PLATELETS AND THE SHWARTZMAN PHENOMENON*

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Plates 17 and 18

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There are many indications that the effects of endotoxin upon blood vessels (1), leucocytes (2, 3), and coagulation (4–6) are important in production of the Shwartzman reaction. The effects of endotoxin upon blood vessels and leucocytes cannot be disputed, but involvement of platelets in the intravascular clotting of the Shwartzman phenomenon has never been established with certainty.

Thrombin (7) and immunologic reactions (8), in addition to bacterial endotoxin (9), can provoke the Shwartzman phenomenon and affect coagulation. Platelets influence the rate of coagulation (15) and must be present in *in vitro* studies to demonstrate acceleration of coagulation by endotoxin or antigen-antibody reactions (16–18). Anticoagulation prevents the Shwartzman reaction, regardless of the anticoagulant used (10–12). Increased fibrinolysis prevents (13), and blockade of fibrinolysis enhances (14), the reaction. These facts suggest strongly that intravascular clotting possibly involving platelets may be essential in production of the Shwartzman reaction.

It has not been possible to produce thrombocytopenia selectively except with specific platelet antiserum. Therefore, studies were done to evaluate the effect of immunologically induced thrombocytopenia upon the local and generalized Shwartzman phenomena. Administration of antiplatelet serum to rabbits prepared with thorotrast produced renal lesions characteristic of the reaction. Immunologic thrombocytopenia did not inhibit the cutaneous hemorrhagic lesion of the local Shwartzman phenomenon produced by sequential injections of endotoxin intracutaneously and intravenously.

Materials and Methods

Animals.—Albino New Zealand rabbits, weighing approximately 5 pounds, and fed standard laboratory food and water *ad lib*, were used in all of the studies.

Thorotrast.—A sterile, stabilized, colloidal suspension of thorium dioxide (24 to 26 per cent by volume) obtained from Fellows-Testagar Co., Inc., Detroit, was given intravenously in a dose of 10 ml per animal.

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Materials given intravenously were dissolved and diluted in sterile, pyrogen-free, 0.85 per cent NaCl immediately prior to injection.

Endotoxin.—Lyophilized lipopolysaccharide of *Escherichia coli* 026:B6 was obtained from Difco Laboratories, Inc., Detroit.

Platelet Antiserum.—Platelet antiserum was prepared by immunization of guinea pigs with thrice washed rabbit platelets prepared from platelet rich rabbit plasma. The platelets were injected initially into the foot-pad after emulsification with complete Freund's adjuvant (Difco Laboratories, Inc.), and subsequently, subcutaneously after emulsification with incomplete Freund's adjuvant (Difco Laboratories, Inc.). The subcutaneous injections were given at weekly intervals for 3 months. At that time, serum was obtained and adsorbed with washed rabbit erythrocytes. Sera were tested for agglutinins against washed rabbit platelets and for the ability to cause thrombocytopenia in normal rabbits. One-half ml of pooled antiserum caused a reduction in the platelet count to less than 10,000 within 30 minutes after intravenous injection.

Guinea Pig Anti-Rabbit Albumin and Antigamma Globulin.—Rabbit albumin (Baltimore Biological Laboratories, West Chester, Pennsylvania) and rabbit gamma globulin (Mann Research Laboratories, New York) were emulsified with incomplete Freund's adjuvant (Difco Laboratories, Inc.) and injected subcutaneously into guinea pigs at weekly intervals for 3 months. Serum was obtained 2 weeks after the last injection and was tested for the presence of the corresponding precipitating antibodies.

Sheep-Anti-Rabbit Gamma Globulin.—Obtained from Baltimore Biological Laboratories.

Platelet Counts.—Platelet counts were determined in duplicate, using phase microscopy, according to the method of Brecher and Cronkite (19).

White Blood Cell Counts.—Total white blood cell counts, were determined in duplicate, using a Coulter Counter, as described by Richar and Breakell (20).

Pathologic Studies.—Animals were killed for pathological examination by the intravenous injection of lethal doses of sodium pentobarbital. Autopsies were performed immediately following sacrifice, and the abdominal organs examined. Tissues were fixed in 10 per cent formalin, and sections were stained with hematoxylin-eosin.

