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Properties of the Reticuloendothelial System of the Cat[1] (38274)

RICHARD J. GORCZYNSKI[2] AND ALLAN M. LEFER[3]

Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia 22901

Physiologic data on the reticuloendothelial system (RES) has been derived primarily from studies in the rat, mouse, and dog (1). These studies have emphasized the importance of serum opsonins in the normal function of the RES (2–5). However, little information is available characterizing the RES of the cat. Since the cat is often used as an experimental model for the study of circulatory shock, and since the RES is known to play an important role in the pathogenesis of hemorrhagic shock, information of RES function in cats during shock is of considerable interest. Therefore, this study was undertaken to determine the normal function of the RES of the cat in terms of clearance kinetics and opsonin dependency. Moreover, data on the effects of hemorrhagic hypotension on RES clearance of particulate matter is also reported.

Materials and Methods. Fasted adult cats, of either sex, weighing 2.0–3.5 kg were anesthetized with sodium pentobarbital (30 mg/kg iv). Polyethylene catheters were placed in the left external jugular vein and right common carotid artery for the measurement of central venous pressure and mean arterial blood pressure, respectively. In addition, catheters were placed in the left femoral vein for the administration of drugs or colloids and in the left femoral artery for sampling of arterial blood. All cats were given 1,500 U of heparin (Upjohn, bovine lung) prior to the experimental procedure. In one group of cats, hemorrhagic hypotension was induced by a reservoir technique previously described (6). These cats were bled to a mean arterial blood pressure of 40–45 mm Hg for 2 hr followed by reinfusion of all shed blood.

Two phagocytic test colloids were utilized in this study. Gelatin-stabilized colloidal carbon was employed in hemorrhagic hypotension experiments and a gelantinized tritiated, "RE-test-lipid emulsion" (2) was used for the demonstration of serum opsonin in both *in vivo* and *in vitro* experiments. Colloidal carbon (C11-143a; Gunther Wagner, Hanover, Germany) was centrifuged at 3,000*g* for 15 min to eliminate large particles. Subsequent to centrifugation, the carbon was diluted with a 1% gelatin solution to yield a solution having a carbon concentration of 50 mg/ml. The carbon solution was then incubated at 37° for 20 min prior to use. The "RE-test-lipid emulsion" was prepared as the anhydrous base, according to previously described techniques (2) with glycerol, peanut oil, and soya lecithin in a weight ratio of 10:10:1, respectively. The peanut oil was labeled with tritiated glyceryl trioleate. Prior to use, a neutralized gelatin (Nutritional Biochemical Corp., Cleveland, Ohio) supplemented with a 5% dextrose and water solution was added to yield either a 1 or 50% emulsion. Emulsions employed *in vivo* had a gelatin concentration of 0.3% and those employed *in vitro* had a 0.1% gelatin concentration. All emulsions were incubated at 37° for 20 min prior to use.

Preopsonization of the 50% test-lipid emulsion was accomplished by the incubation of the emulsion for 15 min at 37° with heparinized autologous plasma. One milliliter of plasma was used for each 200 mg of lipid base.

Further demonstration of the ability of plasma factors to enhance phagocytosis was accomplished by utilizing an *in vitro* tissue-slice technique developed by Saba and coworkers (3, 4). Cat liver slices (200–250 mg) prepared with a Stadie–Riggs tissue slicer were incubated in Krebs–Henseleit bicarbonate buffer (pH 7.35) containing 100 U heparin, 2,000 μg of the labeled lipid emulsion (1% emulsion), and varying amounts of normal heparinized cat plasma in

[1] Supported in part by NIH Research Grant No. HL-14277.

[2] NHLI Predoctoral Trainee.

[3] Present Address: Department of Physiology, Jefferson Medical College, Philadelphia, PA. 19107.

a total volume of 3 ml. All samples were incubated at 37° for 30 min under a gas phase of 95% O_2 and 5% CO_2 in a gyrotory shaker bath. Kupffer cell phagocytosis was measured by extraction of the labeled lipid with a 2:1 chloroform:methanol mixture from homogenized tissue slices and is expressed as the percent of the injected dose phagocytosed per 100 mg tissue. Plasma heated to 56° for 30 min was also tested in tissue slices.

