An Electron Microscopic Study of Thrombin-Induced Disseminated Intravascular Coagulation

By William Margaretten, Ilona Csavossy and Donald G. McKay

The GENERALIZED SHWARTZMAN REACTION is ordinarily elicited in rabbits by two appropriately spaced injections of bacterial endotoxin.1 The lesion which identifies the reaction, renal cortical necrosis, is due to an obstruction of the glomerular capillary circulation by fibrin thrombi.2 The occluding material has been identified as fibrin by immunofluorescent3 and electron microscopic methods.4 An identical lesion can be produced by the slow intravenous infusion of thrombin.5 At the present time the majority of evidence supports the concept that the clotting episode is triggered by an effect of endotoxin on platelets.6 Endotoxin induces aggregation of platelets in vitro7 and in vivo8 to release platelet factor 3.

In the classical Shwartzman reaction the first injection of endotoxin is considered to be the "preparing" dose and the second injection the "provoking" dose. There is some controversy concerning the mechanism of "preparation." Pregnancy,9,10 as well as such diverse agents as Thorotrast®11,12 epsilon-amino caproic acid,13 and steroids14 can be substituted for the first injection. That is, the lesions can then be elicited by one rather than two injections of endotoxin.

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This study was aided by grants HE-05666-06 and 5 T1 GM-865-04 from the National Institutes of Health, USPHS, Bethesda, Md.

First submitted Jan. 17, 1966; accepted for publication Aug. 2, 1966.

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Abbreviations Used in Figures

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>F</td>
<td>Fibrin</td>
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<tr>
<td>EP</td>
<td>Epithelial cell</td>
</tr>
<tr>
<td>END</td>
<td>Endothelial cell</td>
</tr>
<tr>
<td>MS</td>
<td>Mesangial cell</td>
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<tr>
<td>U</td>
<td>Urinary space</td>
</tr>
<tr>
<td>BM</td>
<td>Basement membrane</td>
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<tr>
<td>RBC</td>
<td>Red blood cell</td>
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<tr>
<td>P</td>
<td>Platelet</td>
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<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocyte</td>
</tr>
<tr>
<td>K</td>
<td>Kupffer cell</td>
</tr>
<tr>
<td>HEP</td>
<td>Hepatic parenchymal cell</td>
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<tr>
<td>LY</td>
<td>Lymphocyte</td>
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<tr>
<td>ALV</td>
<td>Alveolus</td>
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<tr>
<td>N</td>
<td>Nucleus of endothelial cell</td>
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<tr>
<td>C</td>
<td>Collagen</td>
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Blood, Vol. 29, No. 2 (February), 1967
Lee maintains that “preparation” is mediated by reticuloendothelial blockade which has already been demonstrated in animals injected with the above agents. According to this theory fibrin macromolecules, which appear in the circulation after a “provoking” injection of endotoxin, cannot be removed by the previously blockaded reticuloendothelial system and lodge in glomerular capillaries and the afferent glomerular arterioles are occluded by fibrin thrombi similar to those seen in the endotoxin Shwartzman reaction. Phosphotungstic acid hematoxylin. × 700.
Fig. 2.—Kidney. Rat sacrificed 30 minutes after the completion of a 30-minute infusion. The glomerular capillary loops are patent and free of fibrin. Endothelial and epithelial cells are large and hyperchromatic, secondary to previous anoxia. Hematoxylin and eosin. × 700.

capillaries. Since reticuloendothelial blockade is of relatively short duration and never complete, it seems likely that the more prolonged circulation of an active macromolecular clotting intermediate (i.e., thromboplastin) is of greater significance than a delayed uptake of fibrin.14

Pregnancy is not associated with reticuloendothelial blockade, thus differing
Figs. 3 and 4.—See legends on facing page.
from other types of "preparation." Pregnant rats can remove carbon particles from the circulation more rapidly than their nonpregnant controls. The mechanism of "preparation" by pregnancy has been studied in rats that were given a prolonged intravenous infusion of thrombin. Fibrin thrombi persisted in renal glomeruli of the pregnant animals compared with their nonpregnant controls to a degree significant for preparation of the Shwartzman reaction. If the nonpregnant rats received, in addition, epsilon-aminocaproic acid, a potent antifibrinolytic agent, glomerular thrombi persisted in a similar manner. This study strongly implicates delayed or impaired fibrinolysis as the mechanism of "preparation" by pregnancy.