RESULTS

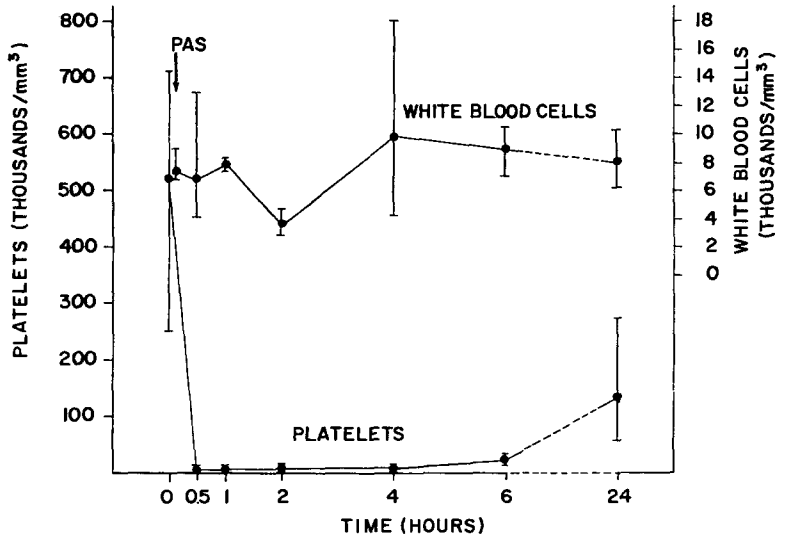
The effect of thrombocytopenia has not been investigated in previous studies on the production of the Shwartzman phenomenon. A method capable of producing thrombocytopenia in the rabbit without inducing concomitant leucopenia is by the employment of specific antiserum against rabbit platelets.

Guinea pig antiserum against rabbit platelets in a dose of 0.5 ml was injected intravenously into a group of rabbits. This produced marked thrombocytopenia within 30 minutes after injection. The platelet count fell below 10,000 per mm^3, remained at this level for about 6 hours, and thereafter gradually increased until at 24 hours it was approximately 30 per cent of the initial count. During this period, no significant leucopenia was observed (Text-fig. 1).

The administration of platelet antiserum did not result in renal cortical necrosis or significant deposition of "fibrinoid" material in the renal glomeruli characteristic of the generalized Shwartzman phenomenon. Occasional scattered glomeruli, however, appeared to contain minimal amounts of hyaline material (Fig. 1 *a* and 1 *b*).

Production of the Generalized Shwartzman Phenomenon.—Preliminary experi-

ments were done to assure an experimental method that would consistently produce the generalized Shwartzman phenomenon. Ten ml of thorotrast was administered intravenously to a group of animals, and 18 hours later each



TEXT-FIG. 1. Effect of 0.5 ml rabbit platelet guinea pig antiserum (PAS) administered intravenously upon the platelet and leucocyte count of normal rabbits; showing prompt development of thrombocytopenia with no appreciable change in leucocyte count.

TABLE I

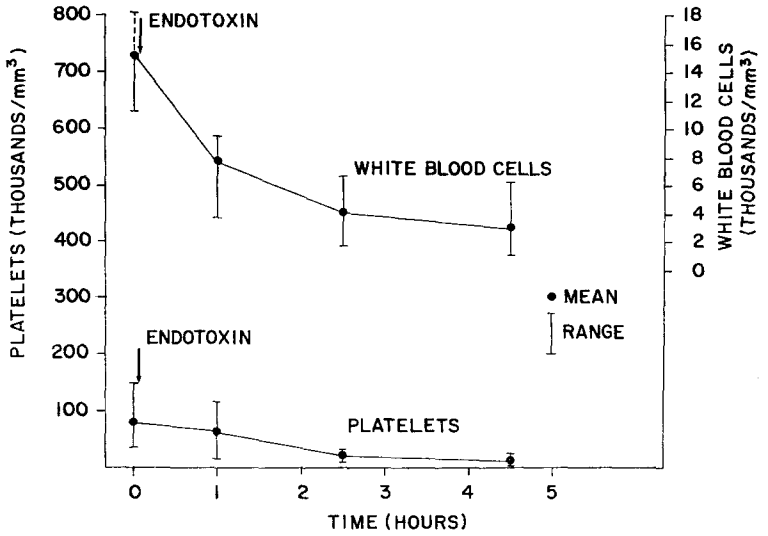
Production of the Generalized Shwartzman Phenomenon

Protocol of i.v. injections	Renal lesions (No. positive total animals)
Thorotrast (0 hrs.), endotoxin (18 hrs.)	14/21
PAS alone	0/7
Thorotrast (0 hrs.), PAS (17 hrs.), endotoxin (18 hrs.)	6/8
Thorotrast (0 hrs.), PAS (18 hrs.)	5/11
Thorotrast alone	0/7
Endotoxin alone	0/7
PAS (0 hrs.), endotoxin (1 hr.)	0/3

animal received 50 μg of *E. coli* endotoxin intravenously. The animals were autopsied at time of death or 24 hours later, and tissues preserved for histologic study.

