Mechanism Mediating Reticuloendothelial System Depression After Surgery[1] (34638)

THOMAS M. SABA
(Introduced by K. Stern)
Department of Physiology, University of Illinois, College of Medicine, Chicago, Illinois 60680

Involvement of the reticuloendothelial system (RES) in bacterial infection (1, 2), neoplastic disease (3, 4), and immunological activity (5, 6) has been well documented. Such studies have emphasized the host defense role of the RES and the need to comprehend factors that induce changes in RE activity.

Recent findings by Donovan (7) in humans, and Saba and Di Luzio (8) in animals have demonstrated that surgical manipulation will depress phagocytosis. Moreover, it has been speculated that the decreased resistance (9–10) and increased incidence of metastasis after surgical stress (11–15) may be due to depressed phagocytosis (8). Although previous findings have revealed the existence of phagocytic changes after surgery, the factors mediating this response have not been determined. Since phagocytic depression can occur as a result of a depletion of circulating plasma factors called opsonins (14, 15), RE depression after surgical stress may reflect a depression of opsonin activity.

In the present study, the involvement of the opsonic system as a factor mediating RE depression after surgery was evaluated. In addition, the time course associated with surgically induced RE alteration as well as the colloid specificity of the response was evaluated.

Experimental Methods. Male Holtzman rats (225–350 g) were maintained on Rockland Lab-Tek chow and tap water *ad libitum* Liver donors were anesthetized with ether and rapidly desanguinated prior to liver removal. The excised liver was chilled in cold isotonic saline prior to liver slice preparation with the use of a Stadie-Riggs tissue slicer (16, 17). Plasma or serum was obtained by vena caval puncture and maintained briefly at 4° prior to evaluation.

Both colloidal carbon (18) and gelatinized "RE test lipid emulsion" (14, 15) were utilized as the test colloids, since they are selectively removed by the process of phagocytosis. Colloidal carbon (C 11/43, Gunther-Wagner, Hanover, Germany) was injected intravenously at a dose of 16 mg/100 g of body weight. The gelatinized ^{131}I "RE test lipid emulsion" was prepared from glycerol, ^{131}I-triolein (Mallinckrodt Nuclear, St. Louis, Missouri), and lecithin mixed in a ratio of 10:10:1 by weight, respectively, as previously described (14, 15, 19). It was injected intravenously at a dose of 50 mg/100 g of body weight. Phagocytic activity was determined by the half-time ($t/2$) for the vascular clearance (8, 14) of either colloidal carbon or gelatinized ^{131}I-"RE test lipid emulsion." Colloidal carbon concentration and blood ^{131}I radioactivity was determined on 5 serial 0.1-ml samples of whole blood by spectrophotometric and isotopic techniques, respectively (14, 18).

Opsonic activity was determined with a liver slice incubation technique (14, 16). The incubation system consisted of 3 ml of heparinized (50 USP units/ml) medium, 2000 μg of the ^{131}I-lipid emulsion (1% lipid emulsion with 0.1% gelatin), and the liver slice (14, 16). All incubation samples were gassed with 95% O_2 and 5% CO_2 and incubated at 37° for 30 min. After incubation, the liver slices were removed, briefly washed in isotonic saline, and analyzed for Kupffer cell colloid uptake. Plasma opsonic activity was evaluated in terms of its ability to stimulate hepatic phagocytosis expressed as either the percentage of the injected dose or micrograms of

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colloid phagocytized per 100 mg of tissue (14–17).

Opsonization of colloidal carbon was accomplished by incubating at 37° equal volumes of colloidal carbon (32 mg/ml) and normal serum with heparin (20 USP units/ml) at pH 7.4 for 30 min (18). Opsonization of the lipid emulsion was accomplished by incubating at 37°, 400 mg of emulsion with 1.0 ml of normal serum at pH 7.4 for 15 min (14). Nonopsonized colloids were incubated under similar conditions with saline.

The surgical stress consisted of a midline laparotomy (5 cm) coupled with gentle intestinal manipulation (30 sec) while the rats were under ether anesthesia. Following surgery, the incision was closed with silk suture. Control rats were exposed to similar conditions of anesthetization.

Determination of ^{131}I radioactivity was accomplished with a Nuclear-Chicago Auto-Gamma crystal scintillation system (Nuclear-Chicago, Des Plaines, Ill.). Data were statistically evaluated with the t test by placement of the confidence level at 95%.