In vivo phagocytic function and the importance of opsonins in phagocytic function was evaluated by determining the rate of intravascular clearance of the 50% lipid emulsion at zero time and again 3 hr later in two groups of cats. The first group of cats received the normal unopsonized lipid emulsion and the second group received the preopsonized lipid emulsion prepared as described above. The emulsion was administered at a dose of 700 mg/kg (16 μCi/kg) via the cannulated femoral vein as a bolus injection, and serial arterial blood samples were obtained at 2, 3, 4, 5, 7,, 9, 12, and 15 min after injection. The lipid radioactivity was determined in each blood sample by extraction in a 2:1 chloroform:methanol mixture, and phagocytic clearance rate was determined semilogarithmically by plotting radioactivity (cpm) of blood against time. Such plots yielded straight lines having a slope which is the phagocytic index (k). Clearance half-times ($t/2$) were determined from the relationship: $t/2 = 0.301/k$.

The lipid radioactivity of the chloroform:methanol extracts from both blood samples and tissue-slice samples was determined after evaporation of the solvents by liquid scintillation spectrometry.

The effects of hemorrhagic hypotension on intravascular clearance of colloidal carbon was determined in two additional groups of cats. Cats subjected to hemorrhagic hypotension underwent a clearance test at zero time (i.e., just prior to bleeding) and again 2 hr later (i.e., just following reinfusion). Nonhemorrhaged control cats underwent clearance tests at equivalent times. Colloidal carbon (40 mg/kg) was injected into the femoral vein, and serial arterial samples from the femoral artery were obtained at 2, 3, 4, 5, 7, 9, 12, and 15 min after each injection. Carbon concentration was determined spectrophotometrically at 675 nm after each blood sample was diluted 1:80 in 0.1% sodium carbonate solution and plotted semilogarithmically against time to determine the phagocytic index and clearance half-time.

Results. Table I summarizes the initial (0 hr) and final (3 hr) phagocytic indices (k) and clearance half-times for animals receiving either the unopsonized or the preopsonized test lipid emulsion. Animals receiving the unopsonized lipid emulsion exhibited rapid clearance rates initially with a half-time of 4.4 ± 0.4 min, but 3 hr later exhibited extremely depressed clearance rates with a half-time of 48.2 ± 13.3 min. This depressed clearance rate at 3 hr could be partially prevented by pretreatment of the emulsion with normal cat plasma (i.e., preopsonization) before injection as shown in Table I for animals receiving the preopsonized lipid emulsion. This normalizing influence of preopsonization of the emulsion occurred despite the fact that preopsonization exerted little effect on the initial clearance rate in this group of animals as compared to the initial values for animals receiving

TABLE I. Phagocytic Clearance of RE-Test-Lipid Emulsion: Effects of Preopsonization.

	Phagocytic index (k)		
	Initial (0 hr)	Final (3 hr)	Δ (Initial - Final)
Unopsonized (5)[b]	0.0714 ± 0.0056[c]	0.0081 ± 0.0025	0.0633 ± 0.006[a]
Preopsonized (4)	0.0587 ± 0.0106	0.0253 ± 0.0052	0.0281 ± 0.0107
		Clearance half-time (min)	
	Initial (0 hr)	Final (3 hr)	Δ (Initial - Final)
Unopsonized (5)	4.4 ± 0.4	52.0 ± 12.0	48.2 ± 13.3[a]
Preopsonized (4)	6.2 ± 1.4	11.8 ± 2.5	6.3 ± 1.8

[a] < 0.01 compared to preopsonized Δ value.

[b] Numbers in parenthesis are numbers of animals in each group.

[c] All values are means ± SE.

unopsonized lipid emulsion.

In vitro phagocytosis was also enhanced by normal cat plasma as shown in Fig. 1, which illustrates the results of the cat liver slice experiments. Incubation of cat liver slices in a medium devoid of normal plasma resulted in minimal phagocytosis whereas incubation of slices in 100% normal plasma resulted in enhanced phagocytosis. Furthermore, the effects of added plasma on phagocytosis was dose dependent over the range studied; progressively larger amounts of plasma in the medium being associated with increased phagocytosis. The results indicate that the factor(s) in plasma responsible for the enhancement of phagocytosis by liver slices was thermolabile. Heat treatment of normal plasma at 56° for 30 min completely blocked the effect of normal plasma on enhancement of phagocytosis. Samples incubated in heated plasma exhibited a degree of phagocytosis which was not significantly different from samples incubated in plasma-free medium.

The effects of hemorrhagic hypotension on intravascular phagocytic clearance of colloid carbon are shown in Table II. The initial (0 hrs) and final (2 hrs) phagocytic index and clearance half-time in nonhemorrhaged controls were not significantly different from each other. This is contrasted to the results obtained with hemorrhaged animals. Although the initial values for clearance taken before hemorrhage were similar to those of the nonhemorrhaged group, the post-hemorrhage clearance rates of these animals was depressed by over 50%. This difference was statistically significant ($P < 0.01$).

