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Plasma Factor Influencing Carbon Phagocytosis in the Isolated Perfused Rat Liver.* (30408)

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The important role of normal or immune serum opsonins in the preparation of bacteria for phagocytosis by leucocytes as well as by the fixed phagocytes of the reticuloendothelial system (RES) has been firmly established (1-4). However, the necessity of plasma factors for efficient phagocytosis of supposedly inert colloids is uncertain.

On the one hand, early experiments of Fenn(5) indicated that leucocyte phagocytosis of carbon and quartz particles was augmented by serum; more recently, Jenkin and Rowley(6) reported that humoral serum factors exert an important influence on intravascular colloidal carbon clearance. On the other hand, Biozzi *et al*(7) maintain that serum factors do not contribute in any way to the rate of phagocytosis of inert particles by the RES.

This paper presents evidence to substantiate the existence of a plasma factor capable of markedly affecting the rate of colloidal carbon gel phagocytosis by the Kupffer cells of the isolated, perfused rat liver.

Methods and procedure. Perfusion technique. The method of liver perfusion used in this study was essentially that described by Brauer(8) and Miller(9). Male rats of the Sprague-Dawley strain maintained on Purina pellets and water *ad lib* were used as both blood donors (rats of 400 to 600 g body weight) and liver donors (rats of 300 to 370 g body weight). The perfusate was either heparinized, whole rat blood or a semi-synthetic medium(10) described in Table I and supplemented with penicillin (20 U/ml) and streptomycin (0.025 mg/ml).

Livers with cannulae in the portal vein, thoracic vena cava and bile duct were rapidly extirpated, inserted into a constant environment chamber, and perfused under a hydrostatic pressure of 18 to 20 cm. Bile was collected and its flow was measured in a Wintrobe tube; blood flow was monitored by a flow tube inserted into the caval outflow.

Paired perfusions were performed by first perfusing one liver for 3 hours and then

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TABLE I. Composition of Semi-Synthetic Perfusion Medium.

Substance	Concentration
NaCl	137 mM
KCl	2.68 "
$CaCl_2$	1.80 "
$MgCl_2 \cdot 6H_2O$	.49 "
$NaHCO_3$	11.9 "
$NaH_2PO_4 \cdot 1\ H_2O$	.67 "
D-glucose	5.45 "
Bovine albumin	25 g/l
Rat blood cells (twice washed)	30–40%

placing a second liver into the identical blood pool and perfusing it for an additional 3 hours.

Carbon clearance tests. Colloidal carbon gel (C11/1431a, Pelikan-Werke, Hanover, Germany) was prepared at a concentration of 32 mg/ml in 2% gelatin (Coleman, Matheson & Bell, Rutherford, N. J.). Test doses of 8 mg carbon were introduced into the blood reservoir and rapidly mixed with a magnetic stirrer. Timed blood samples were withdrawn from the portal supply and diluted in 0.1% sodium carbonate. Carbon concentrations were determined densometrically at 675 mμ on the Beckman Model B spectrophotometer. Phagocytic activity was described as the carbon half-time value obtained from a graph of the logarithm of carbon concentration *versus* time.

Results. Paired perfusions: Carbon tests in both preparations. In a series of experiments 2 livers were perfused in sequence using the identical blood pool and carbon clearances were determined in each member of the perfusion pair. As indicated in the left panel of Fig. 1, the first liver removed carbon at rates comparable to those often reported in *in vivo* studies; however, as shown in the right panel of Fig. 1, a second

