FUNCTION OF THE RETICULOENDOTHELIAL SYSTEM

I. A Study on the Phenomenon of Carbon Clearance Inhibition*

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(Received for publication, May 18, 1965)

The importance of the reticuloendothelial system in clearing the blood of foreign particulate substances, such as bacteria, and altered endogenous materials, such as fibrin aggregates, is generally recognized. Measurement of the activity of the reticuloendothelial system has depended upon estimation of the rate of clearance from the blood of foreign materials, such as colloidal carbon. Under uniform conditions in a given animal species, it has been found that clearance rates of carbon particles follow a definite pattern and are reasonably constant. Biozzi, Benacerraf, and Halpern have found that the clearance of carbon from the blood in several different species of animals is directly proportional to the concentration of carbon (1). Further, these authors found that if a prior injection of the same material or another colloidal material was given during the course of carbon clearance then a reduction in the rate of clearance occurred (2, 3).

The nature of the reduced clearance when two particles are simultaneously presented to the reticuloendothelial system is not understood. Indeed, precise description of the phenomenon with respect to dose, onset, degree, and duration of inhibition has not hitherto been presented. Such information is needed in order to understand the mechanism of this inhibition; therefore, we have assembled this information for carbon clearance and its relationship to heat-aggregated albumin.

Quantitative relationships have been found between the dose of aggregated albumin and the onset and duration of the carbon clearance inhibition. These have been analyzed with regard to the blood carbon concentration and to the amount of gelatin used to stabilize the carbon preparation. Evidence will also be presented which suggests that the rate at which carbon is normally removed from the blood is dependent on the gelatin concentration. Finally, that the relationships found relative to heat-aggregated albumin are also true for foreign red cell impedance of carbon clearance suggests that these relationships may hold for a variety of materials other than carbon that normally are cleared from the blood by the reticuloendothelial system.

* Supported by United States Public Health Service grants 2-T1-GM-100-06, HE-03174 Washington State Initiative 171 Fund.

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Materials and Methods

Animals.—For clearance studies, male Sprague-Dawley rats weighing between 150 and 250 gm were used and anesthetized with intraperitoneal nembutal in a dose of 4 mg/100 gm body weight.

Particulate Material.—Two shellac-free carbon preparations manufactured by Gunther-Wagner were obtained from John Henschel and Co. Inc., New York. One preparation, C-77-702 contained carbon without gelatin whereas the other preparation, C-11-1431a was stabilized with fish gelatin at a reported concentration of 43 mg of gelatin per milliliter. Nitrogen determinations were performed on this latter ink using a modified Nessler's procedure (4) and converted to gelatin using a figure of 18.5 per cent nitrogen for fish gelatin (5). The values derived from these nitrogen determinations were in excellent agreement with the reported 43 mg of gelatin per ml. This preparation contained carbon at a concentration of 168 mg/ml and was used in all experiments unless otherwise indicated.

For those experiments in which gelatin was added to the commercial carbon preparation, U. S. P. gelatin from J. T. Baker Chemical Co., Phillipsburg, New Jersey was used. The gelatin was first prepared as a 10 per cent gelatin solution in 0.15 M NaCl with pH adjusted to 7.2 by addition of ammonium hydroxide. Varying amounts of gelatin were then added to the commercial carbon suspension C-11-1431a at 60°C and mixed for 1 hour. The volume was adjusted by addition of 0.15 M NaCl to yield a final concentration of 16 mg of carbon per ml of solution. In this way carbon preparations containing an added 0.12, 1, and 5 per cent gelatin were made. In computing the ratio of gelatin to carbon the figure of 43 mg of gelatin per ml per 168 mg of carbon in the original Gunther-Wagner ink was included, and all values given are corrected for this original amount of gelatin.

Denatured albumin was prepared from commercial bovine serum albumin (Armour and Company, Kankakee, Illinois) by the method of Benacerraf *et al.* (6) A 1 per cent solution of albumin was heated for 20 minutes at 70°C after which the temperature was raised to 75° to 80°C. The extent of denaturation was monitored by optical density, and denaturation was terminated after an increase of 0.90 OD units in a 1 cm light path at a wavelength of 550 mμ. The denatured protein was collected by isoelectric precipitation and the concentration of the final solution in physiologic saline was determined by the method of Waddel (7). Not all preparations were biologically active, despite control of pH, ionic strength, temperature, and optical density. Of eleven preparations of denatured albumin, nine were active. The active preparations used in these experiments behaved identically and when dissolved in saline were stable for 2 to 3 months at 5°C.

