cording to Lepow *et al*(4), develops in an euglobulin fraction precipitated at pH 5.5 and the inhibitor of C′ 1 esterase is found in small amount only in the euglobulin fraction precipitated at pH 5.5 or pH 5.2. These striking differences in the behavior of C′ 1 esterase and ATEe esterase and their inhibitors cannot be explained readily, especially because of the differences in methods of concentration of the enzymatic activities and of their inhibitors.

We have developed a method of concentration of ATEe esterase activity of human serum which is simpler than previously proposed technics and yields reproducible results. It should facilitate studies in the changes of activity of serum ATEe esterase in pathologic conditions. Preliminary studies have shown a significant decrease in serum ATEe esterase activity in parenchymal liver disease and in long standing diabetes mellitus. It cannot be established whether the decrease of ATEe esterase activity in disease is related to true depletion of enzymatic activity, or to increased inhibitor activity or to a combination of both factors. In any event, these observations agree with those of others who have reported a decrease in BAEe esterase activity in the serum of patients with cirrhosis of the liver(9). Much additional work will be needed, of course, before any conclusion may be reached on the significance of variations in the activity of ATEe esterase of serum in clinical disorders.

Summary. ATEe esterase activity can be precipitated from fresh human serum by dilution of the serum with distilled water and adjustment of pH between 6.8 and 7.4. The activity of this fraction of serum is not enhanced by addition of streptokinase. Its properties are closely similar to those of C′ 1 esterase activity of serum. Human serum also contains an inhibitor, which is precipitated from diluted serum by adjusting the pH to 5.2. The inhibitor combines reversibly with ATEe esterase. Preliminary studies show striking depletion of the activity of ATEe esterase in parenchymal liver disease and in long standing diabetes mellitus.

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The Fate of Foreign Red Cells in Mice with Altered Reticuloendothelial Function.* (30261)

S. H. MORROW† AND N. R. DI LUZIO

Department of Physiology and Biophysics, University of Tennessee Medical Units, Memphis

Previous studies have demonstrated that it is possible selectively to stimulate phagocytic function, due to a pronounced increase in number and activity of Kupffer cells, by intravenous administration of the neutral polysaccharide glucan(1). Conversely, it has been demonstrated that intravenous administration of a methyl palmitate emulsion results in a profound depression of phagocytic function due, in part, to a failure in Kupffer cell phagocytosis(2).

Associated with an enhanced phagocytic function in glucan-treated mice, was a hyper-

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† U.S.P.H.S. Student Research Assistant.

immune state as indicated by the stimulation of the primary and secondary immune responses(2,3). This response was similar to the enhanced immune response observed to occur with other RE stimulants(4). These findings, coupled with the association of a depressed primary and secondary immune response in phagocytically depressed methyl palmitate-treated mice, suggested a unique relation between the functional state of the RES and immune phenomenon(3).

The importance of the RES to immune phenomena was also demonstrated by the inability to transplant effectively homologous and heterologous bone marrow in lethally irradiated mice which had a hyperfunctional state of the RES induced prior to radiation exposure(5). The decreased survival of homologous and heterologous chimeras treated with glucan(6) also supports the concept that the RES is a factor in immune mechanisms. In further support of this concept, Balner has concluded that the accelerated rejection of male skin isografts by BCG treated, RE hyperfunctional female mice was due to an enhanced immune response(7). Diller *et al* have reported that glucan induced marked regression of foreign tumors, but had no effect on spontaneous, or isologous tumors, a finding completely compatible with the hyperimmune state induced by glucan administration(8). Similar observations of retardation in tumor growth and prolongation of survival time of mice have been noted following administration of a variety of RE stimulating agents(9).

In an attempt to ascertain the mechanism of immunological depression in methyl palmitate-treated mice and immunological stimulation in glucan-injected mice, the present study was undertaken to evaluate the behavior of foreign red cells in mice with altered RE function.

Methods. Fifty ml of freshly obtained sheep blood was collected in 10 ml of ACD solution (Abbott Laboratories, Oak Ridge, Tenn.) in the presence of 510 μc of sodium chromate (Cr^{51}). The mixture was incubated for 60 minutes at room temperature after which the blood was centrifuged and the red cells washed with 0.9% NaCl until the activity of the supernatant was negligible.

The red cells were counted and 1×10^9 red cells administered intravenously to either control, RE hypofunctional or RE hyperfunctional C57BL/6J mice. The mice used in this study were not immunized against sheep red cells and no circulating hemolysin was measurable in either control, methyl palmitate or glucan-treated mice.

At predetermined times after administration of sheep erythrocytes, the mice were bled from the retro-orbital venous plexus and 0.1 ml of blood was pipetted into 3.0 ml of 0.1% sodium carbonate solution for counting in a deep well scintillation detector. The mice were killed at various intervals and liver, lung and spleen removed and radioactivity determined.