There was renal cortical necrosis and marked deposition of fibrinoid material in the glomeruli in 66 per cent of the animals tested (Table I). These changes were characteristic of the generalized Shwartzman phenomenon (Fig. 1 *c*).

The animals developed marked leucopenia and thrombocytopenia within 2 hours after receiving the endotoxin (Text-fig. 2). It is noteworthy that many animals demonstrated both leucocytosis and thrombocytopenia 18 hours after receiving thorotrast and prior to receiving endotoxin, although in no instance was the platelet count below 10,000 per mm³ prior to administration of endotoxin. The level of circulating platelets prior to injection of endotoxin did not appear to influence the subsequent production of the renal lesions characteristic of the generalized Shwartzman phenomenon.



TEXT-FIG. 2. Effect of 50 μg *E. coli* endotoxin intravenously upon the platelet and total leucocyte count of rabbits given 10 ml thorotrast intravenously 18 hours previously. Zero hour is 18 hours after injection of thorotrast and immediately prior to injection of endotoxin.

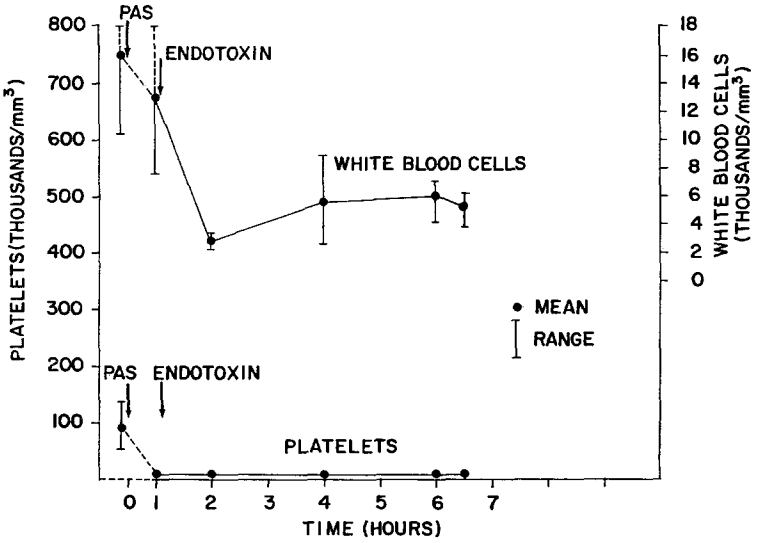
The effect of Thrombocytopenia upon the Generalized Shwartzman Phenomenon. —To determine whether circulating platelets were required for the development of fibrinoid deposits in the glomeruli and renal cortical necrosis in animals prepared with thorotrast and then challenged with endotoxin, the following experiment was performed.

A group of animals was given 10 ml of thorotrast intravenously and 17 hours later each animal received 0.5 ml of platelet antiserum intravenously. These animals developed severe thrombocytopenia without leucopenia immediately after the intravenous administration of the platelet antiserum (Text-fig. 3). One hour following the administration of the platelet antiserum and 18 hours after administration of thorotrast the animals received 50 μg of *E. coli* endotoxin intravenously. Thrombocytopenia persisted and leucopenia developed (Text-

fig. 3). The animals were autopsied at time of death or 24 hours later, and tissues preserved for histologic study.

Severe thrombocytopenia, induced by platelet antiserum prior to administration of a provocative dose of endotoxin did not prevent the appearance of fibrinoid material in the glomeruli and renal cortical necrosis in thorotrast prepared animals. Changes characteristic of the generalized Shwartzman phenomenon occurred in 6 of the 8 animals in this group (Table I).

The Effect of Platelet Antiserum upon Animals Prepared with Thorotrast.—



TEXT-FIG. 3. Changes in total leucocyte count and platelet count in rabbits given 10 ml Thorotrast intravenously, followed 17 hours later by 0.5 ml PAS intravenously. 50 μg endotoxin was given 1 hour after administration of the PAS. Zero hour is immediately prior to the injection of PAS.

The effect of platelet antiserum alone in thorotrast prepared rabbits was evaluated in the following experiments.

