STUDIES ON THE GENERALIZED SHWARTZMAN REACTION

VIII. The Appearance, by Electron Microscopy, of Intravascular Fibrinoid in the Glomerular Capillaries during the Reaction*

By GEORGE D. PAPPAS,‡ Ph.D., MICHAEL H. ROSS, and LEWIS THOMAS, M.D.

(From the Departments of Anatomy and Pathology, New York University-Bellevue Medical Center, New York)

Plates 33 to 38

(Received for publication, September 23, 1957)

The generalized Shwartzman reaction is produced in rabbits by the intravenous administration of two successive doses of endotoxin derived from Gram-negative bacteria, spaced approximately 24 hours apart; the characteristic lesion which identifies the reaction is bilateral cortical necrosis of the kidneys. This lesion results from occlusion of the glomerular capillaries by masses of homogeneous, eosinophilic material with the staining properties of fibrinoid, which appear in the lumen of these vessels within a few hours after the second injection of endotoxin. Similar material is deposited in the vessels in other visceral organs, in association with necrotizing and hemorrhagic lesions (1, 2).

It seems likely that the intravascular fibrinoid is derived from the circulating blood, rather than from altered constituents of the glomerular capillary walls. Histologic studies of glomeruli at different stages in the development of the lesion have shown masses of fibrinoid accumulating on the inner surface of the vessels, or free within the lumen, or entering the capillary tuft still partly contained in the afferent arteriole. Similar material appears in the capillaries in the spleen, liver, and choroid plexus, in the walls of coronary arteries, and in the substance of the mitral and aortic valves, indicating its generalized distribution throughout the body (2). Gamble and Brunson (3) have shown that the renal lesion can be "transferred" from an animal undergoing the reaction to a normal rabbit by perfusing blood from the former through the kidney of the latter.

Treatment with heparin prevents the intravascular deposition of fibrinoid and thus protects against the generalized Shwartzman reaction (4), suggesting the implication of the coagulation mechanism in the reaction. It has been shown that fibrinogen undergoes a change in its stability after an injection of endotoxin, becoming precipitable in the presence of heparin at low temperatures (5). Heparin-like acidic

* Supported in part by funds from the Lillia Babbitt Hyde Foundation.

‡ Present address: Department of Anatomy, Columbia University College of Physicians and Surgeons, New York.

polymers of large molecular size, such as dextran sulfate, sodium polyanethol sulfonate, or sodium polyvinylalcohol sulfonate, which are capable of precipitating fibrinogen from plasma *in vitro*, also have the property of substituting for one of the two injections of endotoxin ordinarily required for the generalized Shwartzman reaction; thus, an injection of acidic polymer 1 hour after a single dose of endotoxin will cause widespread fibrinoid deposition and the typical necrotizing lesions of the reaction. Concurrently with the deposition of fibrinoid, there occurs an abrupt and marked depletion of fibrinogen from the circulating blood, often amounting to complete disappearance of fibrinogen (5, 6). In view of these observations it was suggested that fibrinoid, in the generalized Shwartzman reaction, may represent a precipitated form of fibrinogen in combination with an acid polysaccharide of large molecular size.

The possibility that fibrinogen, or fibrin, may be an important component of fibrinoid in other pathologic situations has been proposed by Gitlin and Craig (7), on the basis of immunohistochemical studies of human tissues with fluorescein-tagged antibody prepared against fibrin. However, Mellors and Ortega (8) have reported that similar techniques reveal the presence of gamma globulin in human fibrinoid, and others have suggested that fibrinoid is derived from altered collagen (9) or from precipitates involving the ground substance of connective tissues (10, 11). Muirhead *et al.* (12) recently proposed on the basis of various histochemical tests that the intravascular fibrinoid of the generalized Shwartzman reaction may originate from necrotic smooth muscle discharged into the blood. It is possible that, as Klemperer (13, 14) has suggested, fibrinoid in different types of pathologic alteration may represent entirely different and unrelated types of material, despite the similarities in staining reactions with conventional histologic techniques.

The present study, employing electron microscopy, was undertaken to learn whether the fibrinoid contained in the glomerular capillaries of rabbits undergoing the generalized Shwartzman reaction possesses any structural characteristics of value for its identification and further investigation.

Material and Methods

Hybrid albino rabbits of both sexes, weighing approximately 1.5 kilos, were used in all experiments. They were maintained on Purina rabbit chow and water.

Two methods were used to produce the renal lesion of the generalized Shwartzman reaction. One group of rabbits were given two intravenous injections of a highly purified lipopolysaccharide endotoxin derived from *E. coli*, generously supplied by Dr. Otto Westphal, of Freiburg. The first dose was 20 micrograms, and the second, given 24 hours later, was 50 micrograms. The animals were sacrificed at various periods of time after the second injection, indicated in the text to follow.

In a second group, the generalized Shwartzman reaction was produced by the combination of a single dose of endotoxin (20 micrograms) with an injection of sodium polyanethol sulfonate (liquoid),[1] as described in a previous publication (6). Each animal received an intravenous injection of 5 mg. of liquoid, prepared as a 1 per cent solution in sterile, pyrogen-free distilled

[1] Liquoid is the commercial designation for sodium polyanethol sulfonate, prepared by Hoffmann-La Roche, Inc., Nutley, New Jersey.

water. Liquoid was injected 1 hour after endotoxin, and the animals sacrificed at the same intervals as the animals in the first group.

Controls included groups of rabbits given single injections of endotoxin or liquoid, or no injections.