Reticuloendothelial stimulation was induced by intravenous administration of a saline suspension of glucan in the amount of 1 mg/20 g for a period of 5 days(10). Control mice received saline intravenously.

A depressed RE functional state was induced by intravenous administration of methyl palmitate (Applied Science Laboratories, State College, Pa.) in the amount of 35 mg/20 g 24 hours prior to red cell administration. The methyl palmitate emulsion was prepared by sonification of a mixture of methyl palmitate suspended in 0.10% Tween 20 in 5% dextrose solution. Sonification materially improved the emulsion in respect to reduced particle size, which was approximately 1 μ, and resulted in a marked reduction or complete absence of toxicity due to pulmonary microembli when compared to emulsions prepared by microhomogenization (2).

The intravascular clearance of colloidal carbon (Gunther Wagner, Hannover, Germany, preparation C11/1431a) was also determined in other control, RE hyperfunctional and RE hypofunctional mice by previously described procedures(11). Statistical analysis of the data was performed at the 95% confidence level by the t-test for difference between means.

Results. The influence of glucan administration on organ weight and phagocytic activity, as indicated by the vascular clearance

TABLE I. Influence of Glucan and Methyl Palmitate on Phagocytic Function as Measured by Vascular Clearance of Colloidal Carbon.*

Group and No.	Body wt, g	Organ wt, as % body wt			Colloidal carbon t/2, min
		Liver	Lung	Spleen	
Control (7)	21.1 ± .4	5.5 ± .1	.7 ± .1	.4 ± .02	17.3 ± 2.4
Glucan (7)	20.0 ± .7	7.8 ± .6	1.2 ± .04	1.5 ± .3	2.2 ± .3
Control (7)	17.6 ± .5	5.8 ± .2	.8 ± .02	.3 ± .01	18.0 ± 1.3
Methyl palmitate (8)	17.8 ± .3	6.5 ± .2	.9 ± .02	.2 ± .01	43.7 ± 5.6

* Values are expressed as means ± standard errors of mean.

of colloidal carbon, is presented in Table I. In agreement with all previous observations (1,3,5,6,10,12,13), it was observed that glucan produced significant increases in the weight of liver, lung, and spleen. This increase in weight of those organs which contain large population of RE cells was associated with a profound enhancement in phagocytic function, as indicated by the 8-fold increase in colloidal carbon removal.

TABLE II. Enhanced Blood Clearance of Cr^{51}-Labeled Sheep Erythrocytes in RE-Hyperfunctional Mice.*

	Blood radioactivity†			t/2, min
	10 min	30 min	60 min	
	(4)‡	(9)	(5)	
Saline	73.6 ± 7.5	36.3 ± 4.2	15.1 ± 1.9	20.8
	(5)	(9)	(4)	
Glucan	25.3 ± 6.2	1.5 ± .6	3.7 ± .4	5.0

* Values expressed as means ± standard error.
† Expressed as % of injected dose per total blood volume.
‡ Values in parentheses are No. of animals per group.

The marked depression of phagocytic activity in methyl palmitate-treated mice is reflected in the colloidal carbon half-time of 44 minutes, in contrast to the 18 minutes half-time observed in the control group, which received the Tween 20-dextrose solution. In essential agreement with previous observations(2) body and organ weights were not significantly altered in methyl palmitate-treated mice.

The vascular clearance of Cr^{51}-labeled sheep red cells was profoundly enhanced in glucan-treated mice, with the intravascular half-time being 5.0 minutes, in contrast to 20.8 minutes in the saline-injected control group (Table II).

The increased vascular removal of the foreign red cells was due to an enhanced liver uptake (Table III). The hepatic uptake of foreign red cells by glucan-treated mice, in relation to controls, was increased 140% at 30 min.

At one hour, the glucan-treated mice showed an 11% decrease in activity, while the control mice showed a 29% increase over their respective 30-minute values. Despite these changes, the hepatic radioactivity was still 63% greater in the glucan-treated group at the 1-hour period. A similar degree of hepatic radioactivity was observed at 24 hours in the RE stimulated and control group.

In contrast to the enhanced liver uptake, lung removal of red cells in RE hyperfunctional mice was decreased approximately 60% at 30 minutes and 1 hour. The uptake of the radioactive foreign red cells by spleen was not altered from control values at any of the time periods in the glucan-treated group, despite a mean 2-fold increase in spleen weight.