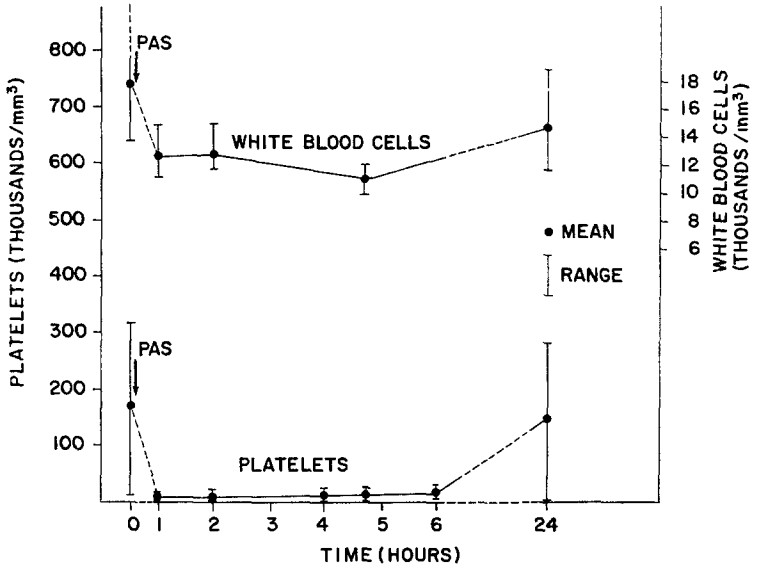
Ten ml of thorotrast was administered intravenously to a group of rabbits and 18 hours later the animals received 0.5 ml of platelet antiserum intravenously. These rabbits developed severe thrombocytopenia within 1 hour after receiving the platelet antiserum, but did not develop leucopenia (Text-fig. 4). The animals were autopsied and tissues preserved for study as before. Five of the 11 animals (45 per cent) developed renal lesions characteristic of the generalized Shwartzman phenomenon (Table I and Fig. 1 *d*).

The administration of 50 μg of endotoxin 1 hour after the intravenous administration of 0.5 ml of platelet antiserum did not result in changes in the

kidney different from those seen after platelet antiserum alone (Table I). The administration of either 10 ml of thorotrast alone or 50 μg of endotoxin alone did not result in the renal changes characteristic of the generalized Shwartzman phenomenon (Table I).

Production of the Local (Cutaneous) Shwartzman Phenomenon.—Preliminary studies were carried out to produce an experimental method that would consistently produce the local Shwartzman phenomenon.

A group of rabbits received 50 μg of endotoxin intracutaneously and 18 hours

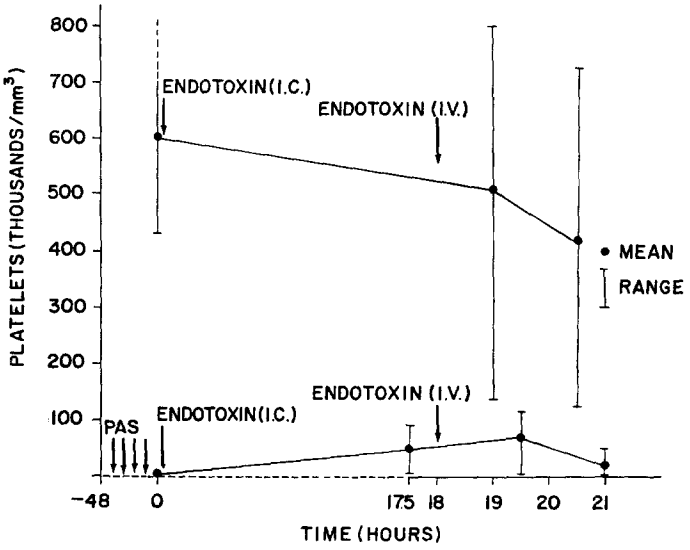


TEXT-FIG. 4. Changes in total leucocyte count and platelet count in rabbits given 10 ml thorotrast intravenously, followed 18 hours later by 0.5 ml of PAS intravenously. Showing thrombocytopenia but no leucopenia attributable to the PAS.

later each animal received 75 μg of endotoxin intravenously. Six hours after the second dose of endotoxin, the skin sites were examined. The animals were sacrificed as before, and the skin biopsied for histologic study as described in Materials and Methods.

Significant leucopenia developed in all animals, but thrombocytopenia developed in only half of the animals in this group after the administration of endotoxin intravenously (Text-fig. 5). Three of the 4 rabbits developed intradermal hemorrhages, intravascular thromboses, and a marked leucocytic infiltration, characteristic of the local Shwartzman phenomenon (Fig. 2 *a*). There appeared to be no relationship between the changes in circulating levels of platelets and white blood cells and the severity of the cutaneous lesions.

