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Lack of Action of Serum Opsonins in Phagocytosis of Inert Particles by Cells of Reticuloendothelial System. (28239)

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Normal or immune serum opsonins play a determinant role in the phagocytosis of bacteria either by isolated phagocytes or by the fixed cells of the reticuloendothelial system (R.E.S.). The situation is completely different as far as the phagocytosis of inert colloid is concerned.

Studies on the kinetics of carbon particles uptake by the R.E.S. have shown that rate of phagocytosis is determined by the balanced interplay of 2 factors acting in opposing directions(1):

1) Actual concentration of particles in the blood, which is proportional to the rate of phagocytosis.

2) Degree of R.E. cell saturation produced by the engulfed particles which slows down the rate of phagocytosis of the colloid remaining in the blood.

These facts have been confirmed with different inert colloids in various animal species (2,3) and in man(4). On the basis of these findings, we were able to establish the principles governing phagocytosis of inert particles by the R.E.S. in terms of the two above mentioned factors, *i.e.,* the amount of particles respectively in blood and in R.E. cells. It appears therefore that the kinetics of phagocytosis can be fully explained by the sole interplay of these 2 factors excluding the intervention of limiting serum factors.

Recently Jenkin and Rowley(5) have suggested that rate of phagocytosis of carbon particles by R.E. cells may be related, at least in part, to the level of serum factors acting as opsonins on the following basis:

a) The rate of carbon phagocytosis in normal mice is increased when the colloid is pre-incubated *in vitro* with different heterologous sera. Adult or fetal pig serum was found to be the most active. A similar effect was also observed with serum from mice treated with a bacterial lipopolysaccharide.

b) Injection of a large amount of carbon (R.E.S. "blockade") reduces the rate of phagocytosis of a second dose of the same colloid. This effect disappears if the carbon

used for the test was incubated with pig serum.

This interpretation, which is obviously contradictory to our observations, prompted further investigation. From the data reported here it does not appear that serum factors contribute in any way to the rate of phagocytosis of inert particles by the R.E.S.

Materials and methods. Experiments were performed on adult male Swiss mice weighing 20-26 g. The phagocytic function of R.E.S. was measured by the rate of blood clearance of colloidal carbon* according to the method previously described(6). R.E.S. activity was expressed by the "phagocytic index" K and by the "corrected phagocytic index" α. Index K measures the function of the total number of R.E. cells in contact with the blood stream and index α corrects the value of K by the variations in size of liver and spleen which contain the majority of R.E. cells involved in carbon phagocytosis. Index K and index α were calculated from the equations:

$$K = \frac{\log Ct - \log Ct'}{t' - t} \qquad \alpha = \sqrt[3]{K} \cdot \frac{W}{Wls}$$

where Ct and Ct' are blood carbon concentrations at times t and t'; W is body weight and Wls is weight of liver and spleen. Doses of carbon used are indicated in the Tables. R.E.S. "blockade" was produced by intravenous injection of 32 mg of carbon/100 g.

Attempts to opsonize carbon particles were performed with the technique used by Jenkin and Rowley(5): 1 ml of carbon at a concentration of 32 or 16 mg/ml was added to 1 ml of the serum to be tested and incubated at 37°C for 20 minutes. The mice received i.v. 0.2 ml/20 g of the mixture corresponding respectively to 16 and 8 mg of carbon/100 g.

The following normal sera were used: human, rabbit, mouse, pig (either adult or newborn). These sera were prepared from freshly collected blood and stored at –20°C. Newborn pig serum was obtained 2-4 hours after birth from piglets deprived of colostrum. Sera obtained from mice in the period of R.E.S. stimulation produced by killed *S. typhi* or living B.C.G. were also studied. A group of 20 mice was injected subcutaneously 3 times at 48 hour intervals with 5×10^9 *S. typhi* T 415 killed by heating at 60°C for 1 hour. Twenty-four hours after the last injection R.E.S. activity was measured in 5 mice, the other animals were bled and pooled serum prepared. Another group of 20 mice was infected i.v. with 40 μg (dry weight) of living B.C.G. from an 8-day-culture in DUBOS medium. Twenty-one days afterward the R.E.S. function was measured in 5 mice and serum prepared from the remaining ones.

Results. The results given in Table I show that the treatment of carbon particles with different sera does not modify the rate of phagocytosis of this colloid by R.E. cells. Likewise sera from mice at the peak of R.E.S. stimulation produced either by *S. typhi* or B.C.G. are unable to accelerate carbon blood clearance. The values of the indices K and α for carbon pretreated with the different sera are comprised within the limits of standard deviation observed for a corresponding dose of untreated carbon.

From Table II it appears that the "blockade" of R.E. cells with a large dose of carbon (32 mg/100 g) produces a marked reduction in rate of phagocytosis of a second dose (8 mg/100 g) of the same colloid (testing dose). This "blocking" effect is similar whether the test is made with normal carbon or carbon treated with newborn pig serum.

Discussion. The data presented here provide evidence that serum opsonins do not intervene as a limiting factor in rate of carbon blood clearance. Results from our preceding experiments demonstrate that velocity of carbon phagocytosis is dependent only on the phagocytic activity of R.E. cells. The principal facts supporting this assertion are:

1) The rate of particles phagocytized in the first minutes after injection tends to a maximal constant value independently of the dose of colloid injected. Subsequently the number of particles fixed by R.E. cells becomes dose dependent, being higher for larger doses(1).

2) The study of competition of a second colloid on the phagocytosis of carbon particles

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TABLE I. Rate of Phagocytosis of Carbon Particles Treated With Various Sera.