Kidney tissue for electron microscopy was obtained in the following manner. The animals were anesthetized with nembutal and the kidneys exposed through flank incisions. The capsule of one kidney was partly removed and the cortex bathed immediately in osmium tetroxide. While circulation was still intact, a slice of cortex was removed and placed in OsO_4. The slice was then sectioned into small blocks approximately 1 mm. square, and washed with OsO_4 before being placed in fresh fixative. The time from stripping the capsule until placing the tissue into fresh fixative was less than 2 minutes. The second kidney was then removed, fixed in Bouin's solution, and stained with hematoxylin and eosin. The fixative used for electron microscopy was 1 per cent osmium tetroxide buffered with veronal-acetate at pH 7.4. After fixation the specimens were washed and dehydrated in alcohol, infiltrated with *n*-butyl methacrylate monomer, containing 10 per cent methyl methacrylate, and finally embedded in the same resin by polymerization at 70°C. Two per cent luperco CDB[2] was used as a catalyst. The embedded specimens were sectioned with a Porter-Blum microtome. The sections were mounted on grids, previously coated with carbon films to provide a supporting membrane (15). Without removal of the embedding plastic, the sections were examined in an RCA microscope, model EMU-2E.

OBSERVATIONS

Fibrinoid was observed within the glomerular capillaries in 12 rabbits in which two successive intravenous injections of endotoxin were given, and in 10 animals receiving combined injections of endotoxin and liquoid. All of these rabbits were sacrificed between 2 and 12 hours after the last injection. The electron microscopic appearance of the fibrinoid was essentially the same in both groups.

Fig. 7 shows several vesicles, swollen areas of endothelium and dense materials amongst these cellular structures. In Fig. 8, a deposit of dense material is seen lying along a swollen but intact endothelial cell membrane. Under higher magnification, as shown in Fig. 9, the material appears to be fibrillar. This preparation was made 5 hours after the second of two injections of endotoxin.

Fig. 10 shows fibrinoid in the glomerular capillary of a rabbit injected with endotoxin and liquoid, 8 hours after the liquoid injection. The material contains trapped blood cells and platelets, and the fibrinoid cannot be well differentiated at this magnification. Under higher magnification, as shown in Fig. 11, the fibrinoid possesses a fibrillar structure. The fibers are not branched, and have a diameter of 200–300 A.

In several micrographs taken at high magnification, the fibrinoid was seen to have an axial repeating structure with a periodicity of approximately 120 A. Fig. 14 illustrates this observation in an animal given two injections of

[2] Luperco CDB is a product of the Novadel-Agel Corporation (Lucidol Division, Buffalo, New York) and contains in equal amounts a catalyst, 2,4-dichlorobenzoyl peroxide, and a plasticizer, dibutylphthalate.

endotoxin, and Fig. 13 shows similar material formed after a combined injection of endotoxin and liquoid. It should be added that very infrequently, a homogeneous deposit was found instead of a fibrillar one (Fig. 12).

In kidneys removed 3 or 4 hours after the second injection of endotoxin, or after combined injections of endotoxin and liquoid, conspicuous balloon-like structures were consistently noted in the endothelial cells, and vacuoles were also seen in epithelial cells (Figs. 4 to 6). These changes were encountered in the preparations before any deposits of fibrinoid had occurred.

The basement membranes of the glomerular capillaries, which in the rabbit measure 600–800 A, exhibited no swelling or other structural alteration during the time when fibrinoid was being laid down in the vessels. In some specimens taken after widespread destruction of tissue had occurred, when cortical necrosis was visible in the gross, the basement membranes were found to be ruptured (see Fig. 15).

No fibrinoid deposits were observed in the control specimens from normal rabbits, or those from animals receiving a single injection of endotoxin or an injection of liquoid without endotoxin. The electron micrographs in Figs. 1 to 3 show normal rabbit glomeruli. Their appearance is essentially the same as that described by Pease (16). Comparison may be made with these electron micrographs and those of the treated animals.

DISCUSSION

In an earlier paper (6), dealing with the capacity of liquoid and other acidic polymers of large molecular size to produce the lesions of the generalized Shwartzman reaction when injected in combination with a single dose of endotoxin, the extreme depletion of circulating fibrinogen accompanying the deposition of intravascular fibrinoid was interpreted as evidence for the precipitation of fibrinogen *in vivo* and thus its participation in the formation of fibrinoid. It was suggested that an unknown acid polymer, perhaps derived from polymorphonuclear leukocytes, might play the same role as liquoid in the classical generalized Shwartzman reaction caused by two successive injections of endotoxin. However, it was not possible to demonstrate the abrupt depletion of fibrinogen in the latter reaction, presumably because fibrinoid deposition occurs more gradually and over a longer period of time in such animals, while the glomerular capillaries become packed with fibrinoid within a few hours in rabbits given the combination of endotoxin and liquoid.

The results of the present study are of some importance in interpreting the earlier experiments with the synthetic acidic polymers, since they provide additional evidence that the underlying basis for the renal lesions is the same in both groups. Fibrillar material, consisting of unbranched fibers of 200–300 A in diameter, with a periodicity of approximately 120 A, occurred in the glomeruli after two intravenous injections of endotoxin, and the same material

was deposited when a single injection of endotoxin was followed by liquoid. It is reasonable to assume that if fibrinogen precipitation is responsible for formation of fibrinoid in the first process, it is also involved in the formation of the same material in the other.

The resemblance of fibrinoid to the electron microscopic appearance of fibrin (17, 18) is noteworthy, the chief differences being the absence of discernible branching of the fibrils in fibrinoid and the difference in periodicity (fibrin has a periodicity of 230 A, as compared with 120 A for fibrinoid). As suggested previously (6), fibrinoid may be derived from fibrinogen in a stage of polymerization part way in its conversion to fibrin. The production of fibrils of collagen with periodicities of varying multiples has been demonstrated by Gross (19), in what may be an analogous situation.

It should be emphasized that the examination under high magnification of fibrinoid associated with the generalized Shwartzman reaction is simplified by the location of the material free in the lumen of capillaries, not obscured by other tissue elements except occasional blood cells and platelets. Even here, the periodicity of fibrils can only be seen when they are longitudinally arranged. Whether similar structure can be perceived in fibrinoid contained in perivascular or connective tissue sites, encumbered by necrotic tissue elements, is problematical; attempts to determine this question as concerns the coronary arteries and heart valves of animals involved in the generalized Shwartzman reaction are now in progress.