The Effect of Thrombocytopenia upon the Local Shwartzman Phenomenon.—To evaluate the effect of thrombocytopenia upon the development of the local Shwartzman phenomenon, a group of rabbits was pretreated with 4 intravenous injections of 0.5 ml of platelet antiserum during the 48 hour period preceding the first injection of endotoxin. These 4 injections of platelet antiserum were spaced approximately 10 hours apart and the last injection was given 4 hours prior to the intracutaneous injection of 50 μg of endotoxin. Eighteen hours later, each animal received 75 μg of endotoxin intravenously. Development of cutaneous lesions was studied as before.



TEXT-FIG. 5. Changes in platelet count in normal rabbits and rabbits made thrombocytopenic with PAS (2 ml), when endotoxin intracutaneously (50 μg) and intravenously (75 μg) was given to produce a local Shwartzman reaction.

Although the rabbits pretreated with platelet antiserum had platelet levels far below those of the control group (Text-fig. 5), and they developed less leucopenia, 75 per cent of the animals (the same per cent as the control group) demonstrated the characteristic gross and histologic picture of the local Shwartzman phenomenon (Table II and Fig. 2 *b*). Included among the animals demonstrating these changes was one whose platelet count remained below 10,000 per mm^3 during the entire experiment.

The administration of 50 μg of endotoxin intracutaneously followed 18 hours later by 0.5 ml of platelet antiserum did not produce the local Shwartzman phenomenon (Table II).

The Effect of Antibodies against Circulating Rabbit Protein in Rabbits Prepared

with Thorotrast.—Since platelet antiserum was shown to produce lesions in the kidney similar to those of the generalized Shwartzman phenomenon, additional experiments were conducted to determine whether antibodies against rabbit albumin or globulin would also produce the changes characteristic of this reaction.

Three groups of 8 rabbits received 10 ml of thorotrast intravenously, and 18 hours later each group received either 0.5 ml of guinea pig anti-rabbit albumin, 0.5 ml of guinea pig anti-rabbit gamma globulin, or 0.5 ml of sheep anti-rabbit gamma globulin. The animals were sacrificed and autopsied as before.

In no instance did the rabbits demonstrate renal lesions after provocation by these various antisera. Each of the antisera were shown to possess precipitins for their respective antigen, and the guinea pig and sheep antigamma globulin

TABLE II

Production of the Local (Cutaneous) Shwartzman Phenomenon

Protocol	Skin lesions (No. positive total animals)
Endotoxin i.c. (0 hrs.), endotoxin i.v. (18 hrs.)	3/4
PAS*, Endotoxin i.c. (0 hrs.), endotoxin i.v. (18 hrs.)	3/4
Endotoxin i.c. (0 hrs.), PAS i.v. (18 hrs.)	0/4

* 4 intravenous injections of 0.5 ml platelet antiserum administered during 48 hours prior to endotoxin injection.

produced hemorrhagic cutaneous reactions when inoculated intradermally in normal animals.

DISCUSSION

Many of the agents used to provoke the generalized Shwartzman phenomenon accelerate coagulation (4–6, 18). In *in vitro* studies (16–18), platelets are required for the demonstration of acceleration of coagulation. Thrombocytopenia without leucopenia has not been previously studied to evaluate the role of the platelet in the Shwartzman reaction. Much attention has been given to the role of white blood cells, however, in the production of this complex phenomenon (2, 3). It seems clear that granulocytes are essential for induction of renal cortical necrosis or local cutaneous hemorrhage characteristic of the local and generalized Shwartzman phenomenon induced with endotoxin. Intravascular coagulation also has been suggested to be important in the pathogenesis of these reactions (4–6, 8, 10–12, 21, 22). In previous studies of the role of granulocytes, the methods employed to induce granulocytopenia may have also interfered with normal platelet function. The investigations reported here show that immunologic thrombocytopenia does not inhibit the local Shwartzman reaction.

In addition, platelet antiserum produces renal lesions similar to that produced by endotoxin in thorotrast pretreated animals.

Local Shwartzman Phenomenon.—Development of the local Shwartzman reaction in thrombocytopenic animals, even when the platelet count was below 10,000 per mm^3, suggests strongly that platelets may be of little importance in the pathogenesis of this reaction. Furthermore, the Shwartzman phenomenon can be prevented by administration of nitrogen mustard, in doses that produce granulocytopenia but minimal or no thrombocytopenia (2, 23).

Intravascular coagulation presumably is involved in the production of the local Shwartzman reaction, since anticoagulation will prevent the local Shwartzman reaction (10, 11). The observation that thrombocytopenia does not prevent the reaction suggests, therefore, that the intravascular coagulation important in the pathogenesis of the reaction may not involve platelets. Additional studies are required to determine the specific clotting factors implicated in the local Shwartzman phenomenon.

