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Mechanism of Gelatin Inhibition of Reticuloendothelial Function.* (31084)

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The functional activity of the reticuloendothelial system (RES) is commonly evaluated by measuring the intravascular clearance of a variety of inert colloids. In addition, RES hypofunction or "blockade" is often experimentally induced by injection of massive doses of particulate matter. Foremost among the agents used to measure and depress RES function are gelatin stabilized colloids such as carbon or gold(1).

Previous studies have demonstrated that high concentrations of gelatin in preparations of chromic phosphate(1,2), carbon(3), or gold(4) markedly retarded intravascular removal of the respective colloid. Prior adminjstration of gelatin also depressed the clearance of subsequent test doses of colloid *in vivo* (5,6,7) as well as in the isolated perfused rat liver(8).

Several explanations have been offered for the mechanism by which gelatin inhibits RES function. It has been postulated that gelatin may retard particle clearance because it either competes with the particle for phagocytic clones or saturates the phagocytic capacity of the RES(3,6). However, it may also inhibit clearance by interaction with, or depletion of, plasma factors or "opsonins" essential for phagocytosis(5,8,9,10). In addition gelatin may directly inhibit the RES(2,7). This study presents evidence that gelatin inhibits colloidal radiogold uptake by rat liver slices due to its interaction with a plasma opsonic system.

Methods. The proceduce employed in evaluating phagocytosis by rat liver slices was essentially as previously described(11). In brief, 300-400 mg slices of liver tissue from male Holtzman rats (300-330 g) were incubated in 3 ml of pooled heparinized (100 U.S.P. units/ml) plasma containing 400 μg of either colloidal radiogold (Abbott Laboratories, North Chicago, Ill.) or 1-5 μ human albumin aggregates labeled with radioiodine (E. R. Squibb and Sons, New Brunswick, N. J.). After 30 minutes of agitated incubation at 37.5°C the liver slices were removed, washed, weighed, and their accumulated radioactivity measured. The uptake was calculated as a per cent of the added dose per 100 mg of liver tissue.

Gelatin was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, and was prepared and neutralized in 0.9% saline immediately prior to use.

Heparinized plasma (100 U.S.P. units/ml) was also obtained from mice (A/J males, Jackson Laboratories, Bar Harbor, Maine), rabbits (3 kg, male, New Zealand Whites), and dogs (5 kg, male mongrels).

Results. Effect of gelatin on colloidal gold and denatured albumin uptakes by rat liver slices. To ascertain whether gelatin would inhibit phagocytosis in the liver slice system, a series of experiments was performed in which varying amounts of gelatin were added to heparinized plasma prior to the addition of either the gold or albumin aggregates and the liver slices. As indicated in Fig. 1, gelatin markedly inhibited gold uptake with practically complete suppression occurring at 320 μg/ml; however, a gelatin dose as low as 10 μg/ml produced a 35% depression in colloidal

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gold phagocytosis. The phagocytosis of colloidal gold in control liver slices was 15.6% of the added dose per 100 mg of tissue. In contrast to the inhibitory effect of gelatin on the phagocytosis of colloidal gold, the presence of gelatin had no appreciable effect on denatured albumin uptake at doses below 80 μg/ml. At higher gelatin concentrations the removal of denatured albumin particles was significantly impaired from a control uptake of 9.95% of the added dose per 100 mg of liver tissue.