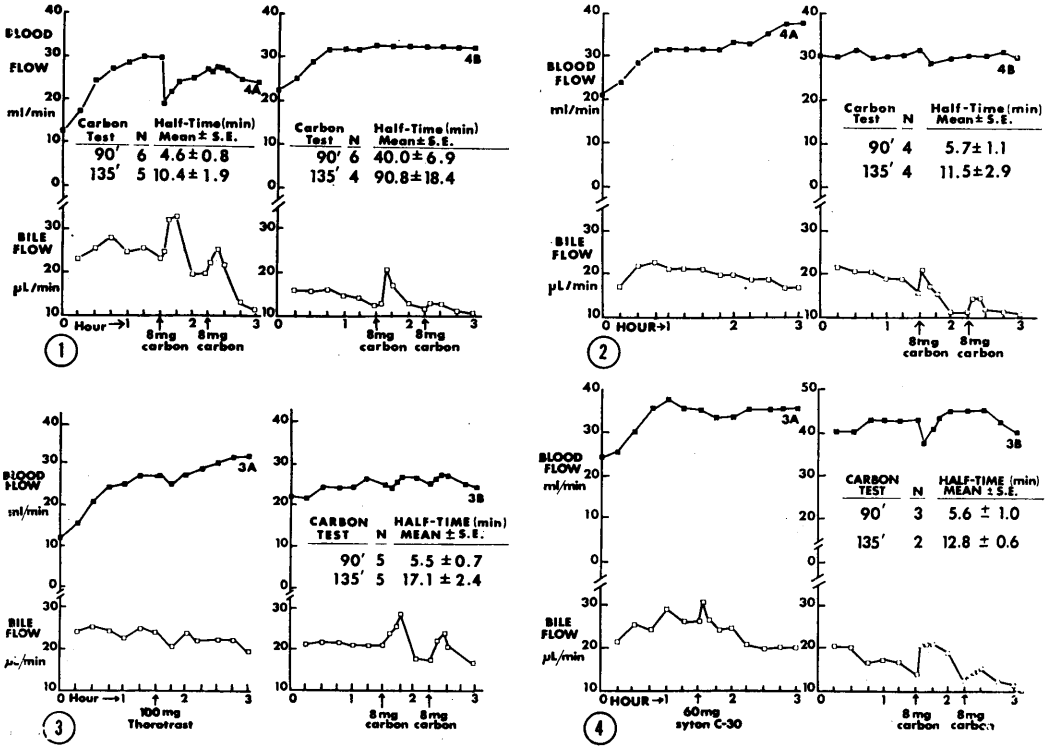

FIG. 1. Carbon clearance tests in liver pairs perfused in sequence with an identical blood pool.

FIG. 2. Carbon clearance tests in Liver 2 of a perfusion pair; Liver 1 untreated.

FIG. 3. Carbon clearance tests in Liver 2 of a perfusion pair; Liver 1 treated with colloidal thorium dioxide.

FIG. 4. Carbon clearance tests in Liver 2 of a perfusion pair; Liver 1 treated with colloidal silicon dioxide.

liver perfused with the same blood as Liver 1 had greatly diminished carbon clearing ability.

Also shown in Fig. 1 are representative blood and bile flow changes accompanying carbon phagocytosis. There was consistently a prompt and striking fall in blood flow after the first carbon injection but this effect was minimal or absent after subsequent injections. The prompt increase in bile flow was also less marked after later injections.

There would appear to be 3 possible explanations for the diminution in carbon clearance in the second liver: 1) non-specific changes in the blood perfusate so that Liver 2 was functionally impaired. 2) release of a phagocytic inhibitor by the cells of Liver 1, due either to exposure to foreign colloid or to phagocytosis *per se*. 3) removal from the perfusate by Liver 1 of a factor or 'opsonin' essential for carbon phagocytosis.

Paired perfusions: Carbon tests in second liver only. To test the first possibility, experiments were performed in which Liver 1 of a pair was perfused for 3 hours without being exposed to carbon or other materials. As indicated in Fig. 2, the carbon half-times of Liver 2 were indicative of relatively efficient phagocytosis and were not statistically different from those of Fig. 1 (left panel). Thus, non-specific changes in the perfusion medium were apparently of minimal importance.