The intravascular thrombi in the hemorrhagic skin lesion of the local Shwartzman reaction are largely composed of packed white blood cells and for this reason are often referred to as "leucocyte thrombi" (24, 25). Anticoagulants may act only to interfere with leucocyte aggregation by an effect on clotting of plasma. It seems possible that the thrombosis in the cutaneous Shwartzman reaction is initiated by procoagulant activity derived from local cellular or tissue damage (26); platelets may be involved secondarily. The preparative skin injection of endotoxin inducing local inflammation with granulocyte infiltration (24) might activate or release the so-called "extrinsic" or tissue thromboplastins, resulting in thrombosis following the intravenous provocative endotoxin injection (27). The findings indicate that platelets are not primarily involved in the thrombosis characteristic of the local Shwartzman reaction.

Generalized Shwartzman Phenomenon.—Renal cortical necrosis was not prevented when the platelet antiserum was administered prior to the provocative endotoxin injection. Indeed, the antiserum itself produced renal lesions typical of the generalized Shwartzman reaction after thorotrast preparation, without a provocative injection of endotoxin. It was not possible, therefore, in the experimental model employed, to induce thrombocytopenia persisting throughout the period of time required for the preparative and provocative injections of thorotrast and endotoxin. Whether or not thrombocytopenia suppresses the reaction is unknown, but thrombocytopenia induced with platelet antiserum a few hours after administration of thorotrast provokes renal lesions indistinguishable from the generalized Shwartzman phenomenon.

It has been shown that heterologous antigen administered to specifically immunized animals can provoke the generalized Shwartzman reaction in appropriately prepared rabbits (8). It is possible that platelet antiserum provoked the reaction in a similar manner but the current studies employed heterologous

antiserum against the platelets of normal recipients, rather than a heterologous antigen in immunized recipients. It might be expected that a heterologous antibody against any circulating antigen of the recipient animal could provoke the generalized Shwartzman reaction. However, it was shown that heterologous antibody (sheep and guinea pig) against rabbit albumin or gamma globulin did not provoke the renal reaction in animals prepared with thorotrast.

Production of the generalized Shwartzman phenomenon with platelet antiserum suggests that the rapid destruction or removal of platelets in appropriately prepared animals is capable of producing this reaction. It is not known whether or not the platelets are lysed or removed from the circulation by the antiserum. Injected radioactive labeled platelets are predominantly sequestered in spleen and lung, but there is suggestive evidence that platelets may become attached to or aggregated along capillary endothelium (28). It is possible, therefore, that platelet antiserum may enable localization of platelets in glomerular capillaries and, in that way, provoke the generalized Shwartzman reaction. Evidence has been presented that platelets are importantly concerned with the initiation and/or acceleration of intravascular coagulation resulting in the changes characteristic of the generalized Shwartzman reaction (4, 6, 16, 17). Furthermore, anticoagulation inhibits the generalized Shwartzman reaction (11, 12).

Granulocytic infiltration of the renal cortex and glomeruli is not a feature of the generalized Shwartzman reaction, but circulating granulocytes are involved as granulocytopenia unassociated with thrombocytopenia the prevents renal lesion (2, 23). Platelet antiserum, however, was capable of producing the generalized Shwartzman reaction in thorotrast prepared animals, but did not cause a marked leucopenia. It is likely that the effects of endotoxin both on circulating leucocytes and platelets are involved in the generalized Shwartzman reaction. Some of the provocative agents used may exert their effect through one cell rather than another. Leucocyte injury with activation of granulocyte lysosomes may affect platelets and/or activate coagulation.

SUMMARY

Studies are reported on the effect of immunologically induced thrombocytopenia upon the local and generalized Shwartzman phenomena. Intravenous injection of antiplatelet serum to rabbits produced profound but transient thrombocytopenia unaccompanied by significant changes in circulating leucocytes. Platelet antiserum alone given to rabbits prepared with thorotrast produced renal lesions characteristic of the Shwartzman reaction. Thrombocytopenia induced by platelet antiserum did not inhibit the cutaneous hemorrhagic lesion of the local Shwartzman phenomenon produced by sequential injections of endotoxin intracutaneously and intravenously. The implications of these observations in the pathogenesis of the local cutaneous and generalized Shwartzman reaction are discussed.