The present experiments, using high resolution electron microscopy, have been designed to elucidate the morphologic characteristics of fibrin deposition and fibrin removal in a typical "fibrination-defibrination" syndrome. The experimental model selected is thrombin-induced disseminated intravascular coagulation in the nonpregnant rat. In this circumstance the animal is slowly defibrinated, but has not been "prepared" for the generalized Shwartzman reaction, and renal cortical necrosis does not result. The analogy to the Schwartzman reaction is based on the assumption that there are similar mechanisms for the localization and disappearance of fibrin in endotoxin initiated intravascular coagulation.

The following electron micrographs clearly illustrate a widespread dissemination of intravascular fibrin during prolonged defibrination. Of more importance, the sudden dissolution of intraluminal thrombi indicates that fibrinolysis is an important factor in the removal of intravascular fibrin. The corollary is suggested that inhibition of fibrinolysis is an effective method of "preparation" for the Shwartzman reaction.

**Materials and Methods**

Virgin female rats of the Columbia-Sherman strain were used in all experiments. Topical thrombin (Parke-Davis Co., Detroit) was reconstituted in sterile, pyrogen-free, isotonic saline and injected intravenously into ten rats via a tail vein catheter. The duration of infusion was varied from 15 minutes to 1 hour. The rate of infusion remained the same for each animal, 1.0–1.2 U./minute. The ten rats were divided into 5 groups of two on the basis of the total dose of thrombin injected and the time interval prior to sacrifice (Table 1). The rats of the first 3 groups were killed immediately after the infusion was completed. Groups 4 and 5 were sacrificed 30 minutes and 1 hour, respectively, after the thrombin infusion had ended, in order to study the mechanism of fibrin removal.

Sections of liver, lung, spleen, and kidney of each animal were prepared for light, phase, and electron microscopy. Routine formalin-fixed sections were stained with hematoxylin-eosin, phloxine-methylene blue, and phosphotungstic acid-hematoxylin. Tissues

**Fig. 3.—Kidney.** Rat sacrificed immediately after a 15-minute infusion of thrombin. Fibrin is present within the lumens of all the glomerular capillaries. An occasional red blood cell and platelet are seen. The endothelial cells, basement membranes, and epithelial cell foot processes are unremarkable. × 8,400.

**Fig. 4.—Kidney.** Rat sacrificed immediately after a 1-hour infusion of thrombin. Large amounts of fibrin occlude the capillary lumens. Intact platelets, identified by their granulomeres, are seen in close proximity to the thrombi. × 4,000.

The periodicity of fibrin (median = 202 Å) is shown at higher magnification in the inset. × 55,000.
Figs. 5 and 6.—See legends on facing page.
Table 1.—Results of Thrombin Infusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats</th>
<th>Duration of Infusion</th>
<th>Total Dose of Thrombin</th>
<th>Time Sacrificed</th>
<th>Thrombi Light Micro. Electron Micro.</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>15 minutes</td>
<td>15–18 units</td>
<td>immediately</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>30 minutes</td>
<td>30–36 units</td>
<td>immediately</td>
<td>+ glomeruli + all organs</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1 hour</td>
<td>60–72 units</td>
<td>immediately</td>
<td>+ glomeruli + all organs</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>30 minutes</td>
<td>30–36 units</td>
<td>30 minutes</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>30 minutes</td>
<td>30–36 units</td>
<td>1 hour</td>
<td>—</td>
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for electron microscopy were fixed in osmium and embedded in araldite. The technical details of the fixation, embedding and staining procedures have been described in a previous publication from this department. An RCA EMU 3 electron microscope was used for all the studies.

RESULTS

The presence or absence of fibrin thrombi in the five groups of rats is listed in Table 1. With the light microscope thrombi are not seen at 15 minutes (Group 1). At the end of the 30-minute infusion (Group 2) and 1 hour infusion (Group 3), fibrin is present in glomeruli to a degree comparable to the generalized Shwartzman reaction (Fig. 1). However, if the animals are not sacrificed immediately, but remain alive for 30 minutes (Group 4) or 60 minutes (Group 5) following completion of the infusion, the glomerular capillary loops are patent again (Fig. 2).

The results obtained with electron microscopy differ in that fibrin, identified by its characteristic periodicity, is present at the earliest time studied, 15 minutes. Furthermore, large amounts of fibrin can be recognized within capillaries of the liver, lung, and spleen, in addition to the glomeruli.