Rabbit erythrocytes were obtained *via* cardiac puncture and used fresh after being washed twice in equal volumes of 0.15 M sodium chloride. When suspended in fresh rat plasma, no visible agglutination of the cells occurred.

Clearance Rates.—Carbon clearance was determined by administering carbon intravenously to individual animals. Blood samples of 0.20 ml each were obtained from the inferior vena cava through a P.E. 10 polyethylene catheter inserted *via* the femoral vein. Three samples were obtained at 2 minute intervals over the first 6 minutes prior to injection of the denatured albumin and nine samples were obtained thereafter at time intervals varying from 1 to 4 minutes, depending on the dose of albumin given. With this schedule the total amount of blood removed from an individual animal was less than 3 ml. No anticoagulant was necessary in the catheter, but syringes rinsed with heparin were used to withdraw the blood. Measured 0.100 ml aliquot of blood were diluted in 2.5 ml of an 0.1 M Na_2CO_3 solution and the concentration of carbon determined photometrically at a wavelength of 650 mμ. The slope of the plot of the log concentration of carbon against time was used to determine clearance rates or the "phagocytic index" K. K is the rate constant in the differential equation expressing the clearance $dC/dT = -2.3\,KC$, where C is the carbon concentration.

Statistical Methods.—The relationship found between the log of the duration of inhibition in carbon clearance and the dose of denatured albumin injected was analyzed using linear regression (8). The regression line had the form: Log time = $A + B$ (dose − $\overline{\text{dose}}$) where A represents an intercept constant estimated by average of log time and B represents the slope of the line. An estimate of B (designated b) was found by minimizing the sums of squares of the deviations from the line. The relationship obtained was then tested for reliability of fit $H:B = 0$ (*i.e.*, Log time is *not* linearly dependent on dose) *via* statistic:

$$t_{(n-2)} = \frac{b - B}{S_{\text{time/dose}}} \sqrt{\sum_{i=1}^{n} (D_i - \overline{D})^2}$$

where $S_{\text{time/dose}}$ is an estimate of the variance about the regression line and D_i is the individual albumin doses. $\overline{D}$ represents the mean of all albumin doses. This statistic has a t distribution with $n - 2$ degrees of freedom and calculation for denatured albumin inhibition of carbon clearance gave $t = 9.5$; the 1 per cent significance level is $t_{11} = 3.1$. Thus it appears firm that the log time is linearly related to the dose.

EXPERIMENTAL OBSERVATIONS

Carbon Clearance.—The carbon clearance method of measuring phagocytic function was standardized using a dose of 8 to 10 mg of carbon per 100 gm of body weight. When the log of the carbon concentration was plotted against time, the clearance was linear and continued to be so until the concentration of carbon in the blood became so low that the photometric method no longer permitted accurate determination of the carbon. The rate at which the carbon was removed from the blood, the phagocytic index K, was 0.036, with a standard deviation of 0.011 on 30 determinations.

The commercial carbon preparation used was stable in serum and plasma but showed a tendency in animals to associate with platelets, forming aggregates in whole blood. The wet mounts of fresh blood, examined under the light microscope, showed the amount of carbon associated with platelets to be small; most of the carbon particles were dispersed and apparently unaggregated. On the other hand, if the carbon preparation used contained no gelatin (C-77-702) massive carbon clumping occurred.

If 10 mg of gelatin were added to 16 mg of carbon, it did not entirely prevent the platelet aggregation as seen in the light microscope yet the rate of intravascular clearance was slowed. In fifteen experiments, using this concentration of gelatin to carbon, the average phagocytic index K was 0.023, with a standard deviation of 0.009. Because the addition of gelatin produced a slower rate of clearance, other concentrations of gelatin were then added to the carbon to see if a relationship might exist between the amount of added gelatin and the rate of carbon removal. When the ratio of total gelatin[1] to carbon was plotted against the phagocytic index K (Fig. 1), a linear relationship emerged. However, if the

[1] It will be recalled that the original carbon suspension contained 43 mg of gelatin per ml per 168 mg of carbon, and hence the ratio of gelatin to carbon in the original preparation was 3.91.

gelatin and carbon are not incubated together under the conditions described but are injected separately but simultaneously there was no slowing in the rate of carbon removal. For these experiments the gelatin was injected as a 5 per cent solution at a temperature of 40° C and up to a dose of 50 mg of gelatin per 100 gm of body weight.