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EXPLANATION OF PLATES

PLATE 17

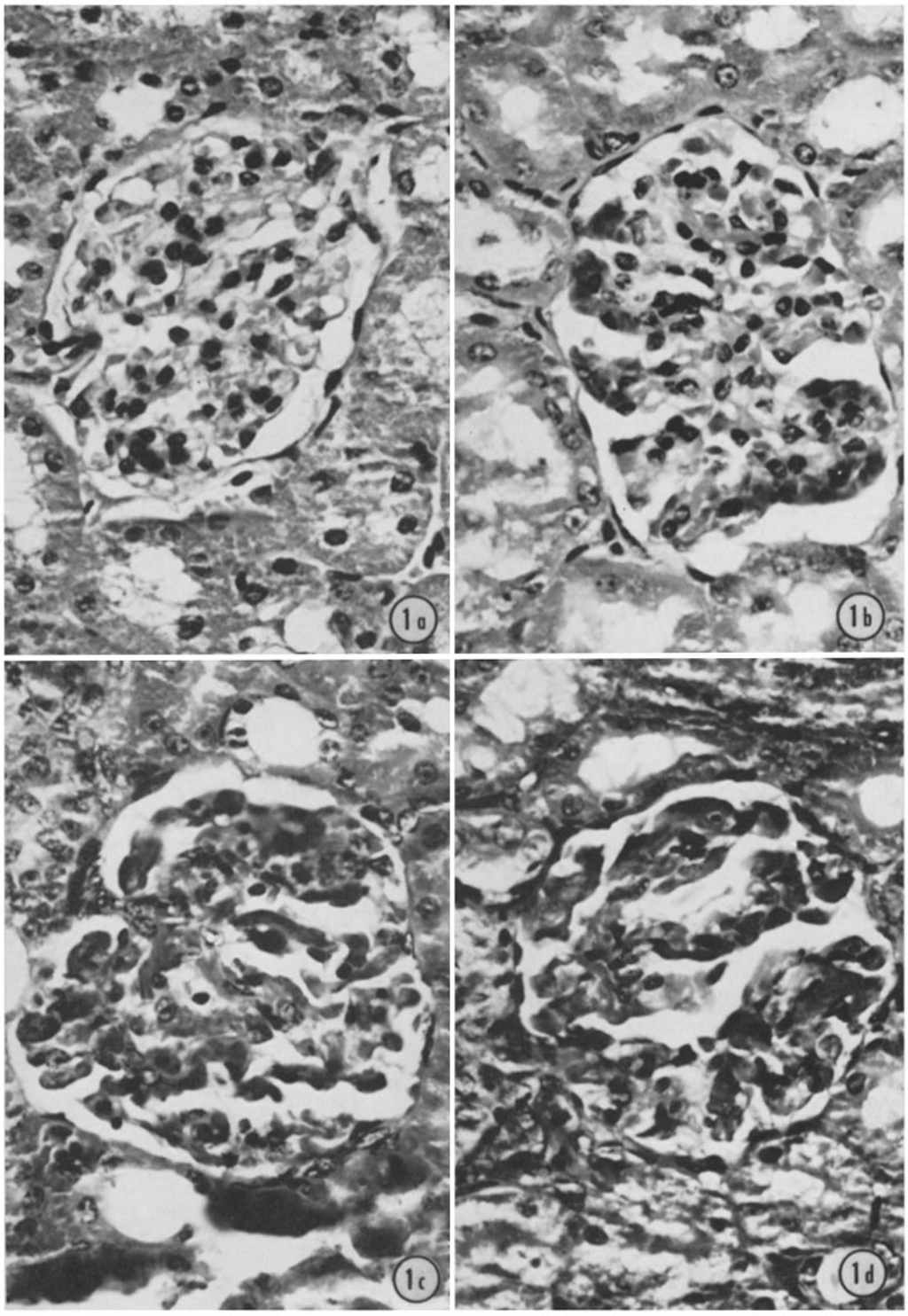
FIGS. 1 *a* to 1 *d*. Effect of platelet antiserum (PAS) upon the generalized Shwartzman reaction.

FIG. 1 *a*. Normal kidney. × 400.

FIG. 1 *b*. Minimal hyaline changes in glomerulus following injection of platelet antiserum alone. × 400.

FIG. 1 *c*. Fibrinoid glomerular change and cortical necrosis produced by preparation of animal with intravenous thorotrast (10 ml) and provocation of reaction by intravenous endotoxin (50 μg) 18 hours later. Animal sacrificed 24 hours after injection of endotoxin. × 400.

FIG. 1 *d*. Fibrinoid glomerular change and some cortical necrosis produced by preparation of animal with intravenous thorotrast (10 ml) and intravenous injection of PAS (0.5 ml) 18 hours later. Animal sacrificed 24 hours after administration of PAS. × 400.