The acute glomerular alterations following thrombin infusion are seen in Figures 3–6. Fibrin with a median periodicity of 202 Å is present in most of the capillaries at 15 minutes (Fig. 3). The periodicity of fibrin in these studies deserves attention. Hall and Slayter found a periodicity of 240 Å units in turtle fibrin that was spread out on a flat surface. In our osmium-fixed sections measurements of the periodicity of various fibrin deposits ranged from 175 Å to 240 Å, with a mean of 202 Å. The difference probably lies in the fact that osmium fixation causes shrinkage of tissue. Such shrinkage coupled possibly with a compression at the time of thin sectioning, as well as tangential cuts, probably accounts for the shorter period in the histologic preparation. Fibrin deposited in the Shwartzman reaction induced by dietary means as well as by endotoxin has the same average periodicity (202 Å units).

At the end of the 1-hour infusion large quantities of granular and fibrillar fibrin...
Figs. 7 and 8.—See legends on facing page.
fibrin occlude the capillary lumens (Fig. 4). Figures 5 and 6 are selected to illustrate representative glomeruli of the animals sacrificed at timed intervals (Groups 4 and 5). Small amounts of fibrin of decreased osmiophilia are occasionally seen in the rats permitted to live 30 minutes (Fig. 5). In rats surviving the infusion by 1 hour, the glomerular capillary loops are completely free of fibrin (Fig. 6). The epithelial and endothelial cells appear swollen and vacuolated. There is fusion of the epithelial foot processes. The mesangial or intercapillary cells are hypertrophied and more prominent.

Electron micrographs of the liver show fibrin in hepatic sinusoids adjacent to the surface membranes of phagocytic Kupffer cells. Strands of fibrin are partially enclosed by the projecting pseudopods of a Kupffer cell in Figure 7. This animal was sacrificed immediately after a 15-minute infusion. The inset of Figure 7 demonstrates fibrin with its characteristic periodicity within the membranes of a phagocytic vesicle. This rat was sacrificed immediately after a 30-minute infusion of thrombin.

Aggregation and viscous metamorphosis of platelets are seen within the capillaries of all the organs studied, although this phenomenon is most prominent in the lungs. A pulmonary capillary in Figure 8 shows viscous metamorphosis of platelets after a 15 minute infusion. Many of the platelets appear to be degranulated and there is a central core of fibrin within the platelet mass. At the end of the 1-hour infusion, large amounts of fibrin occlude the pulmonary capillaries. In Figure 9 fibrin is seen in close proximity to the capillary endothelium which does not display phagocytic properties. In contrast to the ischemic changes of the renal glomerular endothelium, there is little morphologic evidence of capillary damage in the other organs studied.

Fibrin was found in the splenic sinusoids after the 15, 30, and 60-minute infusion of thrombin (Figure 10), completing its demonstration in all the organs studied by electron microscopy.

**DISCUSSION**

Prolonged intravenous infusion of thrombin induces disseminated intravascular coagulation by the enzymatic conversion of fibrinogen to fibrin as well as by aggregation and viscous metamorphosis of platelets. Lee elicited the generalized Shwartzman reaction in rabbits which received a thrombin

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**Fig. 7.—Liver.** Rat sacrificed at the end of a 15-minute infusion of thrombin. Pseudopods extending from the surface of a phagocytic Kupffer cell partially envelop strands of fibrin within the hepatic sinusoid. In general, fibrin was not seen in relation to the surface membranes of the endothelial cells of the hepatic sinusoids. $\times$ 11,200.

The inset is a high magnification of part of a single Kupffer cell. Fibrin with a periodicity of 202 Å is enclosed within a membrane limited phagocytic vacuole. This animal was sacrificed immediately after a 30-minute infusion of thrombin. $\times$ 25,600.

**Fig. 8.—Lung.** Rat sacrificed immediately after a 15-minute infusion of thrombin. Platelets within a pulmonary capillary show budding and degranulation characteristic of viscous metamorphosis. Strands of fibrin are clearly recognized centrally. Many of the platelets have lost their granulomeres. $\times$ 5,600.
Figs. 9 and 10.—See legends on facing page.
THROMBIN-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION

infusion after preparation by reticuloendothelial blockade. In his studies fibrin thrombi were localized in the glomerular capillaries (to the exclusion of other organs) of rabbits pretreated with Thorotrast, endotoxin, or epsilon-amino caproic acid. He attributed the effect of epsilon-amino caproic acid to diminished activity of the reticuloendothelial system as measured by delayed carbon clearances, rather than to inhibition of fibrinolysis.