Centrifugation of the ink at 5200 R.C.F. for 15 minutes caused precipitation of about 50 per cent of the carbon. When injected into animals the rate at which

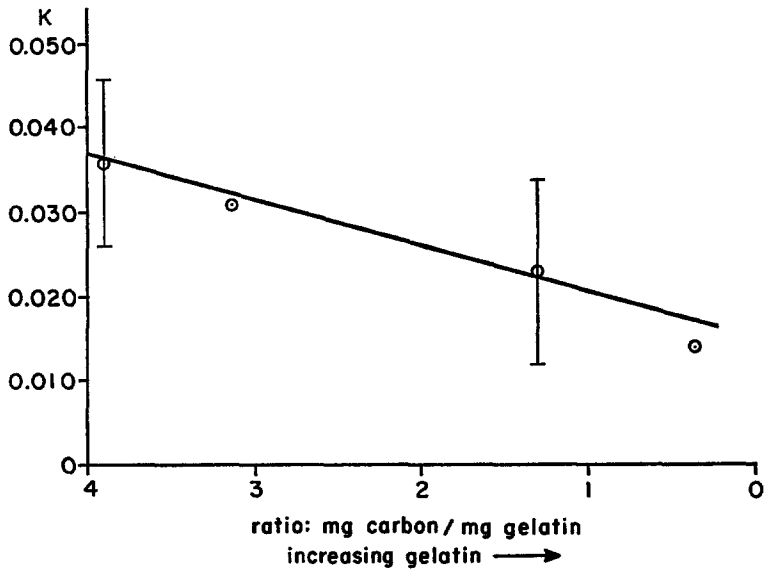

FIG. 1. The phagocytic index K for a standard dose of carbon (8 to 10 mg/100 gm of body weight) in the rat has been plotted against the content of gelatin in each carbon solution prepared as described in the text. The content of gelatin is expressed as the ratio of milligrams of carbon per milligrams of gelatin and it is observed that the phagocytic index is linearly dependent on the gelatin content of the carbon preparation.

the centrifuged (supernate) carbon was removed from blood tended to be slower than the rate of uncentrifuged carbon (Table I). When gelatin was then added to the centrifuged carbon, further slowing in the rate of clearance was observed.

During the course of these experiments two bottles of Gunther-Wagner ink C-11-1431a were utilized. The above graphs and data have been derived from the same bottle of ink. The second bottle showed a somewhat slower rate of normal carbon clearance (K average of 0.030), but despite the slower rate of clearance, the effect of gelatin, centrifugation, and centrifugation plus gelatin was qualitatively similar to the first.

Denatured Albumin Inhibition of Carbon Clearance.—Preliminary experi-

ments confirmed the observation of Biozzi and coworkers (3) that the rate at which a given carbon load is removed from the blood is altered by an intravenous injection of heat-denatured bovine albumin. From these experiments we discovered that, by appropriate selection of the dose of carbon and albumin, a complete curve for the inhibition could be obtained. Such a typical curve, depicting the effect of denatured albumin on carbon clearance, is seen in Fig. 2. Here the initial part of the curve $K-1$ represents the clearance rate of carbon prior to the introduction of denatured albumin. Immediately upon albumin injection the slope flattens out and, in this example, remains so for about 7 minutes. Following this period of inhibited clearance, the curve abruptly re-

TABLE I

The Effect of Gelatin Addition, Centrifugation, and Centrifugation Plus Gelatin on the Rate of Carbon Clearance in Rats

	Uncentrifuged	Uncentrifuged 1 per cent gelatin	Centrifuged 5200 R.C.F. 15 min.	Centrifuged 1 per cent gelatin
Carbon concentration *mg/ml*	168	16	80	16
Gelatin concentration, *mg/ml*	43	14.3*	—	—
Carbon/gelatin, ratio	3.91	1.26	—	—
No. trials	30	15	5	5
Average K	0.036 ±0.010	0.023 ±0.011	0.022	0.014

* This figure includes the 4.3 mg/cc of gelatin associated with the 16 mg/cc of carbon plus 10 mg/cc of added gelatin.

sumes a steeper slope at $K-3$. The slope of this part of the clearance curve $K-3$ has been found in many experiments to return to a value close to that of $K-1$. The general form of this phenomenon is not dependent upon the time at which the denatured albumin is introduced. Thus if the albumin is introduced simultaneously with the carbon a similar degree and duration of depression of the clearance rate is observed.

