Reticuloendothelial Function in the Isolated Perfused Liver
IV. Phagocytosis of Aggregated-BSA and Colloidal Gold. Evidence for Nonspecific Opsonins* (33816)

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Using an *in vitro* isolated perfused liver system it was demonstrated that clearance of colloidal gold (Aurcoloid) and aggregated-BSA by the phagocytic system of the liver led to a blockade of the reticuloendothelial system (RES). This blockade was evidenced in terms of a decreased clearance rate for these particulate materials after several sequential additions of the same material in the perfusate. In our experimental conditions blockade by gold was shown to be related only to depletion of plasma factors (1). By contrast, two factors were found responsible for the blockade by aggregated-BSA: (i) a depletion of opsonins, and (ii) a decreased cellular function of the reticuloendothelial system cells (2).

In an effort to further define the nature of the opsonic factor in plasma, we carried out the present experiments to show that the same plasma factor is depleted by the two foreign particles. Renewal of plasma factors by switching the perfusion to a second perfusate reversed the blockade for one or the other particulate used.

Method. In a previous paper, the *in vitro* double perfusion technique for the isolated liver was extensively presented (1). The liver was maintained in physiological condition as revealed by continuous bile secretion (3), protein synthesis (4), and normal histological appearance by electron microscopy (5). The colloidal gold and aggregated-BSA were prepared as in prior experiments (1, 2) and appropriate radio-labeling permitted their clearance to be analyzed quantitatively in the perfusion system. After this material had been introduced into the fluid perfusing the liver, successive analyses of perfusate samples taken at 5-min intervals (0.5 ml) gave a clearance rate. The same procedure used in prior studies was employed in the present experiments (1, 2).

Two types of experiments were carried out. The first used a unique perfusion system as described previously (3). The liver was perfused by one perfusate in which the two particulates were sequentially added. The second used the double perfusion system which permitted, on the one hand, renewal of the plasma factor involved in RES blockade and, on the other hand, addition of the different particulates in each separate perfusate. Each time perfusate (120 ml) was made of whole heparinized (3000 U/100 ml) pooled rats blood and Tyrode's solution in a ratio of 2:1 to which chlortetracycline (Aureomycin) (Lederle, 20 mg) and dextrose (800 mg) were added.

Results. Summarized in Fig. 1 are observations indicating that establishment of RE blockade with aggregated-BSA produced evidence of exhaustion of plasma factors for gold. As shown, repeated doses of aggregated-BSA resulted in progressively decreasing ability to clear this aggregated protein in spite of increasing concentration of the particulate in perfusate, as a consequence of the addition of new pulses when previous ones were not completely cleared. When RE blockade had been established with aggregated-BSA, 50% of one dose (2.5 mg) of radio-labeled colloidal gold added at time 190 min was cleared in 16 min (in four such identical type experiments T/2 of gold varied between 16 and 20 min). This demonstrated a decrease of clear-

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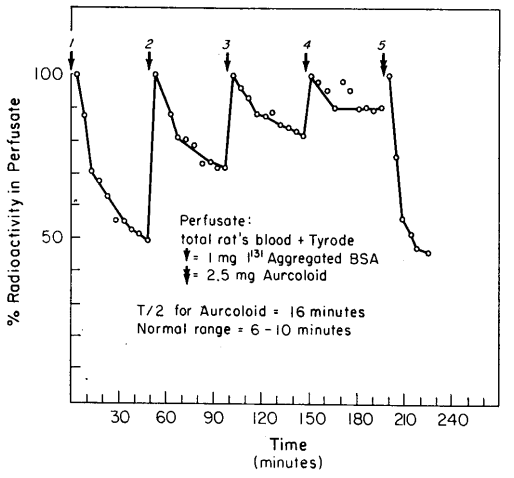

FIG. 1. Clearance of aggregated-BSA and aurcoloid by isolated perfused rat liver. after RES blockade was obtained with 4 sequential challenges of ^{131}I-aggregated-BSA (3 × 1 mg), Aurcoloid (2.5 mg) was added into the perfusate; it's clearance rate was impaired by previous aggregated-BSA challenges.

ance rate for gold. When the first 2.5-mg dose of gold is added without previous challenge of aggregated-BSA, it's half-time clearance is between 6–10 min (1).

In another experiment when colloidal gold was first added and then aggregated-BSA (1 mg) after RE blockade occurred for gold, the amount of aggregated-BSA cleared at this time (0.25 mg or 0.22 mg during 48 min in two different experiments) indicated a cellular uptake for this particulate equal to that observed when aggregated-BSA was added to a perfusate free of plasma factors (2) (see Table I). Indeed, aggregated-BSA added in presence of plasma factors is cleared in a quantity of 0.49 mg in 48 min (See Table I). This lack of facilitation of phagocytosis for aggregated-BSA after challenge of colloidal gold is illustrated in Fig. 2.