Dose of carbon	Serum from	No. of mice	Phagocytic index K	Corrected phagocytic index α	W / Wls
	Norml adult pig ♂	5	.027	5.6	18.5
	" " " "	5	.030	5.9	19
	" " " "	5	.023	5.1	18
	" " " ♀	5	.022	5.3	18.8
	" " " "	5	.024	5.5	19
	" " " "	5	.021	5.3	19.3
	Freeze dried pig serum*	6	.022	5.5	19.6
	Newborn pig	6	.029	5.5	17.9
8 mg % g	" " adsorbed with mouse red cells	6	.028	5.8	19
	Normal rabbit	6	.017	5.5	21.5
	" man	6	.016	5.4	21.5
	" mouse	6	.020	5.4	20
	S. typhi treated mice†	6	.019	5.6	21
	BCG infected mice‡	6	.026	5.5	18.4
	Control mice§	44	.022	5.6	20
			± .011	± .8	± 1.9
	Normal adult pig	6	.012	4.8	20.9
16 mg % g	" mouse	6	.011	4.5	20
	Control mice§	6	.012	4.7	20.3

* Sent by Dr. Rowley.
† Phagocytic index K of donor mice: .075 for dose of 8 mg of carbon % g.
‡ " " " " " .100 " " " " " "
§ Receiving non-treated carbon.

TABLE II. Rate of Phagocytosis of Carbon Particles (8 mg % g) Untreated or Treated With Pig Serum in Mice 4 Hours After R.E.S. Blockade With 32 mg of Carbon % g.

	Carbon used for test	No. of mice	Phagocytic index K	Corrected phagocytic index α	W / Wls
	Not treated	8	.007	3.6	19.1
"Blocked" mice	Treated with newborn pig serum	8	.006	3.3	18.2
Control mice	Not treated	10	.020	5.4	20
			± .009	± .5	± 1.7

shows that the competing effect lasts as long as the second colloid is present in the circulation. Thereafter the carbon phagocytosis resumes its initial value(7).

3) Different normal sera (human, guinea pig, rat and rabbit), injected intravenously in rats and mice, in the course of carbon phagocytosis do not change the rate of carbon blood clearance (unpublished observations).

All these findings show that carbon blood clearance is not dependent on eventual serum factors limiting the rate of phagocytosis in normal animals. The increase in rate of carbon phagocytosis produced either by *S. typhi* or B.C.G. is related to a stimulation of R.E. cell function without demonstrable intervention of serum opsonins.

In animals with R.E.S. "blockade" produced by a large dose of carbon, the depression of R.E. cell function is due to a saturation of phagocytic cells rather than to an exhaustion of serum opsonins. In fact, the results presented in Table II show that the "blocking" effect is the same on the rate of phagocytosis of normal or serum treated carbon particles.

Summary. Incubation of carbon particles with normal sera originating from various animal species and with sera from R.E.S. stimulated mice does not affect the rate of carbon phagocytosis by R.E. cells. These findings show that serum opsonins do not act as a limiting factor in carbon phagocytosis. Depression of R.E.S. phagocytic function by "blockade" with colloidal carbon is due to a saturation of phagocytic cells rather than to a de-

pletion of serum opsonins. These results confirm that the rate of carbon blood clearance is a reliable method for measuring phagocytic activity of R.E. cells.

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ε-Aminocaproic Acid and Urinary Trypsin Inhibitor: Potentiated Inhibition of Urokinase Induced Fibrinolysis.* (28240)

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In a comparative study of some natural protease inhibitors acting upon the fibrinolytic system it was observed that ε-aminocaproic acid (EACA) potentiated the inhibition of the urinary plasminogen activator (urokinase) by the trypsin inhibitor present in human urine. Since this trypsin inhibitor is normally present in the organism, and because EACA is used in the control of hemorrhage caused by excessive fibrinolysis, this observation is of immediate interest in evaluating the effects of EACA in the body.

Materials. 1. ε-aminocaproic acid (reprecipitated from methanol with ether). Stock solution: 20 mg/ml in barbital buffer: 0.05 M; pH 7.75. Further dilutions with barbital-gelatine buffer.

2. Urine inhibitor (mingin) was isolated from human pregnancy urine(1) and purified by methods to be described elsewhere. 1.6 mg completely neutralized 1.0 mg crystalline, pure trypsin.† Solutions were prepared in barbital-gelatine buffer.

3. Urokinase.‡ 2.300 Ploug units/mg. Stock solution: 920 units/ml barbital-gelatine buffer.

4. Thrombin,‡ bovine. 90 NIH units/mg. 10 units/ml barbital-gelatine buffer.

5. Fibrinogen (bovine) prepared after(2) and reprecipitated with phosphate(3).

6. Barbital buffer: 0.05 M; pH 7.75, containing 0.1 M NaCl.

7. Barbital-gelatine buffer: As above with 0.25% gelatine to prevent adsorption to glass.

Method. Clot lysis was followed by means of the thrombelastograph(4,5). This method was used in order to approach physiological conditions in the comparative study. Final reaction mixture contained 0.285% fibrinogen, barbital buffer, appropriate concentrations of inhibitors, urokinase, and was clotted with 1.4 units of thrombin per ml. Clotting time: 1 min. Lysis time was recorded in mm (2 mm = 1 min) measured from the first to the last oscillation on the thrombelastogram.

Results. A reference curve was obtained with varying concentrations of urokinase. The data yielded a straight line in a double logarithmic diagram, Fig. 1 (reference curve for the following experiment). Other experiments showed linearity over a wider range justifying the extrapolation in Fig. 1.

EACA is a weak inhibitor of fibrinolysis and in low concentration even enhances fibrinolytic activity. When tested against urokinase (22 units/ml reaction mixture) it was found that 8.16 mM EACA was the high-

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