A balloon-like fluid-containing vesicle in an endothelial cell lining the afferent glomerular artery has been described in a normal kidney (20). Such vesicles were seen infrequently in the glomerular capillaries of normal animals in the present study. They were, however, consistently seen in glomerular endothelial cells within a few hours after the second injection of endotoxin. The significance of this observation remains to be determined. It is possible that the vesicles may represent an injury to the glomerulus which contributes to the special vulnerability of these vessels to occlusion by fibrinoid. There is, however, no reason to regard the vesicles as a specific lesion.

SUMMARY

The intravascular fibrinoid which is deposited in glomerular capillaries of the rabbit during the generalized Shwartzman reaction has been studied with the aid of the electron microscope. In one group of animals the reaction was produced by two intravenous injections of *Escherichia coli* lipopolysaccharide endotoxin, spaced 24 hours apart. In another, a single dose of endotoxin was followed, 1 hour later, by intravenous liquoid (sodium polyanethol sulfonate).

The appearance of fibrinoid was the same in the two groups. Initially, fibrinoid deposition occurred on the irregular, swollen surfaces of the endo-

thelial cells within the capillary lumen. Subsequently, the fibrinoid mass increased to such proportions that the capillary lumen was completely occluded.

Fibrinoid was found to be composed of unbranched fibrils, having a diameter of 200–300 angstroms and an axial repeating structure of 120 A.

The basement membrane (lamina densa) underwent no change in appearance during the time when fibrinoid was being laid down.

Balloon-like vesicles were consistently encountered in endothelial cells of glomerular capillaries after two doses of endotoxin, and also in animals given one injection of endotoxin followed by liquoid.

The possible significance of the observations are discussed. It is suggested that they are compatible with the hypothesis, proposed earlier, that intravascular fibrinoid, in the generalized Shwartzman reaction, is derived from fibrinogen.

BIBLIOGRAPHY

1. Thomas, L., and Good, R. A., Studies on the generalized Shwartzman reaction, *J. Exp. Med.*, 1952, **96,** 605.
2. Brunson, J. G., Thomas, L., and Gamble, C. N., Morphologic changes in rabbits following the intravenous administration of meningococcal toxin. II. Two appropriately spaced injections; the role of fibrinoid in the generalized Shwartzman reaction, *Am. J. Path.*, 1955, **31,** 655.
3. Gamble, C. N., and Brunson, J. G., Experimental fibrinoid lesions in rabbits, *Arch. Path.*, 1955, **60,** 583.
4. Good, R. A., and Thomas, L., Studies on the generalized Shwartzman reaction. IV. Prevention of the local and generalized Shwartzman reactions with heparin, *J. Exp. Med.* 1953, **97,** 871.
5. Thomas, L., Smith, R. T., and von Korff, R., Studies on the generalized Shwartzman reaction. VII. The role of fibrinogen in the deposition of fibrinoid after combined injections of endotoxin and synthetic acidic polymer, *J. Exp. Med.*, 1955, **102,** 263.
6. Thomas, L., Brunson, J., and Smith, R. T., Studies on the generalized Shwartzman reaction. VI. Production of the reaction by the synergistic action of endotoxin with three synthetic acidic polymers (sodium polyanethol sulfonate, dextran sulfate, and sodium polyvinyl alcohol sulfonate), *J. Exp. Med.*, 1955, **102,** 249.
7. Gitlin, D., and Craig, J. M., Variations in the staining characteristics of human fibrin, *Am. J. Path.*, 1957, **33,** 285.
8. Mellors, R. C., and Ortega, L. G., Analytical pathology. III. New observations on the pathogenesis of glomerulonephritis, lipid nephrosis, periarteritis nodosa, and secondary amyloidosis in man, *Am. J. Path.*, 1956, **32,** 455.
9. Rich, A. R., Voisin, G. A., and Bang, F. B., Electron microscopic studies of the alteration of collagen fibrils in the Arthus phenomenon, *Bull. Johns Hopkins Hosp.*, 1953, **92,** 222.
10. Altshuler, C. H., and Angevine, D. M., Histochemical studies on the pathogenesis of fibrinoid, *Am. J. Path.*, 1949, **25,** 1061.

11. Wolpers, C., Electronenmikroskopische Untersuchungen zur Pathologie kollagener Fasern, *Frankf. Z. Path.*, 1950, **61,** 417.
12. Muirhead, E. E., Booth, E., and Montgomery, P. O., Derivation of certain forms of fibrinoid from smooth muscle, *Arch. Path.*, 1957, **63,** 213.
13. Klemperer, P., Ueber fibrinoide Substanzen, *Wien. klin. Woch.*, 1953, **65,** 713.
14. Klemperer, P., General considerations of collagen diseases, *in* Connective Tissue in Health and Disease, (G. Asboe-Hanson, editor), Copenhagen, Ejnar Munksgaard Publishers, 1953, 251.
15. Watson, M. L., Carbon films and specimen stability, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** suppl., 31.
16. Pease, D. C., Fine structure of the kidney seen by electron microscopy, *J. Histochem. and Cytochem.*, 1955, **3,** 295.
17. Still, W. J. S., and Boult, E. H., Electron microscopic appearance of fibrin in thin sections, *Nature*, 1957, **179,** 868.
18. Porter, K. R., and Hawn, C. J., Sequences in formation of clots from purified bovine fibrinogen and thrombin: study with electron microscope, *J. Exp. Med.* 1949, **90,** 225.
19. Gross, J., Properties and gelation of collagen dissolved in neutral salt solutions, *Fed. Proc.*, 1956, **15,** 82.
20. Bergstrand, A., Electron microscopic investigations of the renal glomeruli, *Lab. Inv.*, 1957, **6,** 191.

