Reticuloendothelial-Depressing Substance: Studies on the Mechanism of Action

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This study was carried out to evaluate the mechanism of action of a reticuloendothelial (RE)-depressing substance. This RE-depressing substance was obtained from the plasma of dogs subjected to 3 hr of intestinal ischemia. RE-depressing substance was partially purified by dialysis and reverse-phase column chromatography. The assay of RE-depressing activity was based on the depression of the rate of clearance of colloidal carbon from the blood of rats or mice. The effect of RE-depressing substance on three other RE system (RES) test particles (gelatinized lipid emulsion, formalinized sheep erythrocytes, and IgM-coated erythrocytes) was determined. RE-depressing substance did not affect the clearance rate or the organ localization of these three test particles. Therefore, RE-depressing substance affected only the clearance of colloidal carbon. Since platelet aggregation has been shown to contribute to the clearance of colloidal carbon, the effect of RE-depressing substance on platelet aggregation was evaluated. RE-depressing substance depressed in vitro platelet aggregation induced by ADP or collagen. It was concluded that the effect of RE-depressing substance on the clearance of colloidal carbon was due to a depression of platelet aggregation rather than to a depression of hepatic macrophage phagocytic function.

Key words: reticuloendothelial-depressing substance, RES clearance function, platelet aggregation

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INTRODUCTION

A reticuloendothelial (RE)-depressing substance has been shown to be present in the circulation following experimental hemorrhagic shock, intestinal ischemia, and thermal injury [4,5,24,26,27]. RE-depressing activity has also been detected in the blood of burned patients [27]. Blattberg et al demonstrated that this RE-depressing substance was a small peptide with a molecular weight of about 700 that depressed the RE system in a dose-related manner [3]. Studies from this laboratory have demonstrated that hydrolysis destroys RE-depressing-substance activity and that injection of RE-depressing substance depresses the RE system (RES) for less than 60 min [26,27]. Injection of RE-depressing substance was found to increase the mortality rate following experimental endotoxin and traumatic shock [6]. Therefore, it has been proposed that this substance contributes to the depression of RES phagocytic function and the impairment of host defense following shock and injury.

The mechanism by which RE-depressing substance acts is unknown. The detection of RE-depressing substance has been based exclusively on the depression of the clearance rate of colloidal carbon from the blood [3-5,24,26,27]. The only attempt to demonstrate any other effect of this substance was carried out by Blattberg and Levy using plasma from dogs subjected to hemorrhagic shock or intestinal ischemia. Dialysates of such plasma depressed the clearance rate of colloidal carbon in rats but did not affect the ability of peripheral blood leukocytes to phagocytose colloidal carbon or Escherichia coli in an in vitro assay [4].

The present study was carried out to evaluate some aspects of the possible mechanism of action of RE-depressing substance. A partially purified preparation of this substance was studied for effects on hepatic blood flow and the clearance rate of four different RES test particles (colloidal carbon, gelatinized lipid emulsion, formalinized sheep erythrocytes, antibody-coated erythrocytes). It was found that RE-depressing substance affected only the clearance of colloidal carbon. Since the clearance of colloidal carbon has been shown to be dependent, in part, on platelet aggregation [16,21], the effect of RE-depressing substance on platelet function was evaluated.

METHODS

Preparation of RE-Depressing Substance

The RE-depressing substance that was used throughout this study was obtained from dogs subjected to intestinal ischemia as previously described [26]. Dogs were anesthetized with 25 mg/kg sodium pentobarbital and the celiac, superior mesenteric, and inferior mesenteric arteries were clamped for 3 hr. Heparin (400 units/kg) was injected and blood was collected from the portal vein immediately after releasing the arterial clamps. Sham animals were treated in the same manner, except that the arteries were not clamped.

The plasma was dialyzed against 10 vol of water overnight to separate the low molecular weight RE-depressing substance from the bulk of the plasma proteins. The dialysate (containing the low molecular weight material) was flash evaporated. This material was then dissolved in distilled water adjusted to pH 2 with trifluoroacetic acid and loaded on a C₈-alkyl silica reverse-phase column. The material that bound to the column was eluted with 50% n-propanol adjusted to pH 2 with trifluoroacetic
acids. The bound fraction was concentrated by flash evaporation and the volume was adjusted to 1/12 the initial volume of plasma. This partially purified preparation of RE-depressing substance was used throughout the study. Ninhydrin-reactive nitrogen amino compound concentration in the fractions was determined by the method of Rosen using tyrosine standards [33]. Sodium content of the fractions was determined with flame photometry.