It seemed reasonable to study, under constant conditions of carbon dose, the effect of varying the dose of denatured albumin. Therefore the quantity of albumin given in a single injection was varied over a wide range, extending from 0.10 to 40.0 mg/100 gm body weight. From this study several interesting points emerged. Irrespective of the size of the dose of denatured albumin the slope of the clearance curve was not reduced to zero. For doses of albumin ranging from 2 to 40 mg per 100 gm of body weight the average value of $K-2$ was 0.011 ± 0.004 (Table II). Although the degree of depression of the clearance rate seemed to be independent of the dose of denatured albumin, the *duration* of this depression showed an interesting and regular dependency on dose. As

can be seen in Fig. 3, the plot of the dose of albumin against the logarithm of the duration of the depressed phase showed a rapid rise between 0.1 and 2 mg of albumin per 100 gm body weight. From 2 to 40 mg of albumin there is an apparent exponential increase in the duration of the depression with increasing doses of albumin. This is statistically a straight line.

Having found a relationship between the dose of albumin and the duration of

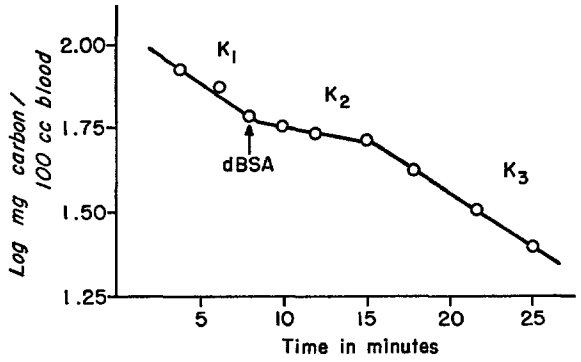

FIG. 2. A typical carbon clearance curve depicting the effect of an injection of heat de natured albumin (abbreviated dBSA) on carbon clearance. Note that three different phago cytic indices (K values) are associated with different portions of the clearance curve.

TABLE II

Carbon Clearance Rates Associated with Injection of Heat Aggregated Albumin. Data from 17 Experiments

	Control (K_1)	Post-albumin injection (K_2)	Recovery control (K_3)
Average	0.035	0.011	0.037
SD	±0.009	±0.004	±0.016

the inhibition in carbon clearance, experiments were then undertaken to examine this relationship with respect to the dose and stability of the carbon.

The effect of increased carbon dose was first examined. If instead of 10 mg of carbon, a dose of 30 mg of carbon per 100 gm body weight was given, then the inhibitory effect of a given dose of denatured albumin was prolonged. When the log of the duration of depressed clearance for this dose of carbon was plotted against the dose of denatured albumin, a curve remarkably similar to the one described for 10 mg of carbon was observed (Fig. 4). The exponential nature of the duration of depressed clearance as a function of albumin dose was again observed for albumin doses greater than 2 mg per 100 gm body weight. In fact this curve was parallel to that observed for 10 mg of carbon. This emphasizes

the point that the effect of an increased carbon dose is to prolong the inhibitory effect of a given albumin dose in a predictable and consistent manner. It is also interesting that for doses of albumin below 2 mg there was a marked fall-off in the duration of carbon inhibition for both the 10 and 30 mg carbon doses, the two curves tending to meet at a common albumin dose.

Centrifugation of the carbon prior to injection into animals tended to slow the rate of carbon removal, but it did not alter the described response of carbon to denatured albumin. For the same dose of albumin, centrifuged and un-

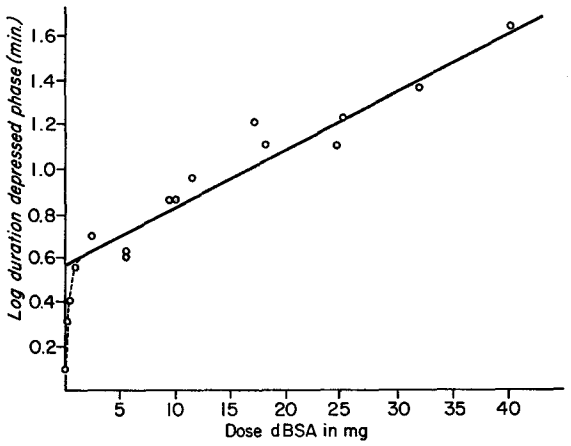

FIG. 3. The log duration of the depressed phase in carbon clearance following injection of denatured albumin has been plotted against the dose of albumin in mg/100 gm of body weight. Note that above a dose of 2.5 mg/100 gm of body weight, an exponential relationship exists between the duration of inhibition and the dose of albumin. In these experiments a constant carbon dose of 8 to 10 mg/100 gm of body weight with a carbon per gelatin ratio of 3.9 was used.

centrifuged carbon produced the same depth of depression and the same duration of inhibition.

The 10 and 30 mg carbon doses employed in the above study were Gunther-Wagner carbon suspensions stabilized in 4.3 per cent fish gelatin. It has already been pointed out that the addition of gelatin may produce a more stable carbon suspension in whole blood with consequent slowing of the rate of clearance. Therefore, it appeared to us that the effect of gelatin on the interaction of carbon and aggregated albumin should be examined.