Paired perfusions: Colloidal thorium or silicon dioxide administered to Liver 1; carbon tests in Liver 2. To test the second possibility experiments were performed in which Liver 1 of a perfusion pair received either colloidal thorium dioxide (Fig. 3; Thorotrast, Testagar Co., Detroit, Mich.) or silicon dioxide (Fig. 4; Syton C-30, Monsanto Chemical Co., Boston, Mass.) while Liver 2 was tested for carbon clearing ability. As indicated in Fig. 3 and 4, neither colloid—as administered to Liver 1—seemingly affected carbon phagocytosis by Liver 2. This finding mitigates against the likelihood of an inhibitor release and demonstrates the specificity of the interaction with colloidal carbon gel. By exclusion, therefore, the most plausible explanation for the data of Fig. 1 appeared to

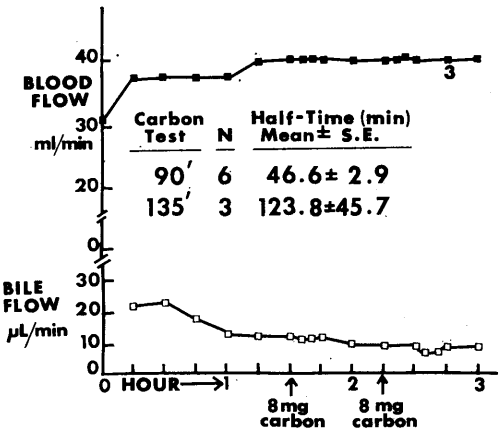

FIG. 5. Carbon clearance tests in livers perfused with semi-synthetic medium.

be an exhaustible factor in the perfusate which was essential for carbon phagocytosis.

Carbon clearance test in livers perfused with semi-synthetic medium. In its most generally accepted meaning, 'opsonic activity' refers to the enhancement of phagocytic rate which occurs in plasma or serum compared to an artificial medium. To test this criterion, a series of perfusions was conducted in which carbon phagocytosis was measured in livers perfused with a semi-synthetic medium (Table I). As indicated in Fig. 5, phagocytosis of colloidal carbon was minimal despite the apparently satisfactory function of the liver as reflected by bile production and a stable blood flow. Thus it would seem that plasma does indeed possess opsonic activity for carbon phagocytosis.

Discussion. It has long been known that blood components often play a determinant role in the engulfment stage of phagocytosis. Many investigators have shown that the specific antibodies of immune serum promote the ingestion of microorganisms by phagocytes. However, equally well established is the fact that normal or non-immune blood factors can behave as opsonins both for bacteria(3) as well as for inert particles such as bentonite (11), starch(12), colloidal gold(13) and wax particles(14). The nature of these non-immune opsonins is uncertain but they probably involve complement, gamma globulin, undefined therm-labile elements and specific or natural antibodies(5). Our data substanti-

ated the existence of a blood factor capable of greatly modifying carbon clearance rates and corroborated the *in vivo* findings of Normann and Benditt(16).

The implications of a plasma factor importantly concerned with carbon gel phagocytosis are significant because of the widespread use of the carbon technique in the study of phagocytosis. It is quite possible that colloidal carbon clearance may be an index, not only of the functional capacity of the phagocyte, but also of the plasma preparatory stage. Some of the reported inconsistencies in the kinetics of carbon phagocytosis may be explainable on the basis of such plasma factors.

The rather marked effect of carbon injection on liver blood flow and bile flow are of interest. The tendency for these changes, particularly in blood flow, to be minimal when the clearance times were prolonged (*e.g.*, Fig. 5) indicate that they are probably associated with the process of phagocytosis rather than with a direct effect of the carbon on the blood vessels or on the formed elements of blood. The mechanism of the bile and blood flow effects is unknown but the abruptness and extent of these phenomena would suggest further study.

Summary. The existence of an exhaustible plasma factor operative in Kupffer cell phagocytosis of colloidal carbon gel was demonstrated in the isolated perfused rat liver. In liver pairs perfused in sequence with an identical blood pool, phagocytosis was minimal in the second preparation compared to the efficient clearance in the first. Neither colloidal thorium dioxide nor silicon dioxide administration to the first member of a perfusion pair affected carbon phagocytosis by the second preparation; carbon clearance was slight in livers perfused with a semi-synthetic medium lacking plasma. It is suggested that an assessment of phagocytic capacity of the rat for carbon should include consideration of such plasma factors.

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