function of the RES provides a regulatory influence on the vascular reactivity in hepatic and extrahepatic microcirculatory beds. This reactivity can be conditioned by either "blockade" or stimulation of the RES and appears to be related to the role of the RES in the removal and detoxification of blood-borne vasoactive substances (12). Transient blockade of hepatic Kupffer cells, as would be induced in the present study by colloid injection (8), could impair the ability for Kupffer cells to remove and detoxify such substances resulting in their effects on the microcirculation. The lag period for the response *in vivo*, coupled with the presence of extrahepatic RE sites *i.e.*, spleen, available for clearance and detoxification in the intact animal compared to the perfused organ would support this concept.

Early studies by Ruttner and Vogel (20) demonstrated bulging of Kupffer cells into the sinusoidal space following phagocytosis. These observations form the basis for the concept that Kupffer cells may participate in the physiologic regulation of sinusoidal resistance and intrahepatic blood flow (21). The possibility that postphagocytic swelling of Kupffer cells into the hepatic sinusoids may be the basis for the resistance changes observed is supported by preliminary electron microscopic findings (Saba *et al.*, unpublished observation), which demonstrate cytoplasmic engorgement and excessive swelling of Kupffer cells in the rat 15–30 min after intravenous injection of this lipid emulsion. Similar observations have been made in humans (22). However, the fact that flow recovery is observed at a time when the cells are still laden with colloid suggests that this is not the total explanation.

Impaired hepatic sinusoidal blood flow following Kupffer cell phagocytosis suggests a possible relationship between RE cell activity and liver blood flow, which may be a critical factor in altered shock resistance induced by colloid injection. The ability to condition vascular reactivity by experimental alteration of the functional state of the RES, coupled with the pivotal role of Kupffer cells and the splanchnic circulation in the development of shock (23), would lend credence to this concept. Thus, colloids themselves may induce vascular changes and alter vascular sensitivity as a consequence of RE storage, which may be a key factor mediating the colloid-induced impairment of host resistance to shock. While the basis for this response remains to be ascertained, the swelling of Kupffer cells or a transient impairment in their ability to clear and degrade vasoactive substances is suggested.

Summary. The phagocytic clearance of both an inert and metabolizable foreign colloid results in a decrease in hepatic sinusoidal blood flow in both the isolated perfused rat

liver and intact animal. While the vascular response was not colloid specific, it was dependent on both the dose injected and the precolloid injection level of stable blood flow. In this regard, the response was directly related to the dose injected and only apparent when the stable flow was within the physiologic range. The temporal relationship between the vascular response and the rate of colloid clearance indicates that the response is a postphagocytic event and dependent on the prior Kupffer cell uptake of the particles. It is suggested that the colloid-induced depression in hepatic blood flow may be a factor mediating the decreased resistance to shock induced by intravenous colloid administration.

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