Assay of RE-Depressing Substance

RE-depressing-substance activity was determined as previously described by comparing the clearance rate of colloidal carbon in groups of rats or mice injected with either saline or a plasma extract [26,27]. Plasma extracts or saline were injected IV at a dose of 0.7 ml/100 g and 15 min later the clearance rate of colloidal carbon was determined. Colloidal carbon was injected at a dose of 4 mg/100 g in rats and at a dose of 8 mg/100 g in mice. Phagocytic index (clearance rate) values were compared by using the Student's t-test with the confidence level set at 95%. A significant decrease in phagocytic index in the assay animals injected with a plasma extract indicates the presence of RE-depressing activity in the plasma extract.

Hepatic Blood Flow

Since the liver is the principal organ that removes colloidal carbon from the blood, a sufficient decrease in hepatic blood flow could decrease its rate of clearance [32]. Hepatic blood flow was determined using the fractional clearance technique in rats [15]. Gelatinized lipid emulsion was used as the test particle and was injected at a dose of 5.0 mg/100 g in rats. At this dose, the rate-limiting factor in the clearance of this test particle is hepatic blood flow and not hepatic phagocytic function [35]. Blood samples were taken every 30 sec for 3 min from a cannulated carotid artery. The clearance rate constant, which is the proportion of the blood volume cleared of the test particle per minute, was multiplied by the blood volume to give the hepatic blood flow. Blood volume was estimated by extrapolating the clearance line to 0 time and assuming even distribution of the test particle throughout the vascular compartment.

Hepatic blood flow was determined in rats 15 min after the injection of 0.7 ml/100 g of partially purified RE-depressing substance or saline.

RES Clearance Function

In order to determine the specificity of the effect of RE-depressing substance on the RES, the clearance of colloidal carbon, gelatinized lipid emulsion, formalinized sheep red blood cells (SRBC), and erythrocytes coated with antieroerythrocyte IgM (EIGM) was determined following the injection of partially purified RE-depressing substance (0.7 mg/100 g) or saline. Each test particle was injected 15 min after the injection of RE-depressing substance. As described above, depression of the clearance of colloidal carbon was used as the basis for the assay of the presence of RE-depressing activity. Gelatinized lipid emulsion was prepared as previously described [35] and was injected at a dose of 50 mg/100 g in rats and 100 mg/100 g in mice. Formalinized SRBC were prepared as previously described [36] and injected at a dose of $3 \times 10^3/100$ g in rats and $5 \times 10^3/100$ g in mice. EIGM were rat erythrocytes coated with the IgM fraction of rabbit antirat erythrocyte serum. The dilution of the
IgM used to coat the erythrocytes was adjusted to obtain a 70–80% hepatic localization of EIgM in controls when injected at a dose of 2.9 × 10⁸/100 g. RES uptake of EIgM was determined only in rats. RES clearance function was assessed from the rate at which the test particles were removed from the blood (phagocytic index), as well as from the organ localization of the test particles. The clearance rate of each of the test particles, except EIgM, was determined over the first 10 min after injection. The clearance rate of EIgM was not determined since this test particle was not cleared in a monoexponential fashion. Localization in the liver, spleen, and lungs of each of the test particles, except colloidal carbon, was determined 10 min after injection. Organ localization of colloidal carbon was not determined.