The Effect of Gelatin on Denatured Albumin Inhibition of Carbon Clearance.— When 10 mg of gelatin were added to 16 mg of the commercial carbon preparation and injected at a dose of 10 mg of carbon per 100 gm of body weight a marked prolongation in the inhibitory effect of denatured albumin was observed. There was again an exponential increase in duration of inhibition with

increasing albumin doses in excess of 2 mg per 100 gm body weight. As seen in Fig. 4 this exponential duration approximately paralleled the effect already described for the 10 and 30 mg carbon dose to which no additional gelatin had been added. When the data were examined by analysis of covariance it was found that the regression lines formed between duration of inhibition and dose of denatured albumin for 10 mg of carbon, 30 mg of carbon, and 10 mg of carbon plus gelatin were indeed parallel.

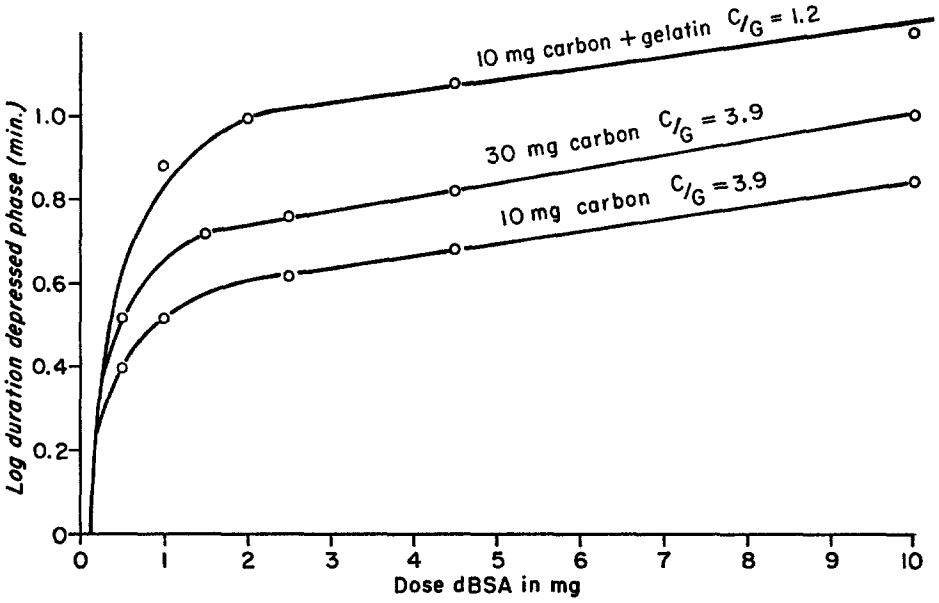

FIG. 4. The log duration of inhibited carbon clearance is plotted against the dose of denatured albumin administered. This graph illustrates the effect of varying carbon concentrations and the amount of gelatin in the carbon preparation. The ratio *C/G* represents the ratio of milligrams of carbon per milligrams of gelatin in each carbon preparation.

A systematic examination was then undertaken to see if a relationship existed between the added gelatin and the increased duration of carbon clearance inhibition. A constant albumin dose of 4.6 mg/100 gm body weight was injected during the clearance of carbon particles previously suspended in various concentrations of gelatin. As the amount of gelatin added to the carbon preparation increased, there was a progressive and dramatic prolongation of the carbon clearance inhibition. For example, when 50 mg of gelatin were added to 16 mg of the commercial carbon suspension, the duration of inhibition was 19.2 minutes compared to 4.2 minutes for the original suspension without added gelatin. When the log of the duration of inhibition was plotted against the ratio of milligrams of carbon to milligrams of gelatin the prolongation of clearance

inhibition resulting from gelatin addition could be expressed linearly as seen in Fig. 5.

The duration of inhibition to a given albumin dose was the same for gelatin added to centrifuged carbon as was observed for gelatin added to uncentrifuged carbon. Moreover, if gelatin in a dose of up to 50 mg per 100 gm of body weight was injected during the course of carbon clearance, there was no observed alteration in the rate of carbon removal.

