The Interaction of Platelets, Salmonella, and Mouse Peritoneal Macrophages[1] (34304)

G. L. MANDELL[2] AND E. W. HOOK[2]

Department of Medicine, New York Hospital–Cornell Medical Center, New York, New York 10021

Thrombocytopenia frequently occurs in humans and animals with bacteremia (1). Intravenous injection of particles such as carbon and latex also results in thrombocytopenia. Mechanisms responsible for the decrease in circulating platelets are not fully understood but probably include platelet aggregation and increased removal of platelets by the reticuloendothelial system (2).

Aggregation of platelets with bacteria *in vitro* (3) and *in vivo* (4) has been well documented. It is possible that phagocytosis of these platelet–bacterial aggregates contributes to the thrombocytopenia observed with bacteremia.

The present studies were undertaken to determine if platelets are ingested by macrophages during phagocytosis of bacteria. In addition, the influence of platelets on the capacity of macrophages to phagocytize bacteria was evaluated.

Material and Methods. All glassware, except coverslips, was baked for 2 hr at 170° and was freshly siliconized prior to use. Coverslips were washed in Microsalv detergent (Microbiological Associates, Inc. Bethesda, Maryland) and rinsed 12 times in tap water and 4 times in distilled water before being autoclaved.

Male Swiss mice (CF1 strain, Carworth Farms, New City, N.Y.) served as the source of peritoneal macrophages and platelets. Mice were killed and the peritoneums were washed immediately with 5 ml of cold medium 199 (Microbiological Associates) containing 20% heat-inactivated fetal calf serum (Microbiological Associates). One-half ml of the peritoneal wash was layered on coverslips in Leighton Tubes (5) and incubated for 60 min in 5% CO_2 in air at 37°. During the incubation period the macrophages settled and adhered to the surface of the coverslips.

Mouse platelet-rich plasma (PRP) was prepared from heart blood anticoagulated with 3.8% sodium citrate (10% by volume). Centrifugation at 300 *g* for 15 min produced a turbid supernatant rich in platelets (count 700,000/mm^3) and poor in erythrocytes and leukocytes. Platelet-poor plasma (PPP) was prepared by centrifugation of citrated mouse blood at 1900 *g* for 25 min. Prior to use in these experiments, PRP and PPP were recalcified and heparinized by the addition of 2 parts of medium 199 containing 15 units of heparin (Connaught Medical Research Labs., Toronto, Canada) and 0.34 mg of $CaCl_2$/ml.

Salmonella typhimurium (strain kk) was used as the test organism. An 18-hr culture in trypticase soy broth was washed twice and diluted 10-fold with normal saline. The organisms were opsonized by adding heat-inactivated rabbit *S. typhimurium* antiserum (5) in a final concentration of 5% and incubating at 37° for 15 min. One-tenth ml of opsonized *S. typhimurium* (10^6 to 10^7 viable organisms) was then incubated with 1 ml of PRP or PPP for 30 min in a shaker bath at 37°. One-half ml of the mixture of opsonized bacteria and PPP or PRP was then placed on a coverslip previously layered with mouse macrophages, and incubated for 60 min in a 5% CO_2 in air incubator at 37°. The coverslips were then fixed in methanol for 1 min and stained with a 5% aqueous solution of Giemsa stain for 30 min. These preparations were examined microscopically to count the numbers of intracellular salmonella and de-

[1] Supported in part by NIH grants TOI AI 00255 and AI 05940 and contract U-1107 with The Health Research Council of the City of New York.

[2] Present address: University of Virginia School of Medicine, Charlottesville, Virginia 22903.