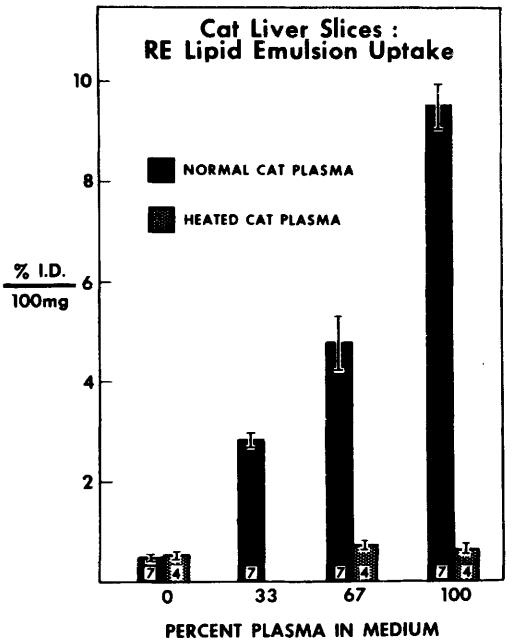

FIG. 1. Cat liver slice phagocytosis of RE-test-lipid emulsion with increasing amounts of normal (solid bar) and heated (56°, 30 min) (hatched bar) cat plasma. Phagocytic uptake is expressed as percent of the injected dose (2,000 μg) phagocytosed per 100 mg tissue. Bar heights represent means ± SEM. Numbers within bars indicate number of slices in each group.

Discussion. Although additional work is necessary to fully characterize the reticuloendothelial system of the cat, the present data indi-

TABLE II. Effects of Hemorrhagic Hypotension on Phagocytic Clearance of Gelatin-Stabilized Colloidal Carbon.

	Phagocytic index (k)		
	Initial (0 hr)	Final (3 hr)	Δ (Initial - Final)
Non-hemorrhaged (5)[d]	0.0376 ± 0.0070[c]	0.0331 ± 0.0066	0.0045 ± 0.0016
Hemorrhaged (5)	0.0439 ± 0.0031	0.0200 ± 0.0028	0.0239 ± 0.0020[a]
	Clearance half-time (min)		
	Initial (0 hr)	Final (3 hr)	Δ (Initial - Final)
Non-hemorrhaged (5)	8.4 ± 1.2	10.4 ± 1.7	2.0 ± 0.8
Hemorrhaged (5)	7.2 ± 0.5	16.6 ± 2.8	9.4 ± 2.8

[a] $P < 0.001$ compared to nonhemorrhaged value.
[b] $P < 0.02$ compared to nonhemorrhaged value.
[c] Values are means ± SE.
[d] Numbers in parenthesis are number of animals in each group.

cate that RES function in the cat does not differ greatly from that of the dog and rat. Thus, the normal values for the phagocytic index and clearance half-time of both the RE-test-lipid emulsion and colloidal carbon are comparable to values obtained by other investigators in the dog and rat (2, 5, 7). Furthermore, the phagocytic response of cat liver slices *in vitro* is similar to that reported for rat liver slices by Saba and coworkers (3, 4).

The phenomenon of blockade of the RES is well-known and has been explained in the past as due to the physical saturation of phagocytic cells by ingested colloid particles (7, 8). Recently, this interpretation has been challenged by investigators who have shown that RES blockade is associated with depletion of serum factors (i.e., opsonins) which support or enhance intravascular phagocytosis (2, 9). Our results are consistent with the opsonin depletion hypothesis. The initial clearance test *in vivo* using the RE-test-lipid emulsion is essentially a blockading dose of colloid as is evidenced by the final depressed clearance rates seen in animals receiving the unopsonized emulsion. If simple physical saturation of RE cells is totally responsible for this blockage, then preopsonization of the emulsion with normal plasma should have no effect on the subsequent clearance of this emulsion when administered 3 hr after the blockading dose. However, preopsonization significantly enhanced clearance of this dose of the lipid emulsion. The fact that preopsonization did not result in complete reversal of the blockade may be explained in a number of ways. First, it is possible that RES blockade is due to a combination of physical saturation of RE cells and to depletion of serum opsonins. If that is the case, prior opsonization of the test colloid would only result in partial improvement of clearance. Another possibility is that the amount of plasma used to preopsonize the emulsion was insufficient to fully opsonize all particles present. Since the colloid can be cleared by opsonin-dependent (4, 10) processes if it is gelatinized, and by opsonin-independent mechanisms if not gelatinized, there is no way to differentiate between these hypotheses using the techniques employed in this study.