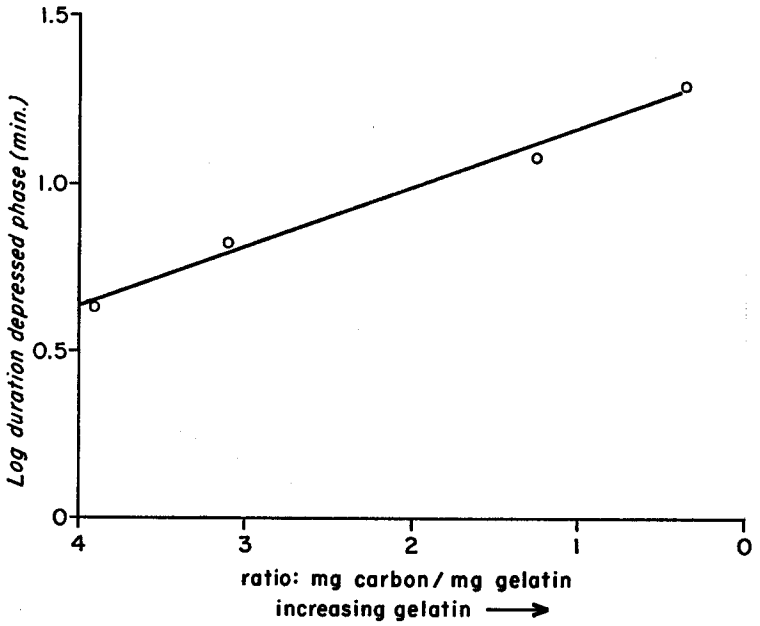

FIG. 5. This figure illustrates the effect of gelatin in the carbon preparation on the duration of inhibition to a constant dose of denatured albumin (4.6 mg/100 gm of body weight). The amount of gelatin is expressed as the ratio of milligrams of carbon per milligrams of gelatin.

Rabbit Erythrocyte Inhibition of Rat Carbon Clearance.—Since the dose of denatured albumin injected during carbon clearance produces a predictable onset, degree, and duration of inhibition in the rate of carbon removal, it was of some interest to explore a different particulate inhibitor of carbon clearance to see how general these relationships might be. For this purpose rabbit erythrocytes were injected during the course of carbon clearance in the rat. Rabbit erythrocytes behave as a foreign particulate in the rat, and are cleared by the rat reticuloendothelial system. When a sufficient number of these erythrocytes was given, they produced inhibition in the rate of carbon clearance. In our experience in order to produce a measurable inhibition in carbon removal at

least 350 million erythrocytes must be given. The onset of inhibition when this dose was exceeded was very rapid but the degree of inhibition was not as great as with heat-aggregated albumin (Table III). Again, the degree of inhibition

TABLE III

Carbon Clearance Rates Associated with Injection of Heat Aggregated Albumin and Rabbit Red Cells Related to the Dose of Carbon and Amount of Gelatin

Inhibitor	Carbon dose mg/100 gm	Carbon/gelatin ratio	Trials	Average K_1	Average K_2	K_1/K_2
Denatured albumin	10	3.91	17	0.036	0.011	3.27
	10	1.26	15	0.023	0.005	4.60
	30	3.91	11	0.021	0.004	5.25
Rabbit red cells	10	3.91	9	0.038	0.017	2.24

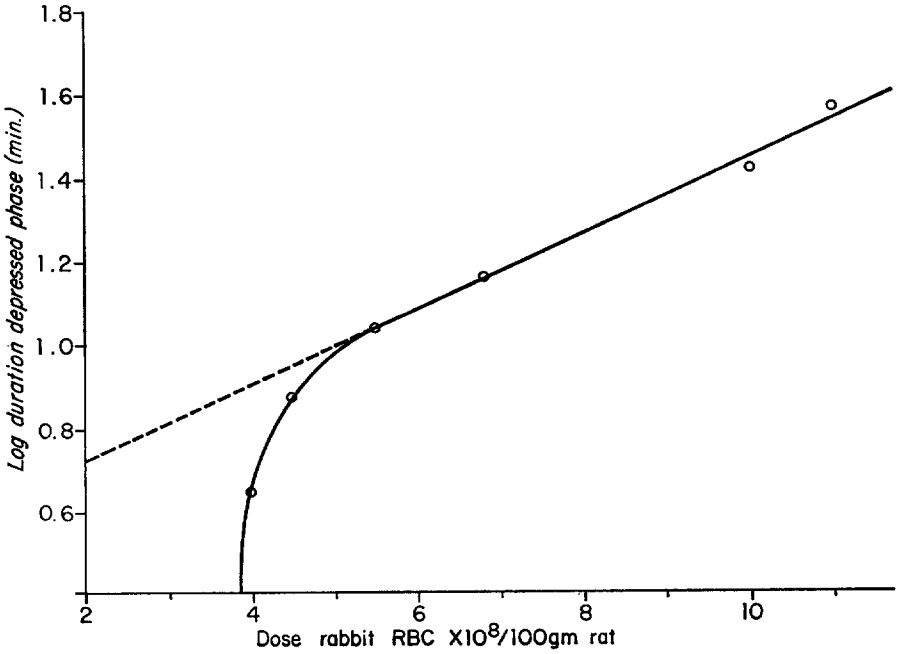

FIG. 6. The log duration of the depressed phase in *rat* carbon clearance following the injection of *rabbit* red blood cells has been plotted against the dose of red cells injected per 100 gm of body weight. Note the similarity between this figure and that of Fig. 3.