TABLE I. Clearance of ^{131}I-Aggregated-BSA by the Isolated Perfused Liver.

	After 1 mg pulse of aggregated-BSA quantity cleared during 48 min	
Exp.	In presence of plasma factors (mg)	In absence of plasma factors (mg)
1	0.50	0.28
2	0.48	0.10
3	0.42	0.20
4	0.56	0.25
5	0.45	0.22
Mean ± SE	0.49 ± 0.03	0.21 ± 0.03

These experiments demonstrated that plasma factors facilitating phagocytosis by Kupfer cells of gold or aggregated-BSA are not specific. To demonstrate that the blockade was, indeed, due to depletion of a plasma factor and not to a cellular blockade, we used a double perfusion system. We added several doses of particulate (aggregated-BSA or gold) in the first perfusate and when blockade was established, we switched to the other perfusate and added the second particulate. Figures 3 and 4 summarize observations made in these models. It will be seen that when blockade occurred with several pulses of aggregated-BSA, colloidal gold was cleared rapidly when perfusate with a fresh supply of plasma factors was renewed (Fig. 3). The same was found to be true when perfusate was changed after blockade occurred for gold and then aggregated-BSA was added to the second perfusate. When, under these circumstances, the plasma factors were available-aggregated-BSA was cleared in an

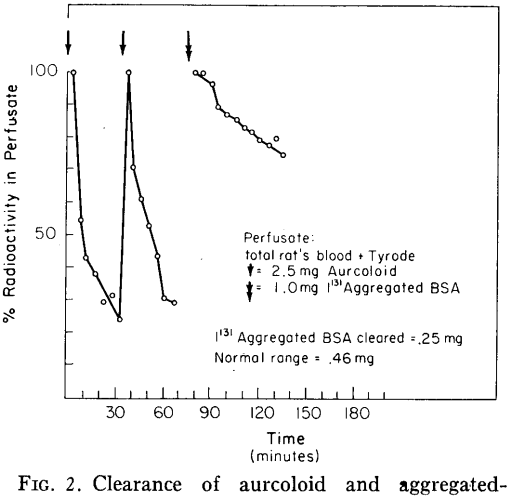

FIG. 2. Clearance of aurcoloid and aggregated-BSA by isolated perfused rat liver: after RES blockade appeared with two sequential pulses of Aurcoloid (2 × 2.5 mg), ^{131}I-aggregated-BSA (1 mg) was added into the perfusate; it's clearance rate was impaired by previous Aurocloid challenges.

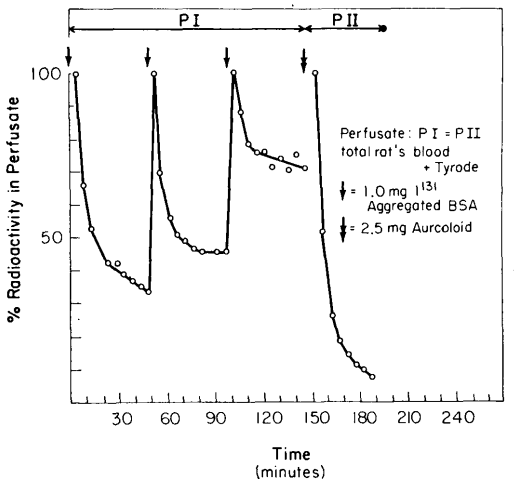

FIG. 3. Double perfusion of the isolated rat liver: when the isolated liver was perfused with perfusate I, RES blockade occurred after 3 pulses of ^{131}I-aggregated-BSA (3 × 1 mg). Renewal of the perfusate (II) with a fresh supply of plasma factors allowed rapid clearance of Aurcoloid solution (2.5 mg).

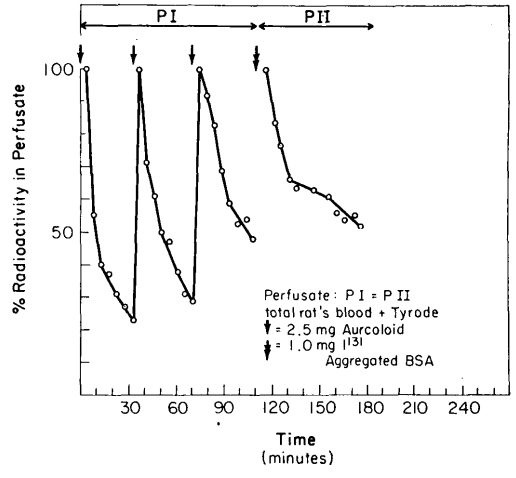

FIG. 4. Double perfusion of the isolated rat liver: when the isolated liver was perfused with perfusate I, RES blockade occurred after 3 pulses of Aurcoloid solution (3 × 2.5 mg). Renewal of the perfusate (II) with a fresh supply of plasma factors allowed rapid clearance of ^{131}I-aggregated-BSA (1 mg). Indeed in these conditions 0.40 mg of ^{131}I-aggregated-BSA were cleared in 45 min in contrast with 0.25 mg in presence of plasma factor depletion as demonstrated by Fig. 2.

amount compatible with the presence of opsonins and unaltered RE cells.