Additional evidence for the existence of phagocytosis promoting factors in normal plasma in the cat can be derived from the cat liver slice experiments. Kupffer cell phagocytosis was minimal in plasma-free medium and was increased by addition of plasma in the medium. Similar results were observed by Saba and coworkers in the rat for phagocytic uptake of both the RE-test-lipid emulsion and colloidal gold (3, 4). Furthermore, heat treatment destroyed the capacity of plasma to promote phagocytosis of the lipid emulsion by the liver slices. Heat treatment probably denatured serum opsonin although it is possible that heat contributed to the activation or formation of factors which inhibit phagocytosis.

Finally, our results on the effects of hemorrhagic hypotension on the *in vivo* clearance of colloidal carbon is comparable to that observed by Blattberg *et al.* (11) in dogs, who also demonstrated significant reticuloendothelial depression after 2 hr of hemorrhage. RES depression is commonly seen in various types of circulatory shock although the mechanism of the RES depression is not clear. One possible mechanism is that shock may result in the depletion of the normal titer of opsonin which then impairs the clearing capacity of the RES. Saba (12) has obtained evidence that this type of mechanism is operative in the case of animals exposed to moderately traumatic surgery. Another possibility for the depressed RES function in circulatory shock is that a reticuloendothelial depressant substance (RDS) isliberated into the circulation which acts to depress phagocytosis. Blattberg and Levy (10, 13) have described such a shock factor in several types of shock and suggest that the substance is a peptide (14).

Since the opsonins are plasma proteins and the RDS a peptide, it is possible that the severe degree of plasma proteolysis (15) which occurs in shock contributes both to a depletion of plasma opsonins and to the production of RES depressant factors. Thus opsonic proteins may be cleared by circulating proteases in shock (15) and the result of this proteolysis may be the liberation of toxic peptides one or more of which depresses RES function.

Summary. Intravascular phagocytic clearance of colloidal carbon and a "RE-test-lipid emulsion" was comparable to that reported for the rat, mouse, and dogs. Clearance of a RE-test-lipid emulsion 3 hr after injection of an initial dose was markedly depressed. However, preopsonization of the emulsion with normal cat plasma prior to injection significantly prevented this depression. Phagocytosis of cat liver slices *in vitro* was found to be dependent on the amount

of normal cat plasma in the incubation medium. As the concentration of plasma in the incubation medium was increased, the degree of phagocytic uptake of the RE-test-lipid emulsion increased proportionately. Heat treatment of normal cat plasma destroyed its phagocytic supporting property. Finally, it was found that hemorrhagic hypotension results in about a 50% depression in the clearance of colloidal carbon as compared to nonhemorrhaged controls. The evidence is consistent with the hypothesis that serum opsonins and/or RES-dependent substances may be involved in depression of RES function in post-oligemic shock.

1. Saba, T. M., Arch. Intern. Med. **126**, 1031 (1970).
2. Saba, T. M., and DiLuzio, N. R., Amer. J. Physiol. **216**, 197 (1969).
3. Saba, T. M., Filkins, J. P., and DiLuzio, N. R., J. Reticuloendothel. Soc. **3**, 398 (1966).
4. Saba, T. M., and DiLuzio, N. R., J. Reticuloendothel. Soc. **2**, 437 (1966).
5. Cornell, R. P., and Saba, T. M., Amer. J. Physiol. **221**, 1511 (1971).
6. Lefer, A. M., Daw, J. C., and Berne, R. M., Amer. J. Physiol. **216**, 483 (1969).
7. Biozzi, G., Benacerraf, B., and Halpern, B. N., Brit. J. Exp. Pathol. **243,** 441 (1953).
8. Murray, I. M., Amer. J. Physiol **204,** 655 (1963).
9. DiLuzio, N. R., and Riggi, S. J., J. Reticuloendothel. Soc. **1**, 136, (1964).
10. Blattberg, B., and Levy, M. N., Amer. J. Physiol. **203**, 409 (1962).
11. Scovill, W. A., and Saba, T. M., Ann. Surg. **178**, 59 (1973).
12. Saba, T. M., Surgery **71**, 675 (1972).
13. Blattberg, B., and Levy, M. N., Amer. J. Physiol. **210**, 312 (1966).
14. Blattberg, B., Frosolono, M. F., Misra, R. P., and Levy, M. N., J. Reticuloendothel. Soc. **12**, 371 (1972).
15. Leffler, J. N., Litvin, Y., Barenholz, Y., and Lefer, A. M., Amer. J. Physiol. **224**, 824 (1973).

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