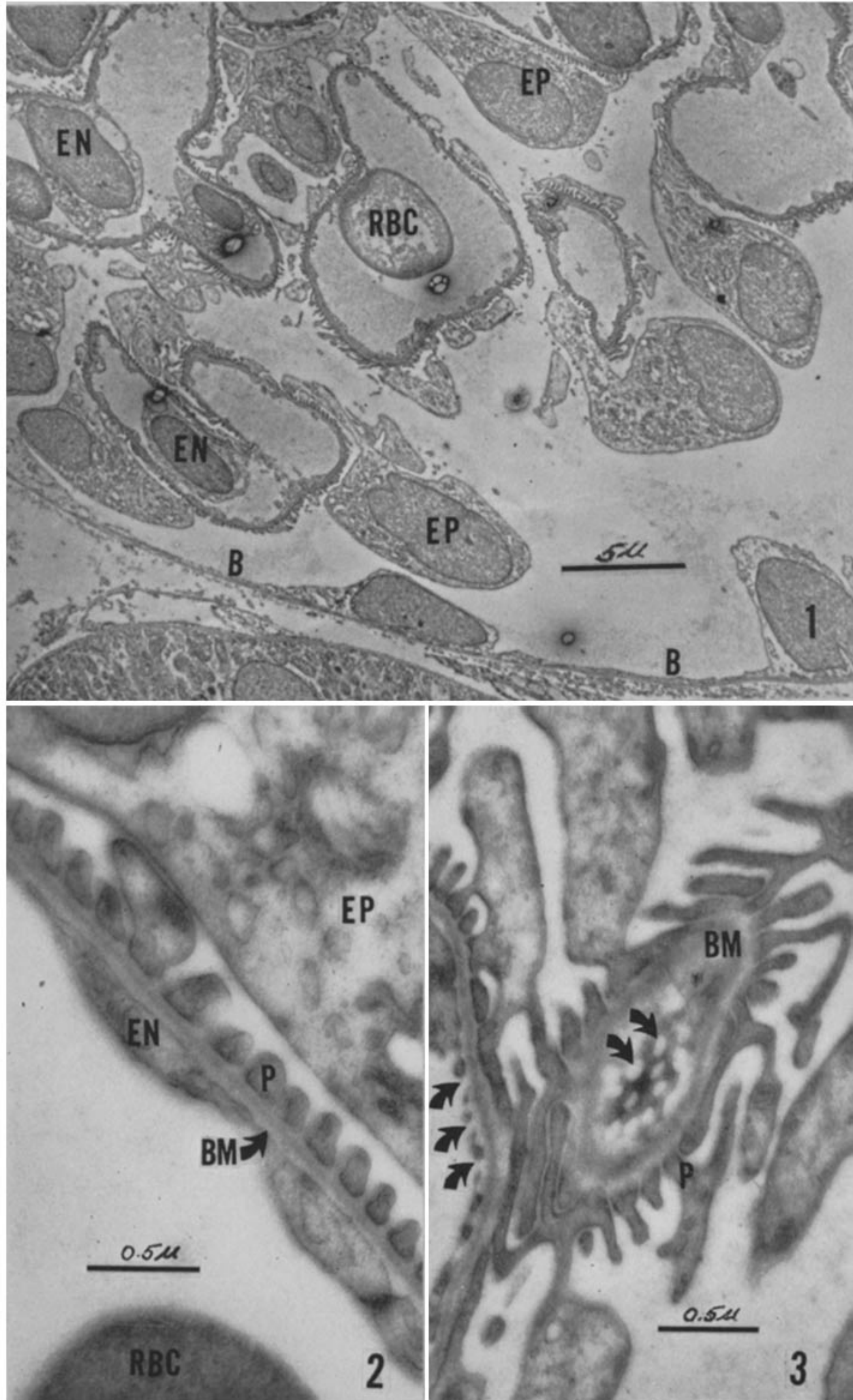
EXPLANATION OF PLATES

Plate 33

Fig. 1. A low power electron micrograph showing a portion of a normal rabbit glomerulus. A red blood cell (*RBC*) is seen in one of the capillary loops. At (*B*) is a segment of Bowman's capsule. The basement membrane separates the endothelial cells (*EN*) from the epithelial cells (*EP*) and their cytoplasmic extensions, the podocytes. Magnification, 3,500.

Fig. 2. At higher magnification a cross-section of the capillary basement membrane (*BM*), showing its characteristic dense inner zone. Resting upon the basement membrane are the podocytes (*P*) which are cytoplasmic extensions of the epithelial cell (*EP*) seen at the upper right. The endothelium (*EN*) does not show any discrete fenestrations in this section. Magnification, 33,600.

Fig. 3. A tangential section through a normal glomerular capillary wall. The pores in the endothelial lining are marked by arrows. The basement membrane (*BM*) is cut obliquely in this section. Surrounding the basement membrane are the podocytes (*P*) which are here seen to be extensions of the epithelial cytoplasm. At the extreme left a second capillary wall is seen in cross-section. Again the endothelial fenestrations are marked by arrows. Magnification, 30,000.



(Pappas *et al.*: Generalized Shwartzman reaction. VIII)

PLATE 34

FIG. 4. Four hours following the second endotoxin injection. The endothelium exhibits marked localized swellings (*S*). Vacuolization of the epithelial cells (*V*) is also conspicuous. At this stage there are no other apparent morphological changes. Magnification, 18,000.

FIG. 5. Two balloon-like structures are seen protruding from the endothelial lining of the capillary. Note the greater density of the contents of the "balloon" as compared with the lumen. The membranes show no indication of any openings or rupture. Magnification, 20,000.

FIG. 6. Balloon-like structures similar to those in Fig. 5. Because of the plane of section there is no apparent attachment of these structures to the capillary endothelium. The contents of the balloons at this stage appear to be of equal density to that of the material in the capillary lumen. Magnification, 13,500.