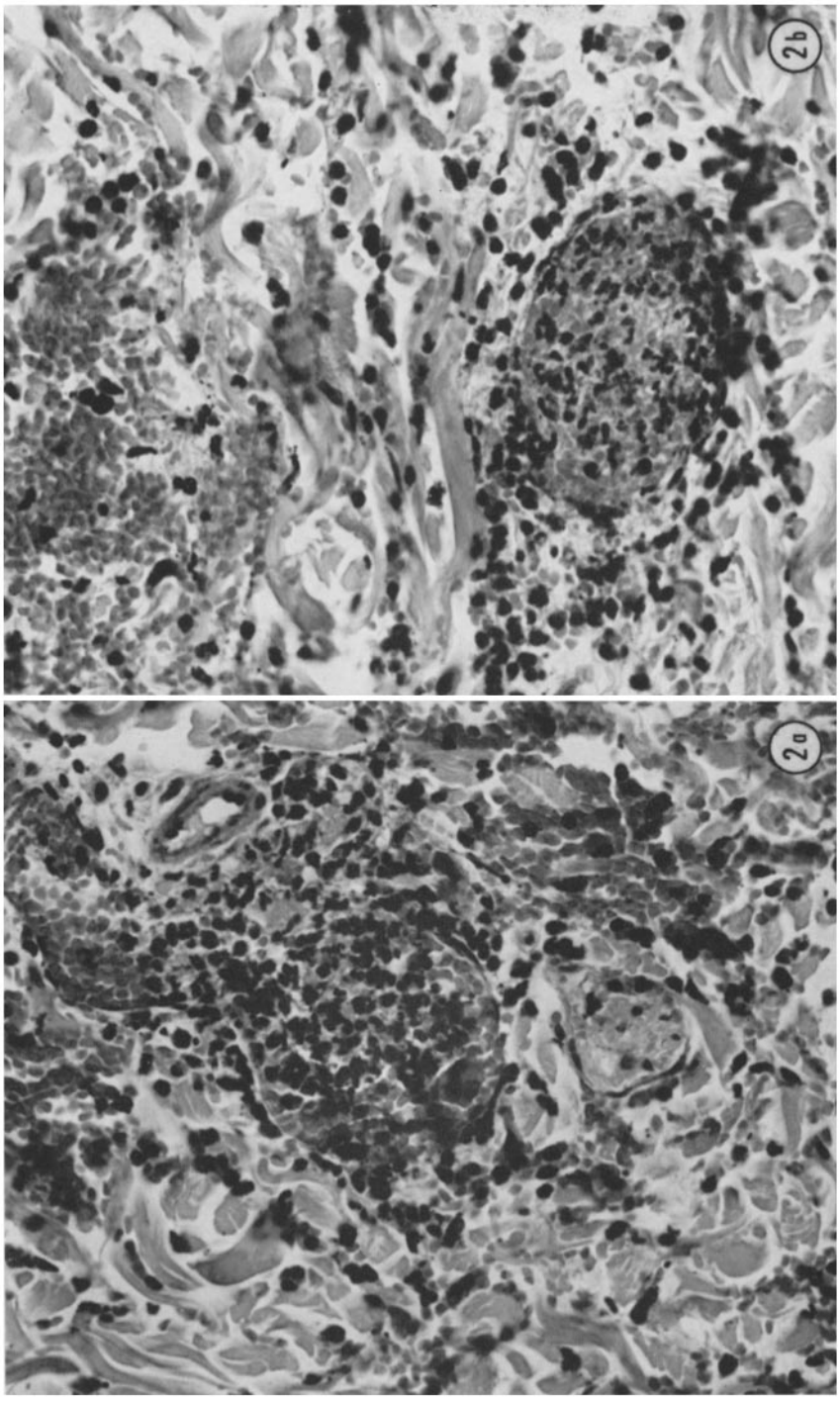
(Levin and Cluff: Platelets and Shwartzman phenomenon)

PLATE 18

FIGS. 2 *a* and 2 *b*. Local Shwartzman phenomenon.

FIG. 2 *a*. Thrombosis, leucocytic exudate, and hemorrhage in rabbit skin prepared by intracutaneous injection of 50 μg endotoxin followed 18 hours later by intravenous injection of 75 μg endotoxin. Skin biopsied 24 hours after the intravenous injection. × 400.

FIG. 2 *b*. Skin lesion identical with Fig. 2 *a*, produced with endotoxin intracutaneously and intravenously as in Fig. 2 *a*, in rabbits made thrombocytopenic by 4 repeated injections of PAS over a 48 hour period prior to the intracutaneous and intravenous injection of endotoxin. × 400.



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