Results. Presented in Fig. 1, is the half-time ($t/2$) for the phagocytic clearance of colloidal carbon at various intervals after surgery. A significant ($p < .001$) depression in vascular clearance of colloidal carbon was observed at 15 min postsurgery as indicated by the mean 31% increase in the half-time ($t/2$) as compared to control values. Thereafter, the $t/2$ for carbon clearance was increased 47% at 30 min, 37% at 60 min, 63% at 90 min, 41% at 120 min, and returned to control values at approximately 2.5-hr postsurgery. RE stimulation relative to colloidal carbon clearance occurred at 3- and 4-hr postsurgery. Also presented in Fig. 1, is the vascular clearance of the lipid emulsion after surgery. A slight increase in the rate of vascular clearance of the lipid emulsion was observed at 15 min postsurgery. Comparable to carbon clearance alterations, RE depression ($p<.001$) was manifested by 30-min postsurgery as indicated by the 82% increase in the $t/2$ for the clearance of the lipid emulsion. The depression in phagocytosis of the lipid emulsion was also transient, with recovery observed at approximately 2.5 hr after surgery (Fig. 1). RE stimulation in regards to

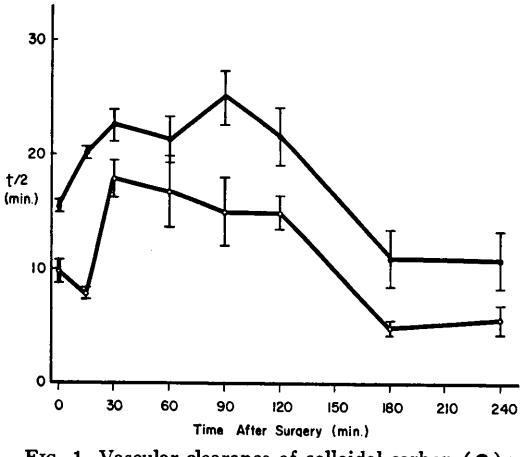

FIG. 1. Vascular clearance of colloidal carbon (●); and ^{131}I-lipid emulsion (○) as a function of postsurgery interval. Each point represents the average $t/2$ of 4–13 experimental animals. Data is presented as means ± standard error.

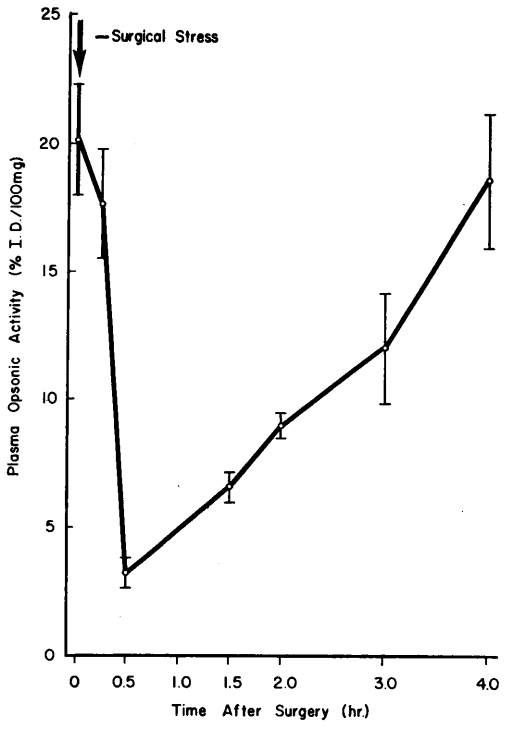

FIG. 2. Effect of surgery on circulating plasma opsonic activity as assayed by the ability of plasma to enhance hepatic phagocytosis. The zero-time point represents plasma opsonic activity prior to surgery. Each point represents the mean ± standard error of 4–6 experiments.

TABLE I. Comparative *in Vitro* Hepatic Phagocytosis[a] in Krebs–Ringer Phosphate, Normal Plasma, and Postsurgery Plasma.

Liver slice donor	Incubation medium	No. of incubation samples	Hepatic phagocytic uptake[b]	
			% ID/100 mg	Emulsion (μg/100 mg)
Normal	Krebs–Ringer phosphate	6	0.61 ± 0.12	12.2
	Normal plasma	10	13.45 ± 1.80	269.4
	Postsurgery plasma	6	3.22 ± 0.87	64.4
Postsurgery	Krebs–Ringer phosphate	6	0.43 ± 0.04	8.6
	Normal plasma	6	13.99 ± 1.88	279.8
	Postsurgery plasma	6	4.35 ± 0.87	87.0

[a] Incubations were conducted in 3 ml of heparinized medium with an incubation duration of 30 min. Postsurgery plasma was obtained 30 min after surgery.

[b] Injected dose (ID) was 2000 μg of the labeled lipid emulsion. Values are expressed as means ± standard error.

clearance of the lipid emulsion also occurred at 3- and 4-hr postsurgery.

In an attempt to evaluate the role of opsonin activity in the observed RE depression, plasma opsonic activity was determined at various intervals after surgery (Fig. 2). Plasma opsonic activity was slightly altered at 15 min postsurgery, fell rapidly to 16% of control values by 30 min, and remained significantly ($p<.001$) depressed during the entire period of phagocytic depression (Fig. 1). Recovery of phagocytic activity (Fig. 1) was closely associated with a restoration of circulating opsonin activity (Fig. 2).