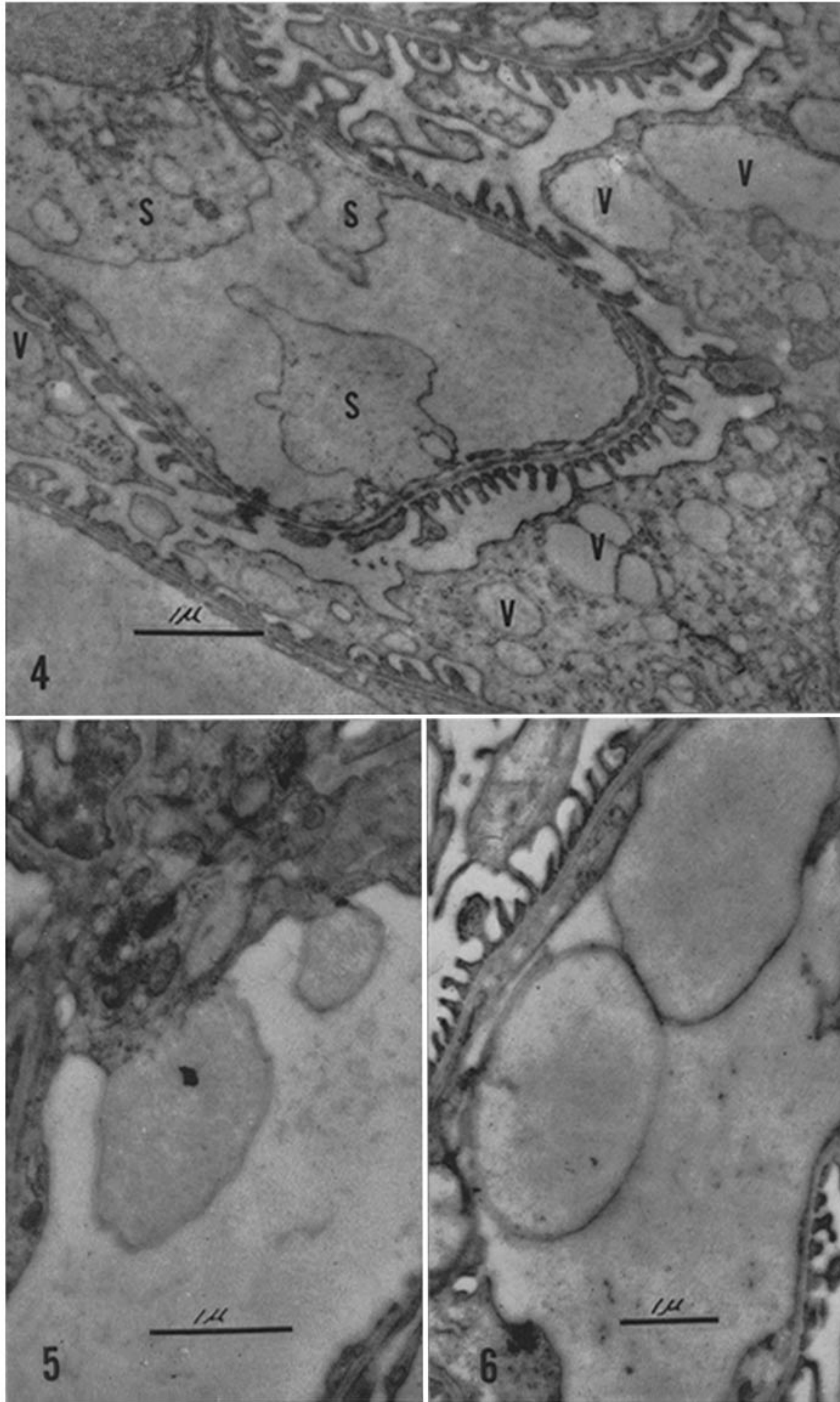
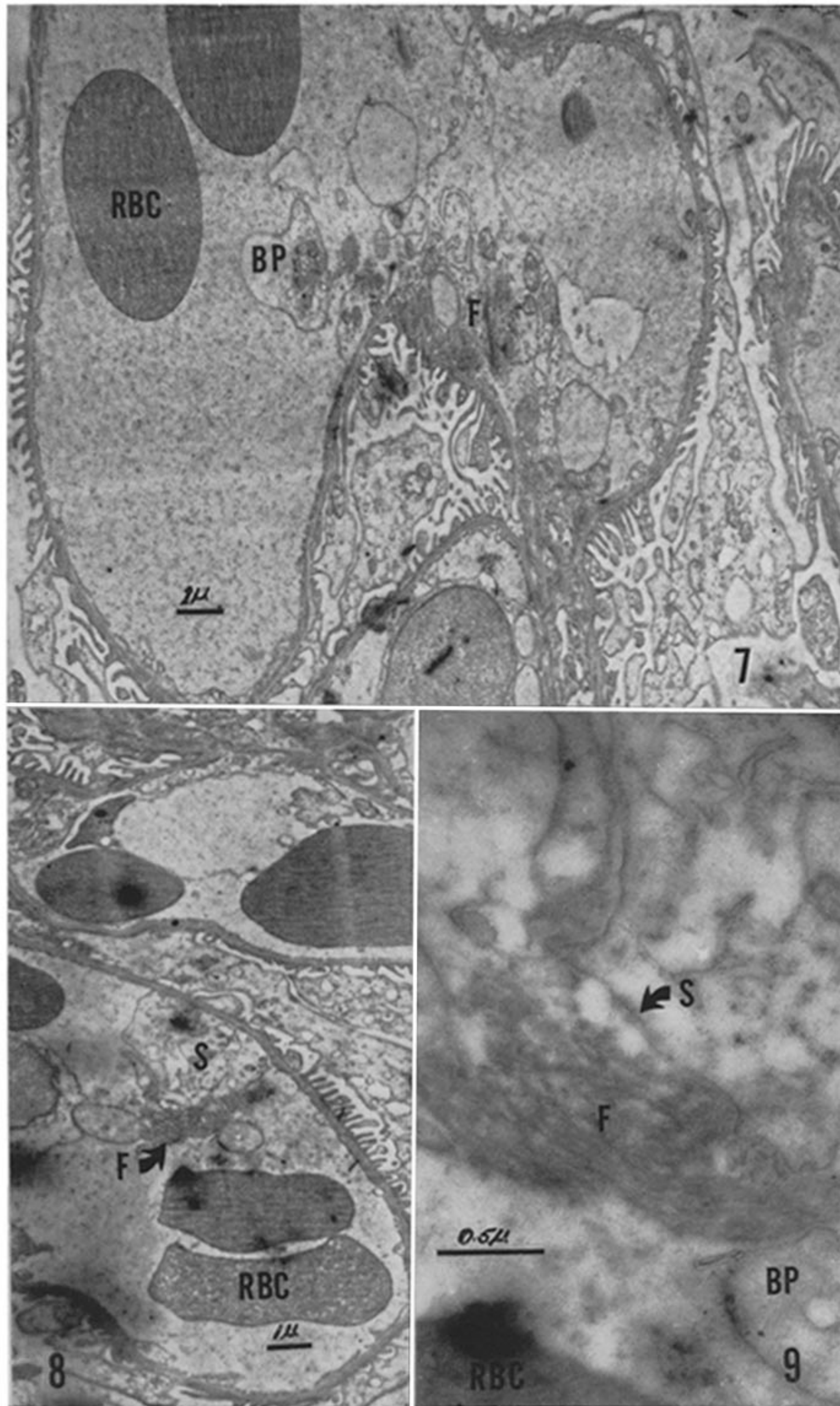