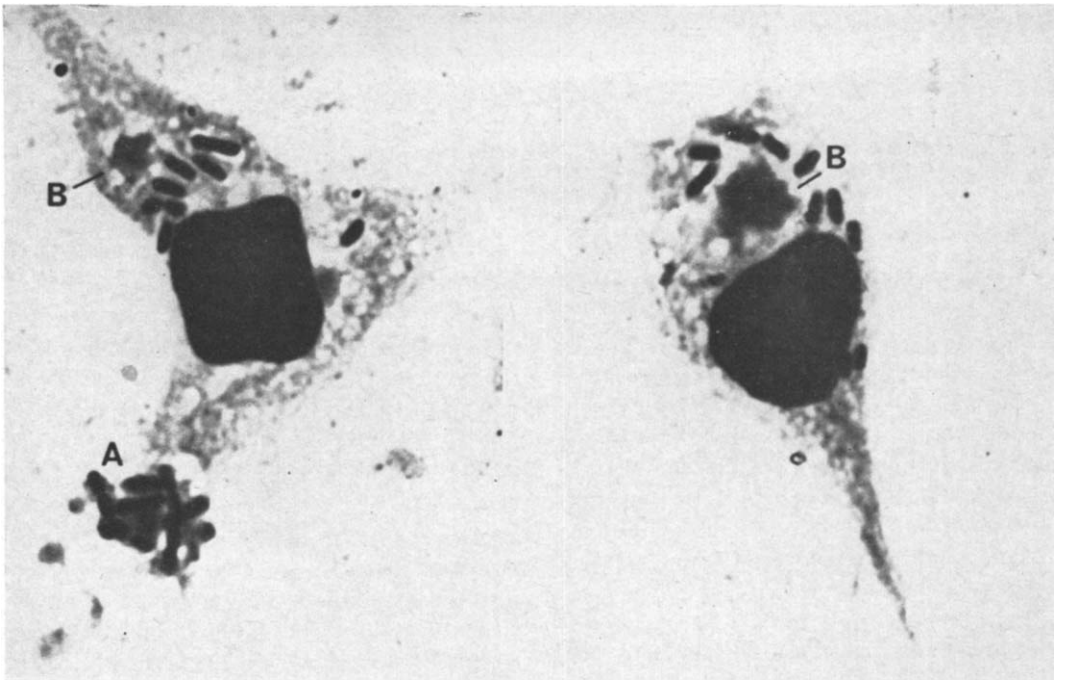

FIG. 1. Phagocytosis of platelets (B) and salmonella by macrophages in the presence of PRP; Many extracellular platelet–bacterial aggregates (A) were observed in these preparations.

termine the presence or absence of platelet phagocytosis by macrophages.

Results. Many platelet–bacterial aggregates were observed in coverslip preparations of macrophages, salmonella, and PRP. Almost all of the macrophages contained intracellular salmonella and, in addition, numerous platelets (Fig. 1). Platelets were not phagocytized when macrophages were exposed to PRP without bacteria.

Quantitation of phagocytosis of bacteria showed that twice as many organisms were ingested by macrophages when the bacteria were suspended in PRP as when bacteria were suspended in PPP (Table I). Frozen and thawed PRP was ineffective in increasing phagocytosis by macrophages.

TABLE I. Phagocytosis of Opsonized *S. typhimurium* in PRP or PPP.

	Bacteria per 100 macrophages		
Expt.	I	II	III
PRP	540	488	330
PPP	252	232	120

Discussion. Our observations show that phagocytosis of bacteria by macrophages is accompanied by active phagocytosis of platelets. Although several mechanisms are probably involved in the thrombocytopenia associated with bacteremia, platelet phagocytosis *in vivo* may play a role.

It is clear that the interaction of platelets and bacteria results in marked alterations in platelets, including changes in morphology and release of platelet constitutents (3). It is likely that these alterations render platelets more susceptible to phagocytosis. Alteration or damage of platelets by a number of different mechanisms has been shown to be associated with phagocytosis of platelets. Movat and colleagues found that platelets altered by exposure to latex particles are phagocytized by polymorphonuclear leukocytes and mononuclear cells *in vitro* (6, 7). In addition, altered platelets in thrombi are phagocytized *in vivo* by polymorphonuclear leukocytes and macrophages (8, 9). Immunologic injury may also make platelets more susceptible to phagocytosis (10).

It is also of interest that intact platelets, but not frozen and thawed platelets, enhanced phagocytosis of bacteria *in vitro*. The most likely explanation of this phenomenon is that the clumping of bacteria in platelet–bacteria aggregates increased the number of organisms coming in contact with phagocytizing macrophages.

Summary. Mouse peritoneal macrophages phagocytize platelets along with opsonized *Salmonella typhimurium,* but do not phagocytize platelets in the absence of bacteria. The presence of intact platelets results in an increase in the number of bacteria ingested by macrophages *in vitro*.

1. Cohen, P. and Gardner, F. H., Arch. Internal Med. **117**, 113 (1966).

2. Cohen, P., Braunwald, J., and Gardner, F. W., J. Lab. Clin. Med. **66**, 263 (1965).

3. Des Prez, R. M., Horowitz, H. I., and Hook, E. W., J. Exptl. Med. **114**, 857 (1961).

4. Bull, C. G. and McKee, C. M., Am. J. Hyg. **2**, 208 (1922).

5. Gill, F. A., Kaye, D., and Hook, E. W., J. Exptl. Med. **124**, 173 (1966).

6. Movat, H. Z., Weiser, W. J., Glynn, M. F., and Mustard, J. F., J. Cell. Biol. **27**, 531 (1965).

7. Mustard, J. F. and Packham, M. A., Ser. Haematol. **1**, 2, 168 (1968).

8. Poole, J. C., Quart, J. Exptl. Physiol. **51**, 54 (1966).

9. Jorgensen, L., Rowsell, H. C., Hovig, T., and Mustard, J. F., Am. J. Pathol. **51**, 681 (1967).

10. Barker, B. E., Farnes, P., and LaMarche, P. H., Lancet **1**, 437 (1967).

Received June 24, 1969. P.S.E.B.M., 1969, Vol. 132.