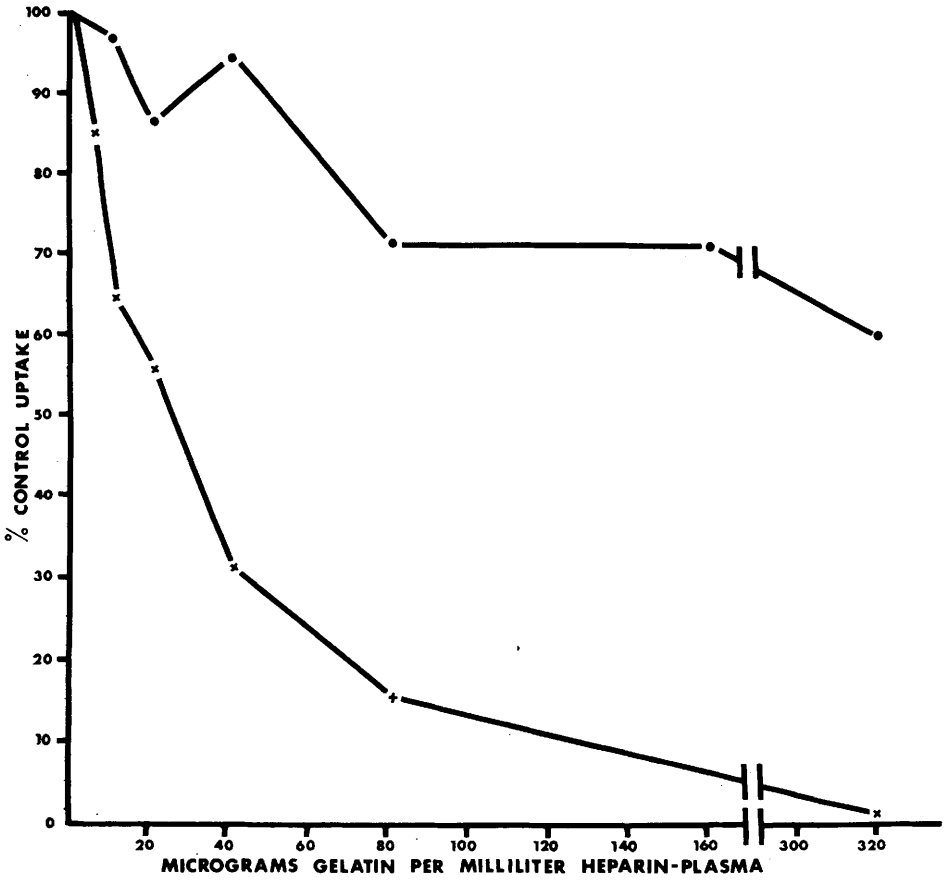

FIG. 1. Effect of gelatin on phagocytosis of colloidal gold and human albumin aggregates by rat liver slices. x——x colloidal gold; ●——● albumin aggregate. Each point represents average of 6 samples from 3 livers. Average standard error approximated 3% of indicated uptake.

TABLE I. Effect of Pre-Incubation of Colloidal Gold (10 Min at 25°C) with Heparinized Rat Plasma on Gelatin Inhibition of Phagocytosis by Liver Slices *in vitro*.

Group	Treatment	No. livers	No. samples	% Injected dose/100 mg liver tissue* (mean ± S.E.)
1	Gold incubated with heparinized plasma; no gelatin added	8	27	8.60 ± .53
2	Gold incubated with heparinized plasma prior to addition of gelatin (20 μg/ml)	8	29	8.25 ± .56
3	Gold incubated with heparinized plasma after addition of gelatin (20 μg/ml)	9	46	3.47 ± .19†

* Value expressed as per cent of added radioactivity (% I.D.) recovered from liver slices after 30 min incubation at 37.5°C.

† $P < .001$ as compared to Groups 1 or 2 using Student's "t" test.

TABLE II. Comparative *in vitro* Phagocytosis of Radiogold.

Liver donor	Plasma donor	No. livers	No. samples	% I.D./100 mg liver tissue (mean ± S.E.)
Mouse	Mouse	8	8	20.1 ±4.5
Rat	Rat	4	12	18.0 ± .70
Dog	Dog	5	39	11.5 ± .66
Rabbit	Rabbit	7	43	.84 ± .10

TABLE III. Phagocytosis of Colloidal Gold by Combinations of Rat and Rabbit Liver in Presence of Isologous or Heterologous Plasma.

Liver donor	Plasma donor	No. livers	No. samples	% I.D./100 mg liver tissue (mean ± S.E.)
Rabbit	Rabbit	3	13	.79 ± .06
"	Rat	3	13	11.5 ±1.04
Rat	Rabbit	3	15	.66 ± .06
"	Rat	3	12	8.77 ± .59

Effect of pre-incubation of gold with heparin plasma on gelatin effect. In a series of experiments colloidal gold was incubated with heparinized plasma prior to and after addition of gelatin. As shown in Table I, gelatin had no inhibitory effect on the phagocytosis of gold particles which were pre-incubated in plasma (Group 2). In contrast, marked depression was observed when gelatin was added to plasma prior to addition of colloidal gold (Group 3). These findings negate a significant inhibitory effect of gelatin *per se* on the phagocytes and support the concept that gelatin interacts with an opsonin(5,8).