It is apparent at the present time that "preparation" for the Shwartzman reaction can be mediated by more than one pathway. The state of "preparation" induced by pregnancy is related to the persistence of fibrin thrombi in glomerular capillaries for a significant time interval. Although it has not been possible to demonstrate a reduced proteolytic or fibrinolytic activity of the plasma of pregnant rats, Epstein has reported diminished tissue activator of plasminogen in the glomeruli of pregnant compared to nonpregnant rats. Beller has been able to produce the Shwartzman reaction in thrombin-infused rabbits treated with a wide variety of antifibrinolytic agents. The incidence of renal cortical necrosis was proportional to the concentration of the antifibrinolytic drug.

In the present experiments the thrombin-infused animals (rats) were not "prepared" for the generalized Shwartzman reaction, so that renal cortical necrosis did not occur. The greatest proportion of fibrin, by far, was present within the capillary lumens of all the organs studied by electron microscopy. Its abrupt disappearance from intraluminal sites after the infusion was completed implicates the process of intravascular fibrinolysis. If the reticuloendothelial system was effectively clearing the circulation of fibrin monomers and polymers during the conversion of fibrinogen to fibrin, a widespread distribution of intravascular thrombi could not be possible. The granuloplectic activity of the Kupffer cell during the uptake of fibrin is illustrated in the electron micrographs of the liver (Fig. 7). However, the amount of fibrin removed from the circulation in this manner lags far behind the rate of intravascular deposition. These morphologic observations, taken in conjunction with previous experiments in pregnant animals and with antifibrinolytic agents, support the concept that inhibition of fibrinolysis constitutes a method of "preparation" for the Shwartzman reaction.

There is some question whether the proteolytic enzymes and/or phagocytic activity of polymorphonuclear leukocytes play a role in the dissolution of intravascular fibrin. It is well-known that exudative neutrophils participate in the removal of extravascular fibrin. In addition an increasing number of white blood cells appear within intravascular thrombi as a function of time. However, the electron micrographs of the present paper give no support to their involvement in the early stages of intravascular dissolution of fibrin.

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Fig. 9.—Lung. Rat sacrificed immediately after a 1-hour infusion of thrombin. Fibrin with its characteristic periodicity is partially surrounded by platelet remnants. The capillary endothelium is unremarkable. × 11,200.

Fig. 10.—Spleen. Rat sacrificed immediately after a 15-minute infusion of thrombin. Fibrin is present in the lumen of a splenic sinusoid in close anatomic relation to degenerating platelets. × 11,200.
Vassalli has presented the fine structure alterations of the renal glomeruli which follow an episode of intravascular coagulation. The acute glomerular lesions, such as endothelial and epithelial cell vacuolization, probably represent an effect of ischemic capillary obstruction, although protracted fibrin deposition within the capillary wall itself could have a damaging action. Nevertheless, as a mechanism of fibrin removal, the transglomerular passage from the capillary lumen into the urinary space can hardly be effective compared with the rate of intravascular dissolution.

SUMMARY

Fibrin thrombi due to slow intravascular coagulation appear simultaneously in rat kidney, liver, lung, and spleen, although the kidney is the only organ to show fibrin by light microscopy. The strands of fibrin are frequently associated with aggregation and viscous metamorphosis of platelets, particularly in the lungs. Some fibrin is eliminated by the reticuloendothelial system and through damaged glomeruli, but the major mechanism of removal is intravascular dissolution. Ischemic changes secondary to thrombosis are more prominent in glomerular capillaries than in other tissues. The morphologic observations are discussed in relation to "preparation" for the generalized Shwartzman reaction.

SUMMARIO IN INTERLINGUA

Thrombos de fibrina causate per un lente coagulation intravascular occurre simultaneemente in le ren, le hepate, le pulmon, e le splen del ratto, ben que le ren es le sol organo que monstra fibrina in microscopia optic. Le filos de fibrina es frequentemente associate con aggregatos e un metamorphose viscose de plachettas, particularmente in le pulmon. Un certe quantitate de fibrina es eliminate per le systema reticuloendothelial e via lesionate glomerulos, sed le mechanismo principal del elimination es le dissolution intravascular. Alterationes ischemic secundari a thrombose es plus prominent in capillares glomerular que in altere tissus. Le observationes morphologic es commentate in relation al "preparation" pro le reaction generalisate de Shwartzman.

REFERENCES

THROMBIN-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION


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