Discussion. In prior studies, several investigations have brought forward evidence that blockade of the reticuloendothelial system can be attributed to depletion of opsonins (6–8). Others have argued that reticuloendothelial blockade is due to an alteration of the function of the RE cells (9–11). Our own previous studies have shown that for clearance of colloidal gold, RES blockade, as studied in an isolated perfused liver system is attributable entirely to depletion of plasma opsonins (1). By contrast, we showed that RES blockade for aggregated-BSA involves both exhaustion of a cellular function and depletion of a plasma factor (2).

In the present studies we showed that the plasma opsonin operating to facilitate RES function, which is depelted when blockade of this system is established, is to some extent nonspecific. We found, for example, that depletion of the plasma opsonin by phagocytosis of colloidal gold depletes the opsonic factor facilitating RES phagocytosis of aggregated-BSA. On the other hand, depletion of the plasma opsonin by phagocytosis of aggregated-BSA depletes the plasma factor facilitating RES phagocytosis of colloidal gold. Fresh plasma reverses, for aggregated-BSA, the RES blockade induced by phagocytosis of successive pulses of colloidal gold.

The nature of the plasma opsonins still remains enigmatic. Observations indicate that among the factors at least some do not possess a high degree of serological specificity. That these substances which may be depleted to provide one form of RES blockade are not entirely nonspecific has, however, been shown in a recent study from our laboratory (12). Here we found that production of RES blockade in the isolated perfused rat liver with repeated pulses of *Salmonella typhosa* or *Brucella melitensis*, which could be reversed with fresh plasma, did not deplete the plasma opsonin that facilitates RES phagocytosis of colloidal gold. Our findings must be considered in light of the observations of Normann and Benditt (13). These investigators showed that clearance of denatured BSA *in vivo* depletes a plasma factor facilitating clearance of carbon. Here again, lack of spe-

cificity of a presumed opsonin was demonstrated. The present observations would be entirely in concert with Normann's view for the rat. In the rabbit, however, Lirenmann *et al.* (14) could find no evidence for the circulating factor defined by Normann for the rat. Differences in both species and differences from one particulate matter to another make it essential when speaking of those opsonins operative in RES function to define precisely the system under study in all particulars.

The analysis of the relationships of the opsonins for aggregated-BSA and colloidal gold presented here illustrate, once again, the usefulness of the isolated perfused rat liver and the double perfusion system for assaying many questions concerning the function of the reticuloendothelial system.

Summary. Plasma opsonins facilitating RES phagocytosis of aggregated-BSA and colloidal gold were studied in a double isolated perfused rat liver system. Activity of the nonspecific plasma opsonin for both colloidal gold and BSA was demonstrated. The relationship of this factor to other plasma opsonins is considered.

1. Jeunet, F. S. and Good, R. A., RES, J. Reticuloendothelial Soc. **4**, 351 (1967).

2. Jeunet, F. S. and Good, R. A., RES, J. Reticuloendothelial Soc. **6**, 94 (1969).

3. Jeunet, F. S. and Quitt, J., J. Physiol. **54**, 625 (1962).

4. Koblet, H., Diggelmann, H., and Jeunet, F., Helv. Med. Acta **31**, 169 (1964).

5. Clawson, C. C., Jeunet, F. S., and Good, R. A., in preparation.

6. Jenkin, C. R. and Rowley, D., J. Exptl. Med. **114**, 363 (1961).

7. Murray, I. M., J. Exptl. Med. **117**, 139 (1963).

8. Filkins, J. P., Chase, R. E., and Smith, J. J., RES, J. Reticuloendothelial Soc. **2**, 287 (1965).

9. Halpern, B. N., Biozzi, G., Mene, G., and Benacerraf, B., Ann. Inst. Pasteur **80**, 582 (1951).

10. Biozzi, G., Halpern, B. N., Benacerraf, B., and Stiffel, C., *in* "Physiopathology of the RES," p. 204. Blackwell, Oxford (1957).

11. Biozzi, G., Stiffel, C., Halpern, B. N., and Mouton, D., Proc. Soc. Exptl. Biol. Med. **112**, 1017 (1963).

12. Jeunet, F. S., Cain W. A., and Good, R. A., RES, J. Reticuloendothelial Soc. **6**, (1969).

13. Normann, S. J. and Benditt, E. P., J. Exptl. Med. **122**, 709 (1965).

14. Lirenmann, D. S., Fish, A. J., and Good, R. A., RES, J. Reticuloendothelial Soc. **4**, 34 (1967).

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