**Preparation of Platelets**

Platelets for aggregation studies were obtained from male, ex-breeder Sprague-Dawley rats. Whole blood was drawn from animals anesthetized with ether via the inferior vena cava into a plastic syringe containing either 2 units/ml heparin (Upjohn Corp) for experiments requiring platelet-rich plasma (PRP) or 19% sodium citrate (2 parts citrate to 100 parts blood) for experiments with isolated platelets. Heparinized PRP was prepared by centrifuging the whole blood at 1,000g for 3–5 min. For isolated platelet studies, PRP was prepared by adding 2 ml of a buffered saline glucose solution (BSG-citrate: 0.117 M NaCl, 0.0136 M sodium citrate; 0.011 M glucose, 0.0086 M Na₂HPO₄; and 0.0016 M KH₂PO₄, pH 7.4) to 8 ml blood and centrifuging at 1,000g for 3 min. This procedure was repeated three to five times until more than 95% of all the platelets were isolated. The BSG-citrate-diluted PRP was layered over a discontinuous gradient consisting of 4 ml 20% Stractan II (St Regis Paper Co) which is an isosmotic arabinogalactan solution, and 3 ml 10% Stractan II in a 15-ml conical centrifuge tube (Falcon). The tube was centrifuged for 15 min at 3,500g, following which the plasma/BSG and 10% Stractan layers were aspirated and discarded. The platelet layer was then removed and washed free of Stractan by centrifugation for 8 min at 1,200g in excess BSG-citrate. The platelet pellet was resuspended in modified Tyrode solution (0.137 M NaCl, 0.031 M KCl, 0.012 M NaHCO₃, 0.006 M glucose, and 0.004 M NaH₂PO₄, pH 7.4). This isolation procedure is a modification of the procedure of Corash et al [12,13].Platelets were counted by the method of Bjorkman [2] on a hemocytometer with a phase-contrast microscope. All procedures were performed at room temperature in plastic or siliconized glassware.

**Platelet Aggregation**

Platelet aggregation was measured in vitro according to the method of Born [7] using a Payton Single Channel 300-B Aggregation Module (Payton Assoc, Inc) and recorded with an Omniscirbe B-5000 chart recorder (Houston Instruments). Aggregation was performed at a block temperature of 37°C and a stir bar speed of 900 rpm using a platelet concentration of 3 × 10⁸ cells/ml. A typical aggregation assay consisted of 100 μl of isolated platelets in Ca⁺⁺/Mg⁺⁺-free modified Tyrode solution, 10 μl CaCl₂ (ie, 0.001 M final concentration), 100 μl partially purified RE-depressing substance or sham solution, and appropriate volumes of aggregating agents (eg, 10 μl ADP). The assay mixture was brought up to 500 μl final volume using modified Tyrode solution. The platelet suspension in Tyrode with RE-depressing substance and calcium added was allowed to equilibrate during stirring for 2 min.
prior to the addition of the aggregating agent. Adenosine 5'-diphosphate (ADP) grade IX isolated from equine muscle was purchased from Sigma. A concentrated stock solution was prepared using phosphate-buffered normal saline and was frozen. Purified human α-thrombin was kindly provided by Dr John Fenton of the New York State Department of Health, Albany, NY. Arachidonic acid in the form of sodium arachidonate was obtained from BioData Corp. Homologous type I collagen for platelet aggregation was purified after the manner of Morin et al [31] from rat-tail tendons. Collagen purity was assessed via SDS polyacrylamide electrophoresis on a gradient gel (5-15%) under reducing conditions. Stock solutions of collagen were prepared in 0.01 M acetic acid, pH 4.3, and stored frozen at −20°C. For ADP-induced aggregation studies of isolated platelets, 200 μg (37 μl) of purified rat fibrinogen was added to each cuvette prior to ADP addition. Rat fibrinogen was purified by the method of Kazzal et al [22].

RESULTS
Partial Purification of RE-Depressing Substance

Plasma extracts from five dogs subjected to intestinal ischemia were combined and used for this study. Prior to fractionation with the reverse-phase column, the combined plasma extracts contained 57.6 μM/ml of ninhydrin-reactive material and 1,340 mEq/liter of sodium. The fraction that was retained on the reverse-phase column contained 2.4 μM/ml of ninhydrin-reactive material and 20 mEq/liter of sodium. RE-depressing activity was present in the plasma dialysates of each dog and was also detectable in the material that was retained on the column, but it was not present in the nonretained material. Material from three sham animals had no detectable RE-depressing activity.