Radiogold uptake in plasma of other animal species. Previous investigations of the effect of gelatin on the RES have employed diverse animal species(1,2,3,5,6). To evaluate the general significance of the opsonin for gold, plasma of 4 common laboratory animals was evaluated for its opsonic activity. As indicated in Table II, heparinized plasma of the mouse, rat and dog supported the efficient phagocytosis of radiogold by the respective liver slice. In contrast, heparinized rabbit plasma failed to promote efficient phagocytosis of gold by rabbit liver slices. However, as shown in Table III, rabbit liver slices incubated in heparinized rat plasma accumulated gold to a significant degree. In addition, rat liver slices in the presence of rabbit plasma displayed minimal uptake. These findings suggest the rabbit lacks a plasma factor essential for *in vitro* gold phagocytosis which is present in the plasma of dogs, rats, and mice. Furthermore, non-specific protein absorption onto the surface of the gold colloid as the sole mechanism of gold opsonization is minimized by the inefficacy of rabbit plasma to opsonize colloidal gold.

Discussion. The inhibitory effect of gelatin on the hepatic phagocytosis of a gelatin stabilized colloid is again clearly illustrated in these studies. In addition, the postulated interaction of gelatin with plasma opsonin (5,8) is supported by data showing gelatin has no effect on the phagocytosis of pre-incubated gold particles which would have been opsonized prior to addition of gelatin. The present findings are consistent with *in vivo* data regarding the importance of opsonins in the phagocytosis of gelatin-stabilized colloids (5,10). The relatively slight but significant inhibitory effect of gelatin on the phagocytosis of denatured human albumin aggregates may reflect either a direct effect of gelatin on the RES or some interaction between the opsonic systems for gelatin-stabilized colloids and albumin aggregates(11). The finding that albumin aggregates inhibit carbon clearance *in vivo* (3,12) lends credence to this suggestion. Indeed, an opsonic system may interact with surface chemical groupings which are similar in the degraded protein, gelatin, and denatured proteins.

The finding that rabbit plasma lacks the opsonin for gold is of importance in evaluating the detailed studies of Koenig *et al*(7) on the dynamics of reticuloendothelial blockade. Their interpretation of RES blockade as not representing opsonin depletion may be limited to the rabbit. Indeed, opsonin depletion probably represents the mechanism of blockade induced by gelatin containing colloids in most other species.

The nature of the opsonin for gelatin-stabilized colloids is uncertain. It may be a natural antibody to gelatin *per se,* since it has been shown that rats and dogs but not rabbits possess such an antibody(13,14). It is conceivable that the same factor may be involved in opsinization of all gelatin-stabilized colloids and its role should be considered in

evaluating numerous studies equating RES function with rates of particle phagocytosis.

Summary. Gelatin markedly inhibited the uptake of colloidal gold by rat liver slices incubated in heparinized rat plasma. In contrast, the phagocytosis of albumin aggregates was affected only at high gelatin doses. Gelatin had no inhibitory effect on gold particles previously incubated with heparinized plasma. Plasma of mice, dogs, and rats enhanced uptake by the respective liver slice. Rabbit plasma failed to support phagocytosis by rabbit or rat liver slices; however, rat plasma facilitated phagocytosis by rabbit liver. It is suggested that rabbit plasma is deficient in a factor essential for *in vitro* hepatic phagocytosis. It is also indicated that gelatin interacts with plasma opsonin in rat plasma and thereby induces reticuloendothelial depression.

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Effect of Hypertrophy on Myocardial Distensibility. (31085)

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The development of ventricular hypertrophy has long been recognized to be a fundamental adaptive mechanism utilized by the heart subjected to a chronically increased work load. However, only recently has detailed attention been given to the physiologic, biochemical and morphologic changes which are associated with the development of an increased myocardial mass. While distinct alterations at the cellular level have been demonstrated during and following the development of ventricular hypertrophy(1-4), the relations between these changes and the function of the myocardium remain to be elucidated. It has been reported that the development of hypertrophy permits the ventricle to attain a greater level of performance than normal, as reflected in the maximum pressure (5) or cardiac output it can achieve(6). Kerr *et al* found that hypertrophied papillary muscle was capable of developing greater than normal levels of active tension. This augmented tension was not due solely to an increase in the mass of the hypertrophied muscle, since the maximum developed tension per mg of hypertrophied myocardium also exceeded the normal(7). In contrast, Grimm and associates reported that hypertrophied and normal papillary muscles developed similar peak tensions when corrections were made for differences in muscle mass(8).

It is widely appreciated that cardiac hypertrophy decreases the apparent distensibility of the ventricle. A number of clinical studies have demonstrated marked elevations of left ventricular end-diastolic pressures with little or no increase of ventricular end-diastolic volumes in patients with marked ventricular hypertrophy(9-12). The problem of whether these changes are merely due to an increase in the ventricular muscle mass without a change in the distensibility of each unit of myocardium