S
S
S
V
V
V
V
V
1μ
4
1μ
5
1μ
6

PLATE 35

FIG. 7. Low power electron micrograph showing an agglomeration of materials at the junction of two capillaries. A blood platelet (*BP*) is seen as well as several vesicles and swollen areas of the endothelium. Dense materials (*F*) are seen amongst these cellular structures. Magnification, 6,200.

FIG. 8. A capillary at a magnification similar to that of Fig. 7, again showing a dense material (*F*) which is adhering to the swollen endothelial (*S*) membrane. This dense material, marked by arrow, is presumably an early stage in the formation of fibrinoid. Magnification, 5,800.

FIG. 9. Electron micrograph at higher magnification of the same dense material seen in Fig. 8. The swollen endothelial membrane (*S*) is completely intact. Note the fibrillar appearance of the fibrinoid. (*F*) A portion of a blood platelet (*BP*) is seen at the lower right. Magnification, 29,000.

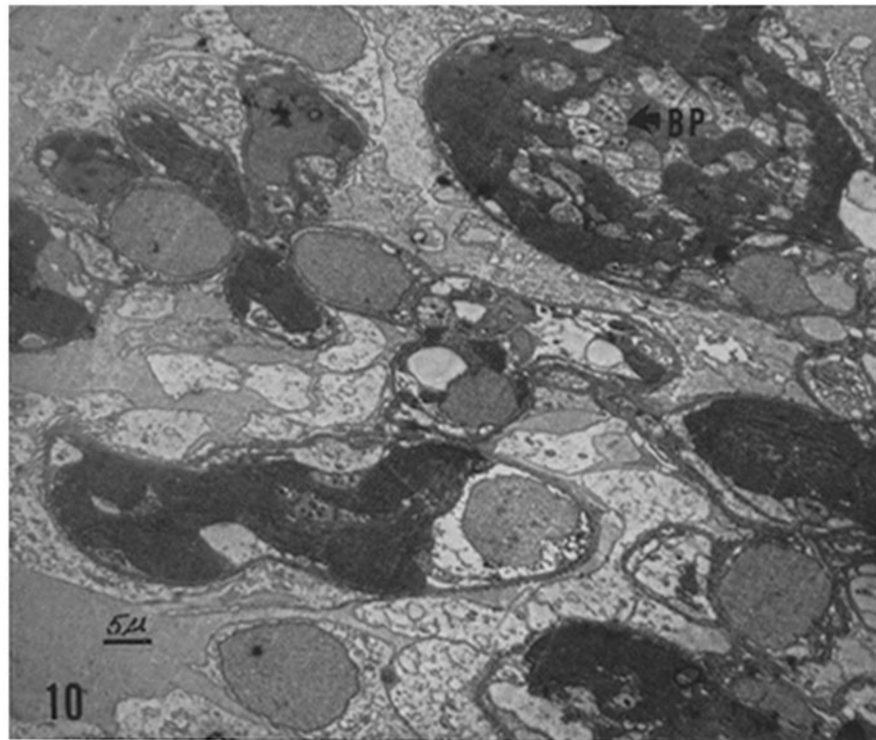


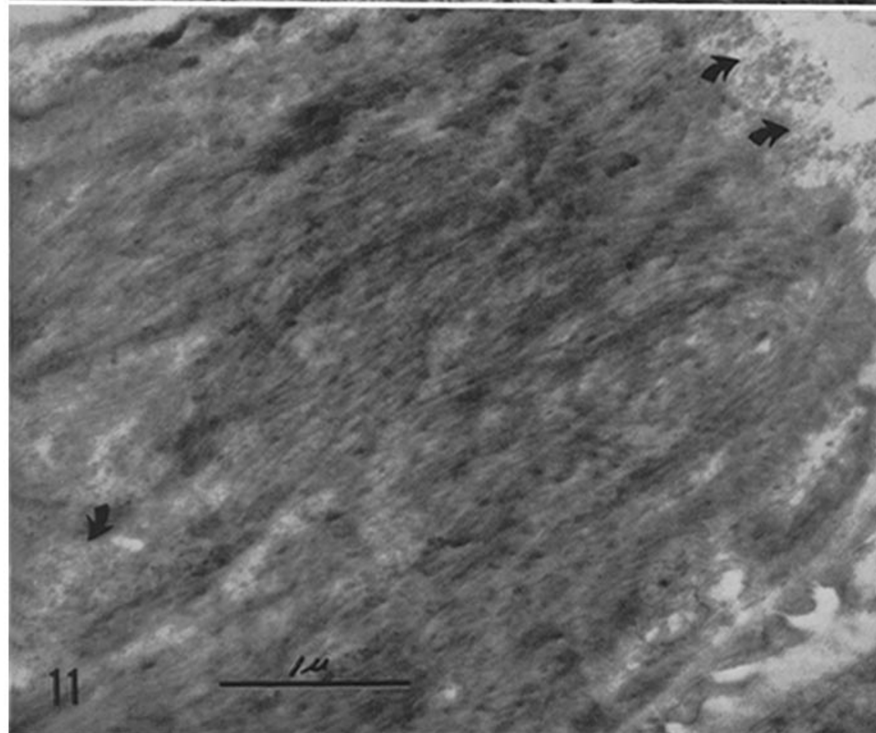
(Pappas *et al.*: Generalized Shwartzman reaction. VIII)

Plate 36