was independent of the dose of erythrocytes given. However, as seen in Fig. 6, the duration of inhibition was markedly dose-dependent. When the number of erythrocytes injected exceeded 575 million per 100 gm of body weight, there

was an exponential increase in the duration of inhibition with increasing dose of erythrocytes. Below 575 million, however, there was a marked fall-off in duration which was not measurable below 375 million cells. Thus, whereas the degree of inhibition achieved with foreign erythrocytes was not as great as with denatured albumin, the duration of inhibition above a critical dose was exponentially dependent on dose. This suggests that the phenomenon of carbon inhibition by competing particles follows certain fundamental patterns relative to dose, onset, degree, and duration of inhibition.

DISCUSSION

The rate at which colloidal particles, exemplified by carbon, are removed from the blood stream appears to be proportional to the number of carbon particles present in the circulation and therefore to follow a simple exponential function expressed by the following equation: $dC/dT = -2.3\ KC$. When one modifies the carbon clearance rate by the addition of gelatin to the carbon suspension prior to injection, or by injection *in vivo* of heat-aggregated albumin, a change occurs in the constant of proportionality, indicating a change in rate without any evident change in the form of the relationship. Exactly how this effect on the clearance rate is brought about is unknown.

Gelatin is known to confer suspension stability on a variety of colloidal materials and for this reason is frequently employed in the preparation of a number of different particle suspensions used to measure phagocytic function. Of these preparations, colloidal carbon is the most often used and yet little information is available on how the gelatin present in the preparation affects the rate of carbon removal. That gelatin might have such an effect is suggested by a previous study by Dobson using chromium phosphate in which he found that the rate at which these particles were removed from blood was inversely proportional to the amount of gelatin added (9). The experiments presented above show that carbon clearance is also dependent on the gelatin concentration in the carbon suspensions. As we have seen, the ratio of the carbon concentration to gelatin concentration when plotted against K shows a linear relationship. This suggests that it is the amount of gelatin relative to carbon that is critical and not the total or absolute amount of gelatin. This effect of gelatin is present, although not of the same magnitude, even when a more homogeneous suspension of particles is prepared by centrifugation.

It appears that gelatin mediates its effect by a direct action on the carbon particle surface to alter either its relationship to blood constituents (its stability in blood) or the affinity of the reticuloendothelial cells for the particle. The evidence that supports this conclusion is the observation that equivalent or excess amounts of gelatin when injected during the course of carbon clearance do not alter the rate of carbon removal. In order to observe an effect due to gelatin, the carbon particle must be associated with the gelatin prior to its introduction into the blood stream.

That the observed effect of gelatin may in part be due to an alteration in the interaction between the carbon particle and blood components is supported by the observation that carbon, in the complete absence of gelatin, immediately and massively aggregates in blood. The addition of gelatin to the carbon suspension prior to injection into the blood stream prevents gross carbon clumping. That an interaction between particle, blood proteins, and gelatin may occur has already been suggested for radiogold colloid by Murray and Katz (10). In their experiments, however, the gelatin was injected 15 minutes prior to the trace dose of radiogold, resulting in delayed clearance and evidence was advanced to suggest that this was the result of a complex between the gold, gelatin, and plasma proteins. On the other hand, we have failed to observe a significant slowing in the removal of carbon particles when gelatin was injected during carbon clearance. Whether this failure of carbon to complex with gelatin in the circulation may be due to an inherent difference between gold and carbon or due to a difference in the relative concentration of the particles and gelatin is not readily apparent. It is possible that the slower rate of clearance induced by gelatin injection and interpreted as "R.E. blockade" that has been reported for trace colloids including aggregated human serum albumin (11) may be resulting from just such an interaction between the gelatin in the circulation, the trace colloid and the blood proteins. This would be consistent with the slower rate of carbon removal that we have observed as the gelatin coating of the carbon increased.