Hepatic Blood Flow

Hepatic blood flow was not affected by RE-depressing substance. Hepatic blood flow in rats receiving saline was 2.08 ± 0.23 ml/min/g and in animals receiving RE-depressing substance was 2.12 ± 0.26 ml/min/g (n = 6 in each group). Similar results were obtained when plasma dialysates that contained RE-depressing activity were tested.

RES Clearance Function

RE-depressing substance depressed the clearance of colloidal carbon from the blood of rats and mice (Tables 1, 2). Neither the clearance rate nor the organ localization of gelatinized lipid emulsion, formalinized sheep erythrocytes, or ElgM were affected by RE-depressing substance (Tables 1, 2). RES uptake of ElgM was evaluated in rats only. Similar results were obtained when plasma dialysates that contained RE-depressing activity were tested.

Platelet Aggregation

In vitro aggregometry with heparinized rat PRP demonstrated an inhibitory effect of 100 μl RE-depressing substance (final total assay volume was 500 μl) on ADP- and collagen-induced platelet aggregation. Typical examples of these results are shown in Figures 1 and 2 for ADP and collagen, respectively. The inhibitory effect of RE-depressing substance was most apparent at low (0.1–1.0 μM final
TABLE 1. Effect of RE-Depressing Substance on the RES Clearance of Four Different Test Particles in Rats

<table>
<thead>
<tr>
<th>Test particle (dose)</th>
<th>Phagocytic index</th>
<th>Liver (% ID/organ)</th>
<th>Spleen (% ID/organ)</th>
<th>Lungs (% ID/organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colloidal carbon (4 mg/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.059 ± 0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RE-depressing substance</td>
<td>0.039 ± 0.002*</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Gelatinized lipid emulsion (50 mg/100 g)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.076 ± 0.009</td>
<td>66.28 ± 5.40</td>
<td>2.67 ± 0.15</td>
<td>10.21 ± 1.25</td>
</tr>
<tr>
<td>RE-depressing substance</td>
<td>0.081 ± 0.018</td>
<td>66.75 ± 1.11</td>
<td>1.97 ± 0.32</td>
<td>10.91 ± 1.19</td>
</tr>
<tr>
<td>Formalinized sheep erythrocytes (3 x 10⁹/100 g)</td>
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<tr>
<td>Control</td>
<td>0.110 ± 0.016</td>
<td>52.75 ± 1.74</td>
<td>2.58 ± 0.59</td>
<td>7.03 ± 0.86</td>
</tr>
<tr>
<td>RE-depressing substance</td>
<td>0.120 ± 0.008</td>
<td>48.07 ± 2.73</td>
<td>3.78 ± 0.41</td>
<td>5.97 ± 0.86</td>
</tr>
<tr>
<td>ElgM (2.9 x 10⁹/100 g)</td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td></td>
<td>70.90 ± 2.06</td>
<td>2.28 ± 0.15</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>RE-depressing substance</td>
<td></td>
<td>74.02 ± 1.65</td>
<td>2.43 ± 0.30</td>
<td>0.41 ± 0.23</td>
</tr>
</tbody>
</table>

*Partially purified RE-depressing substance was injected at a dose of 0.7 ml/100 g 15 min prior to the injection of the test particles. Organ localization was determined 10 min after injection of the test particles and is expressed as the percent of the injected dose (ID) per organ. Values are the mean ± SE with five to six animals per group.

*P < .05.

TABLE 2. Effect of RE-Depressing Substance on the RES Clearance of Three Different Test Particles in Mice

<table>
<thead>
<tr>
<th>Test particle (dose)</th>
<th>Phagocytic index</th>
<th>Liver (% ID/organ)</th>
<th>Spleen (% ID/organ)</th>
<th>Lungs (% ID/organ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colloidal carbon (8 mg/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.120 ± 0.022</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RE-depressing substance</td>
<td>0.047 ± 0.005*</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gelatinized lipid emulsion (100 mg/100 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.027 ± 0.005</td>
<td>40.80 ± 3.32</td>
<td>3.86 ± 0.62</td>
<td>7.72 ± 0.61</td>
</tr>
<tr>
<td>RE-depressing substance</td>
<td>0.028 ± 0.005</td>
<td>41.34 ± 2.80</td>
<td>4.59 ± 0.44</td>
<td>6.75 ± 0.42</td>
</tr>
<tr>
<td>Formalinized sheep erythrocytes (5 x 10⁹/100 g)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.041 ± 0.005</td>
<td>28.39 ± 1.86</td>
<td>2.17 ± 0.16</td>
<td>13.07 ± 0.84</td>
</tr>
<tr>
<td>RE-depressing substance</td>
<td>0.042 ± 0.004</td>
<td>28.03 ± 1.36</td>
<td>2.26 ± 0.26</td>
<td>14.23 ± 1.63</td>
</tr>
</tbody>
</table>