TABLE III. Distribution of Cr^{51}-Labeled Sheep Erythrocytes in RE-Hyperfunctional Mice.*

Time	30 min		1 hr		24 hr	
Group and No.	Saline (6)†	Glucan (5)	Saline (5)	Glucan (4)	Saline (6)	Glucan (7)
Body wt, g	22.2 ± 1.2	21.0 ± .6	22.4 ± .8	20.0 ± .5	20.0 ± .7	18.7 ± .8
Liver	30.8 ± 2.9	73.4 ± 1.5	40.0 ± 2.3	65.0 ± 2.4	47.2 ± 3.4	47.5 ± .7
Lung	7.7 ± .6	2.7 ± .1	6.2 ± .9	2.6 ± .5	.2 ± .1	.9 ± .1
Spleen	9.7 ± .9	8.0 ± 1.0	8.8 ± .5	8.4 ± .8	6.7 ± .6	9.7 ± .5

* Values, as % of injected dose per organ, are expressed as means ± standard error.
† Values in parentheses are No. of mice/group.

TABLE IV. Blood Clearance of Cr^{51}-Labeled Sheep Erythrocytes in RE-Hypofunctional Mice.*

Group	Blood radioactivity† 5 min	20 min	30 min	60 min	t/2, min
Tween	(5)‡ 81.9 ± 3.7	(4) 44.0 ± 3.7	(4) 28.0 ± 5.6	(4) 16.8 ± 5.3	16.5
Methyl palmitate	(4) 98.8 ± 3.3	(5) 95.8 ± 4.9	(3) 86.0 ± 2.5	(4) 81.5 ± 4.8	209

* Values expressed as means ± standard error.
† Expressed as % of injected dose per total blood volume.
‡ Values in parentheses are No. of animals/group.

TABLE V. Distribution of Cr^{51}-Labeled Sheep Erythrocytes in RE-Hypofunctional Mice.*

Time	1 hr		24 hr	
Group and No.	Tween (5)†	Methyl palmitate (5)	Tween (6)	Methyl palmitate (5)
Body wt, g	20.2 ± .2	19.0 ± .8	19.2 ± .7	19.4 ± .8
Liver	37.4 ± 4.3	7.7 ± .5	24.1 ± 2.0	35.8 ± 2.0
Lung	3.3 ± .6	4.8 ± .2	.3 ± .02	4.6 ± 1.2
Spleen	10.3 ± 1.8	.6 ± .1	8.9 ± .9	3.2 ± .5

* Values, as % of injected dose per organ, are expressed as means ± standard error.
† Values in parentheses are No. of mice/group.

In general, a converse situation occurred in methyl palmitate-treated mice, where a 12-fold increase in the vascular half-time of sheep red cells was observed (Table IV). The decreased vascular clearance was associated with an extreme inhibition in the removal of the foreign red cells by liver and spleen (Table V). Liver radioactivity relative to control values was decreased 79% and 49% at 1 and 24 hours respectively in the RE impaired group. The splenic uptake of red cells in methyl palmitate-treated mice was, in respect to control values, decreased 94% at 1 hour and 64% at 24 hours. The localization of the labeled erythrocytes in lung was significantly greater in the methyl palmitate group at both intervals. Relative to the 1-hour values, a 90% decrease was observed in lung radioactivity at 24 hours in the control group; however, the RE depressed mice showed no loss in activity at 24 hours.

In an effort to determine the duration of splenic depression, additional groups of 5 Tween and 5 methyl palmitate-treated mice were killed either at 48 or 72 hours after red cell administration. Relative to control values, spleen radioactivity continued to manifest depression in phagocyticly incompetent mice with the mean and standard error at 48 hours being 7.7% ± 0.6 of the injected dose in the spleen of the control group, and 2.3% ± 0.4 in the methyl palmitate group. At 72 hours the radioactivity in the spleen of control mice was 5.9% ± 0.3 and the RE depressed mice 3.5% ± 0.5. In contrast, liver radioactivity was not significantly different at 48 and 72 hours, being 21.1% ± 2.9 and 19.8% ± 2.3 of the injected dose in Tween control mice. Similar values were obtained in the RE depressed group with 25.1% ± 0.5 and 22.7% ± 0.5 of the injected activity recovered in liver at 48 and 72 hours respectively.

Discussion. In agreement with previous observations, administration of a methyl palmitate emulsion intravenously produced a profound depression in the phagocytosis of of colloidal carbon(3,5), while glucan enhanced its vascular clearance(1,12). The present studies indicate that viable non-sensitized foreign red cells have a fate similar to colloidal carbon(12) or a lipid emulsion(12, 13) with an enhanced liver uptake in RE hyperfunctional mice. These studies further denote that the enhanced vascular clearance of colloids and particulate material observed in glucan-treated animals is primarily associated with an enhanced hepatic activity.

Halpern *et al*(14), employing pigeon red cells found that Kupffer cells of the liver

played a major role in the phenomenon of foreign red cell removal. They also demonstrated that the intravascular clearance rate of pigeon red cells in mice followed an exponential function similar to that observed with other colloids. The present studies with incompatible sheep red cells are in complete accord with these observations.