In contrast to gelatin, heat-aggregated albumin readily impedes carbon clearance (decreases K) when injected *during* the course of carbon clearance. In order to observe accurately the inhibitory effect of a given albumin dose, it is essential to have a sufficiently fast rate of normal carbon clearance, so that the termination of the inhibited phase of the clearance can be adequately judged. Moreover, the dose of albumin given in order to prolong the duration of inhibition must be sufficient to permit measurement by the technique employed.

The question of whether a threshold for inhibition exists is raised by the observation that the smallest dose of albumin that can be administered and yet produce any noticeable effect is 0.2 mg of albumin per 100 gm of body weight. Despite the fact that a prolongation of inhibition for a given albumin dose can be achieved by gelatin addition to the carbon suspension or by increasing the carbon concentration, this minimal dose necessary to produce observable inhibition remains unchanged. Thus the fact that the quantity is greater than zero may be important and imply that a threshold for inhibition exists. However, the amount is so small that it is difficult to verify this point in the case of albumin using the present techniques. A stronger case for the existence of an inhibition threshold can be made by using foreign red cells to impede carbon clearance, because here, in order to produce an observable effect, at least 350 million rabbit cells must be given per 100 gm of rat body weight.

The initial observation that heat-aggregated albumin injected during the course of carbon clearance impedes the clearance was made by Biozzi and coworkers (12). Although these authors did not systematically examine the effect of varying the dose of aggregated albumin on the impedance in carbon clearance they did suggest that a relationship might exist between the dose of aggregated albumin and the degree of inhibition. However, over a wide range of albumin doses, we have found that the degree of inhibition is independent of the dose of denatured albumin. On the other hand, it is the duration of inhibition that is markedly dose dependent: above a certain albumin dose the duration of inhibition becomes exponentially related to the dose. These two observations on the degree and duration of inhibition seem to be generally true for variations in the concentration of carbon, for centrifuged and gelatin stabilized carbon, and for foreign red blood cells. This suggests that what we are observing pertains to general relationships between carbon clearance and substances that impede carbon clearance.

As the amount of carbon is varied or as the amount of gelatin used to stabilize the preparation is increased, a family of curves is derived that expresses the relationship between duration of inhibition and dose of competing colloid. The fact that an increase in concentration of either carbon or denatured albumin similarly prolongs the inhibition suggests that both are mediated by a very closely related if not identical mechanism. If this be true, it would imply that carbon interferes with the clearance of denatured albumin in a manner identical to denatured albumin impedance of carbon clearance.

The fact that foreign red cells inhibit carbon clearance in a manner nearly identical to denatured albumin implies that the inhibition can occur between dissimilar particle surfaces and therefore involves a relatively non-specific type of interaction. This does not imply, however, that the surface properties of the particles are not important because as we have seen an apparent change in the gelatin coat on the carbon particle markedly influences the inhibition. Thus an exponential increase in duration of inhibition to a given albumin dose was observed as a function of the carbon to gelatin ratio. That such changes in the gelatin coat also influence the rate of carbon removal underscores the importance of the particle surface in relation to blood proteins for an understanding of both clearance and clearance inhibition.

Although the exact nature of the mechanism of carbon clearance inhibition by denatured albumin is unknown, several possible explanations can be advanced: (*a*) saturation of the phagocytic cell, (*b*) direct competition for phagocytic sites, (*c*) direct interaction with carbon to form a carbon-albumin complex, and (*d*) competition between carbon and denatured albumin for some component of serum necessary for maximum rate of phagocytosis. Experiments to be reported separately indicate that the inhibition does involve a serum component, limited in amount, and responsible for maximum rate of carbon phagocytosis.

SUMMARY

The inhibitory effect of heat-aggregated albumin on carbon clearance has been studied with respect to the dose-dependent relationships involved. It has been found that the dose of aggregated albumin does not affect the degree of inhibition but does affect the duration of inhibition. Above a critical dose the duration increases exponentially with increasing albumin dose. The addition of gelatin to the carbon suspension was found to produce two effects: (*a*) it prolonged the inhibitory effect of a given aggregated albumin dose, and (*b*) it produced a slowing in the rate of carbon removal. The fact that foreign red cells inhibit carbon clearance in a manner similar to denatured albumin suggests that the relationships observed relative to denatured albumin may be generally applicable to carbon clearance and indeed may have application to phagocytic particles other than carbon.

The authors wish to express appreciation: to Dr. David Lagunoff for his helpful suggestions in the planning of the experiments and for reviewing the manuscript, to Miss Alison Ross for her help in the preparation of the manuscript, and to Mr. Emile Keene for his technical assistance.

A preliminary report of the material presented here was made at the 49th annual meeting of the Federation of American Societies for Experimental Biology, *Fed. Proc.*, 1965, **24,** 683.

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