*Partially purified RE-depressing substance was injected at a dose of 0.7 ml/100 g 15 min prior to the injection of the test particles. Organ localization was determined 10 min after injection of the test particles and is expressed as the percent of the injected dose (ID) per organ. Values are the mean ± SE with six to seven animals per group.

*P < .05.
RE-Depressing Substance: Mechanism of Action

Fig. 1. Effect of RE-depressing substance (REDS) on ADP-induced platelet aggregation. Typical in vitro platelet aggregation curves for platelet-rich plasma are shown. RE-depressing substance or similarly prepared material from sham animals (100 μl) was added 2 min before the addition of ADP. The final volume was 500 μl. ADP was added (arrows) to obtain final ADP concentrations of 0.1 μM, 1.0 μM, and 10 μM. Platelet aggregation was inhibited by REDS at ADP concentrations of 0.1 μM and 1.0 μM.

correlation) concentrations of ADP (Fig. 1A, B). With rat PRP, unlike human PRP, these concentrations of ADP trigger a platelet aggregation response that reverses; ie, platelets disaggregate with time [37]. When higher concentrations of ADP (10 μM final) were used, inhibition of platelet aggregation by RE-depressing substance was not seen (Fig. 1C). The presence of 100 μl RE-depressing substance during aggregation induced by 0.1 μM ADP or 1.0 μM ADP resulted in a 17% and 10% inhibition of maximum aggregation, respectively.

Collagen-induced aggregation of rat PRP was also inhibited by RE-depressing substance (Fig. 2). The presence of 100 μl RE-depressing substance resulted in a 15% inhibition of the maximum aggregation induced by 20 μg collagen. Furthermore, platelets in RE-depressing substance showed a tendency to disaggregate after several minutes. Platelet disaggregation was not observed in control aggregations performed in the presence of sham material. When platelet aggregation was induced with higher doses of collagen (30 μg), the inhibitory effect of RE-depressing substance was lost or overwhelmed by an excess of aggregating agent.

To determine if the inhibitory effect of RE-depressing substance on platelet aggregation was dependent upon plasma factors, platelets were isolated from plasma for study. RE-depressing substance (100 μl/cuvette) inhibited ADP-induced aggregation of isolated platelets (Fig. 3). Therefore, RE-depressing substance appeared to have a direct effect upon the platelet. No inhibition was detectable when either purified thrombin (0.5 units) or sodium arachidonate (50 μg) was used to induce aggregation.

DISCUSSION

Previous studies from this and other laboratories have demonstrated that an RE-depressing substance is present in the blood following intestinal ischemia, hemorrhagic shock, and thermal injury [3–5, 24, 26, 27]. RE-depressing substance has been previously partially purified by gel filtration [3, 24], and in the present study it was
Fig. 2. Effect of REDS on collagen-induced platelet aggregation. Typical in vitro platelet aggregation curves for platelet-rich plasma are shown. REDS or similarly prepared material from sham animals (100 μl) was added 2 min before the addition of the collagen. Type I collagen from rat-tail tendons was added (20 μg; arrow). REDS inhibited collagen-induced platelet aggregation under these conditions.

partially purified by reverse-phase column chromatography. However, this low molecular weight substance has not been fully isolated or chemically identified.

The effect of depressing substances on the RES has been evaluated exclusively on the basis of their effects on the clearance rate of colloidal carbon. We have shown that a dose of RE-depressing substance that caused a depression in the clearance of colloidal carbon did not affect the clearance rate or hepatic uptake of three other RES test particles: gelatinized lipid emulsion, formalinized sheep erythrocytes, and ElgM. The inability of RE-depressing substance to depress the RES clearance of test particles other than colloidal carbon makes it unlikely that this substance acts through a general depression of macrophage phagocytic function.