The removal of the spleen is associated with elimination of the hemolysin response to intravenously injected red cells(15,16). The present data indicate that the enhanced immune response to sheep red cells in glucan-treated mice was not due to increased spleen uptake since the localization of tagged red cells in spleen was essentially unaltered in RE hyperfunctional mice. The rapid loss of radioactivity from the liver of glucan-treated mice is, however, suggestive of an enhanced processing of the particulate antigen by increased intracellular digestion of the red cell(17,18). However, since antibody formation to intravenously administered sheep red cells is absent in RE hyperfunctional splenectomized animals(15,16), the ultimate contribution of the spleen to enhanced antibody formation in glucan-treated mice is obvious.

The effect of methyl palmitate induced RE depression on the vascular clearance of unaltered sheep red cells is similar to the results obtained by Buchanan and MacGregor(19). These investigators, employing ethyl palmitate to induce RE depression and studying the survival of human red cells in mice, found an 8-fold prolongation in the vascular clearance of Cr^{51} tagged red blood cells which was associated with a reduction of liver and spleen activity.

Since splenectomy profoundly reduces the hemolysin response to intravenously injected red cells(15,16), it is suggestive that failure of antibody formation previously observed in methyl palmitate-treated mice(2,3) may be due not only to a failure in hepatic macrophage uptake and processing of antigen(17, 18) but also a co-existing splenic defect.

These studies demonstrate that alterations in hemolysin formation previously observed in mice with altered RE function are associated with at least two events; namely, an alteration in vascular clearance and the organ distribution of the foreign cell.

Frei *et al* have reported that the fraction of the antigen susceptible to phagocytosis by macrophage induces the immune response while the least phagocytizable fraction induces tolerance. These studies also indicated that antibody formation can be induced only when the antigen is metabolized in the macrophage and a hypothetically active fragment is transferred to the small lymphocytes(20).

Schoenberg *et al*(21) have demonstrated a direct cytoplasmic connection between macrophages and potential antibody producing cells, and suggested a transfer of cytoplasmic substance from macrophages to cells of the lymphocytic series. Whether in RE hyperfunctional states such as interaction of macrophages and potential antibody forming cells is enhanced, accounting for the hyperimmune state, or that a converse situation occurs in RE depressed states, resulting in depression in the transfer of antigenic material, remains to be evaluated.

Jenkin and Rowley(22,23), demonstrated that the phagocytosis of a variety of particles requires the presence of serum factors or opsonins. Similarly, they observed that serum from RE stimulated mice possessed greater opsonic activity for both inert and living particles(23). Whether the present findings of altered phagocytosis and localization of foreign red cells in mice with altered RE function reflect changes in opsonin levels remains to be ascertained.

While additional studies will be essential to elucidate further the altered immunological behavior of RE stimulated or RE depressed mice to particulate antigens, the present studies demonstrate the ability to alter the removal and site of deposition of foreign colloidal and particulate antigenic materials by chemical means which alter the metabolic behavior of macrophages. This technique should be of appreciable value in the understanding of a variety of metabolic and immune processes.

Summary. The vascular removal and organ uptake of incompatible sheep red cells labeled with Cr^{51} was studied in mice with either normal, hyperfunctional or hypofunctional

phagocytic states in an effort to ascertain the mechanism of their altered immune responses. RE hyperfunction, induced by glucan, was associated with an enhanced vascular removal of the foreign red cells due exclusively to an increased liver uptake. Conversely, RE depression, induced by methyl palmitate administration, was associated with decreased vascular clearance due to a failure in phagocytosis by both liver and spleen. The present studies not only indicate that the previously observed alterations in hemolysin formation in mice with altered RE activity is associated with both a change in vascular clearance and organ distribution of the foreign red cell, but also demonstrate the ability to control the fate of colloids and particulate antigens by purified chemicals which influence the behavior of macrophages.

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Use of Heparin in Distinguishing Plasma Kallikrein from Pf/dil. (30262)

DAVID J. MCCONNELL, LAWRENCE J. KAGEN AND ELMER L. BECKER
(Introduced by L. S. Baron)
Department of Immunochemistry, Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

The plasma permeability globulins, kallikrein and Pf/dil, have recently been isolated from human serum and shown to be distinct from each other(1,2). Plasma kallikrein is a gamma globulin while Pf/dil is a beta globulin. The activity of both is inhibited by soybean trypsin inhibitor, but not by antihistamines. Both plasma kallikrein and Pf/dil are apparently members of the plasma kinin-forming system(3). Kallikrein releases kinin from both heated and unheated plasma by acting directly on kininogen. Pf/dil will release kinin only from unheated plasma apparently by activating plasma kallikrein or some other substance which then releases kinin.