Fig. 10. Low power of a portion of the glomerulus 8 hours after the second injection of liquoid. The capillaries here are completely occluded. Because of the almost equal density of the fibrinoid and red blood cells it is difficult to distinguish one from the other at this magnification. A large number of platelets (*BP*) can be seen trapped with the red blood cells in the fibrinoid mass. Magnification, 3,100.

Fig. 11. At higher magnification the fibrinoid is clearly shown to be fibrillar. The fibrinoid seen here is from the same section as Fig. 10. In the upper right hand corner near the basement membrane as well as in the lower left corner, fibrinoid is seen in cross-section (indicated by arrows). Magnification, 25,200.



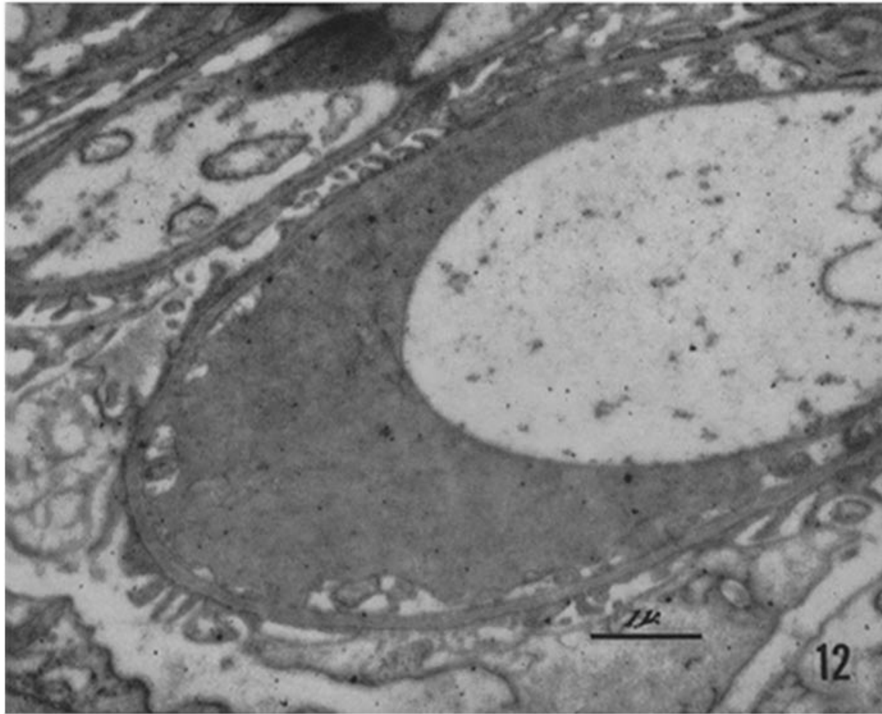


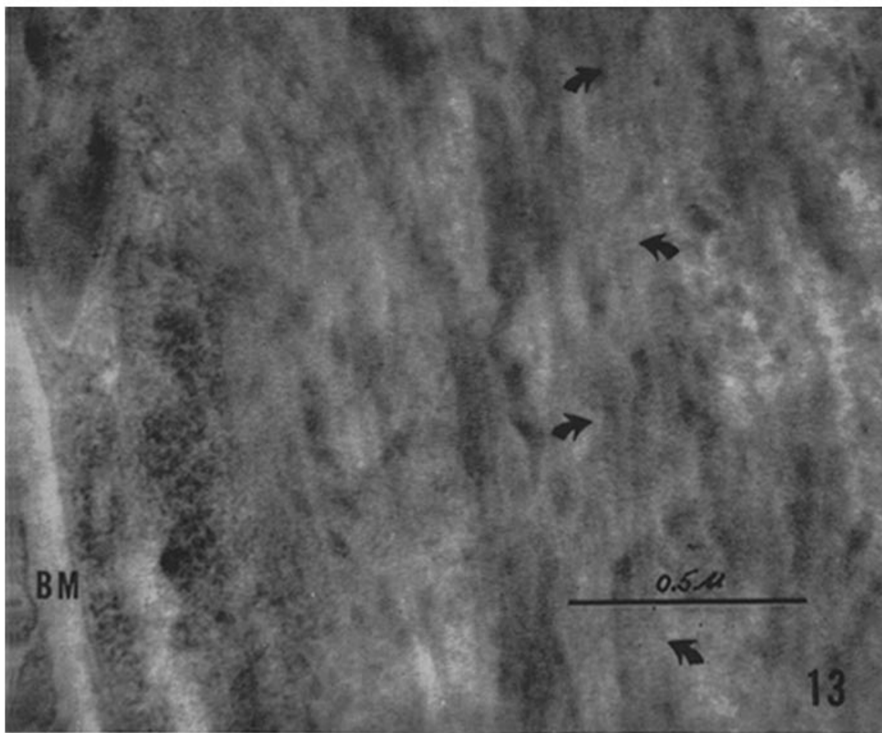
(Pappas *et al.*: Generalized Shwartzman reaction. VIII)

PLATE 37

FIG. 12. A portion of a capillary loop is shown here in which the fibrinoid appears completely homogeneous. As in the preceding two figures the animal was sacrificed 8 hours after the second liquoid injection. This homogeneous appearance of fibrinoid was infrequently encountered. Magnification, 14,700.