The action of RE-depressing substance appears to be rather specific for the RES clearance of colloidal carbon. While the exact mechanism for the clearance of colloidal carbon is not fully understood, platelets contribute to the clearance, and the carbon particles are phagocytized by the hepatic macrophages [16,21]. The RES clearance of gelatinized lipid emulsion is highly dependent on the binding of this test particle to plasma fibronectin [34]. Formalinized sheep erythrocytes and ElgM activate complement and are probably cleared by interacting with complement receptors on the hepatic macrophages [17,28,30]. However, the formalinized sheep erythrocytes also interact with other macrophage receptors because this test particle is phagocytized, while ElgM that bind only to the complement receptors are released back into the blood through the action of C3b inactivator [17]. Therefore, each of these test particles is cleared by different mechanisms, and RE-depressing substance affects the clearance of only colloidal carbon.
RE-Depressing Substance: Mechanism of Action

It has been postulated that the elaboration of RE-depressing substance following injury and shock contributes to the depression of RES function that is observed under these conditions [3–6, 24, 26, 27]. The presence of RE-depressing substance could mediate the depression of the clearance of colloidal carbon, which is well known to be depressed following injury and shock [1, 40]. However, the RES uptake of gelatinized lipid emulsion, formalinized sheep erythrocytes, and E1gM has also been shown to be depressed after shock and injury [23, 25, 27, 29, 34]. Therefore, the RE-depressing substance used in the present study could not have been responsible for the depression of the RES uptake of these latter test particles following injury and shock. Since no attempt was made to remove all of the RE-depressing substance from the starting plasma, it is possible that some protein-bound or labile substances with RE-depressing activity could contribute to the RES depression following injury and shock. An example of such a labile substance is prostacyclin, which can depress the RES and which would have been inactivated by the extraction procedures employed in the present study [8, 39].

The mechanism of action of RE-depressing substance was further investigated in terms of effects on platelet aggregation, which participates in the clearance of colloidal carbon [16, 21]. In vitro aggregation studies showed that RE-depressing substance was capable of inhibiting ADP-induced platelet aggregation of both rat PRP and washed rat platelets. RE-depressing substance also inhibited collagen-induced aggregation in PRP (isolated platelets were not tested). The platelet response to thrombin and arachidonate was unaffected by the amounts of RE-depressing substance tested. However, dose-response studies have not yet been performed. Therefore,

![Graph](image_url)

**Fig. 3.** Effect of REDS on ADP-induced aggregation of isolated platelets. Typical in vitro platelet aggregation curves are shown. REDS or similarly prepared material from sham animals (100 μl) was added 2 min before the addition of ADP. ADP was added (arrow) to obtain a final concentration of 1.0 μM. REDS inhibited ADP-induced platelet aggregation under these conditions.
inhibition of platelet aggregation could have contributed to the effect of RE-depressing substance on the clearance of colloidal carbon from the blood.

The mechanism for the inhibition of platelet aggregation by RE-depressing substance has not been identified. However, based on the purification procedure employed, RE-depressing substance appears to be a hydrophobic molecule, thus allowing its insertion into the platelet plasma membrane, and thereby perturbing membrane function. Such membrane events may include receptor-ligand binding, subsequent signal transduction, the platelet dense-granule-secretion reaction, and platelet-platelet binding as mediated by surface glycoproteins.

While RE-depressing substance does not cause a generalized depression of RES clearance function, it did depress the clearance of colloidal carbon and platelet aggregation. Therefore, RE-depressing substance could increase susceptibility to infection with bacteria, which appear to be dependent upon platelet interactions for their clearance [10,11,14]. Platelets also aggregate in response to many types of foreign material including bacteria, endotoxin, viruses, and antigen-antibody complexes [18-20,38]. Additionally, platelet aggregation leads to the release of vasoactive amines, prostaglandins such as thromboxane A2, and chemotactic factors [9]. These observations suggest that a substance that interferes with platelet function could contribute to the impairment of host defense caused by injury.

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