Phagocytosis by normal liver slices in postsurgery plasma was significantly less than uptake in normal plasma (Table I). Furthermore, liver tissue obtained from RE depressed rats after surgery manifested minimal phagocytosis in postsurgery plasma, and a 33-fold stimulation of phagocytosis in normal plasma (Table I).

Presented in Fig. 3, is the reversal of RE depression after surgery with the intravenous injection of opsonized colloids. As shown, the $t/2$ for the clearance of a test dose of opsonized colloid in 30-min postsurgery rats was significantly faster than its clearance in a nonopsonized form. In this regard, opsonization reversed the carbon clearance depression and initiated a significant ($p<.001$) degree of recovery with regards to the vascular clearance of the lipid particles.

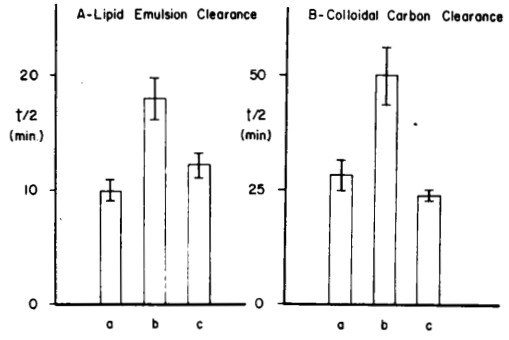

FIG. 3. Effect of opsonization on the $t/2$ for the vascular clearance of lipid emulsion (A) and colloidal carbon (B) at 30-min postsurgery. Means ± standard error for the following groups are presented: (a) controls injected with nonopsonized colloids; (b) surgically stressed injected with nonopsonized colloids; (c) surgically stressed injected with opsonized colloids. Each bar represents 5–13 separate *in vivo* experiments.

Discussion. Recent findings have adequately demonstrated the intimate role played by serum and plasma factors in the regulation of phagocytosis (14–17, 20). These studies have emphasized the role of opsonins in RE activity and the need to more fully comprehend the nature of opsonins and factors which can affect their biological activity.

The present investigation demonstrates a profound and transient depression of phagocytosis after surgery. These studies confirm and extend the previous observations by Saba

and Di Luzio (8) on RE alterations after operative stress. The observations by Donovan (7) on impaired clearance of denatured albumin in humans after surgery coupled with present findings support the concept that this response is neither species specific nor specifically related to a particular foreign colloidal surface.

Depression of carbon clearance was detectable sooner after surgery as compared to altered clearance of the lipid emulsion. The demonstration that opsonins for colloidal carbon are possibly different than those influencing phagocytosis of the lipid emulsion (14), suggests that the differential response may be related to a differential depletion of circulating plasma opsonic activity. Opsonin specificity for colloid phagocytosis (14, 21) is well documented.

Decreased circulating opsonic activity during a period of RE depression after surgery suggests an opsonin deficit as a major factor mediating the phagocytic depression. Indeed, the demonstration of normal hepatic phagocytosis in normal plasma by liver tissue removed from surgically stressed–RE depressed rats, and the reversal of RE depression *in vivo* by opsonization lends credence to this concept.

RE depression as a result of depressed circulating opsonic activity parallels the observations on starvation induced RE alteration (15) and colloid induced RE "blockade" (14, 20) which have been shown to be due to a depletion of opsonins. Restoration of opsonin activity during the RE recovery phase after surgery may reflect the synthesis and/or release of this factor into the blood from a specific tissue compartment (14). While RE depression is related to opsonin depression, RE stimulation at 3- and 4-hr postsurgery appears to reflect an altered functional state of the phagocytic cells, since an elevation of the circulating opsonin activity above normal levels did not exist at that time.

Depression of the reticuloendothelial system after surgery has important implications. Increased host susceptibility to tumor metastasis and infectious disease after surgery (9–13) may well reflect impaired macrophage recognition and ingestion of foreign matter due to the depression of opsonic activity. It is speculated that the level of host resistance to disease during the early postsurgical interval may be related to the blood level of opsonins and the reticuloendothelial system.

Summary. Surgery induced a significant and transient depression of the reticuloendothelial system (RES) as characterized by the impaired phagocytic clearance of colloidal carbon and the gelatinized "RE test lipid emulsion." RE recovery was observed at approximately 2.5-hr postsurgery, and a phase of RE stimulation existed by 3- and 4-hr postsurgery. Circulating opsonic activity was decreased during RE depression after surgery, and recovered toward control levels during the period of RE recovery. Comparative phagocytic and opsonic determinations suggested that RE depression after surgery was due to the decrease in opsonin activity. These findings support the concept that the increased host susceptibility to disease after surgery may be mediated in part by an alteration of the opsonic system and reticuloendothelial function.

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