FIG. 13. At high magnification fibrinoid is seen to have an axial repeating structure. The periodicity is most striking in the region of the arrows, owing to its orientation. The periodicity is in the order of 120 A. The basement membrane (*BM*) may be seen in the lower left hand corner. The fibrinoid here was formed following endotoxin-liquoid injections. Magnification, 72,900.



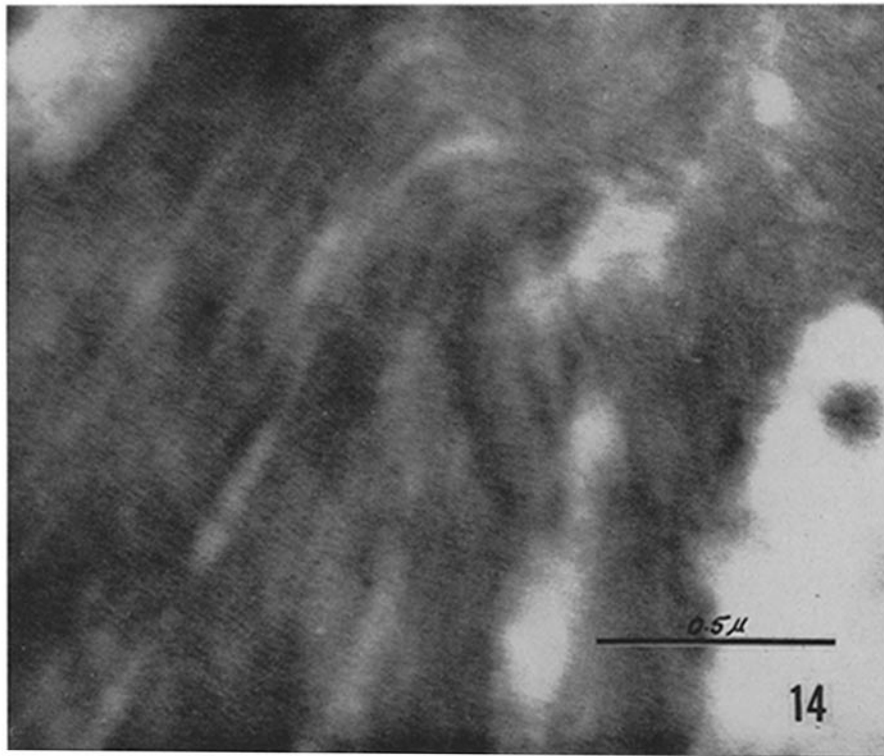


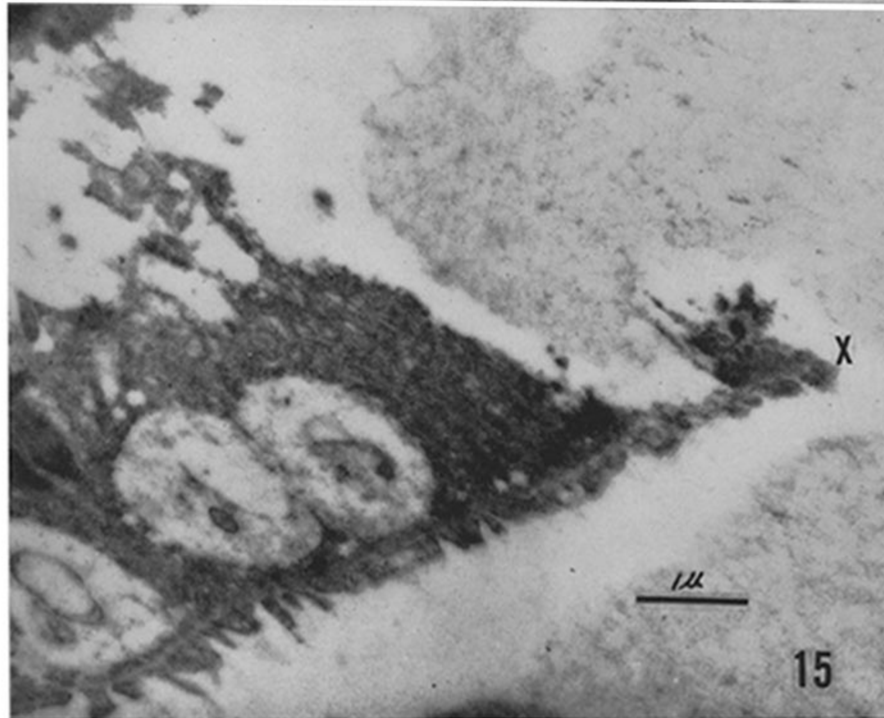
(Pappas *et al.*: Generalized Shwartzman reaction. VIII)

PLATE 38

FIG. 14. The periodicity (120 A) of fibrinoid produced after two injections of endotoxin is clearly seen in this electron micrograph. The upper right hand corner lacks any suggestion of a periodic structure owing to the orientation of the fibrinoid in this area. Magnification, 63,250.

FIG. 15. A ruptured capillary 12 hours after the second injection of endotoxin. This stage represents a kidney that displays gross cortical necrosis and hemorrhage. At the rupture (*X*) most of the contents of the capillary have escaped. The three oval profiles are the remains of platelets. It should be noted that there is no change in the thickness of the basement membrane preceding this rupture. Magnification, 14,500.





(Pappas *et al.*: Generalized Shwartzman reaction. VIII)