THE GLOMERULUS IN EXPERIMENTAL RENAL DISEASE IN RATS AS OBSERVED BY LIGHT AND ELECTRON MICROSCOPY*,‡

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Large or potent doses of nephrotoxic serum prepared by sensitizing rabbits to rat kidney produce in rats a disease of immediate onset. Uremia and oliguria occur promptly. Proteinuria, hypoproteinemia, hyperlipemia, and edema follow in a few hours (1). The elevated blood urea decreases in 7 to 14 days. The edema also subsides in this interval but may recur in the terminal stages of renal failure (2, 3). 20 to 50 per cent of these diseased animals show manifest signs of progressive renal insufficiency.

Because potency of the nephrotoxic serum (4, 5) and species (6), age and sex (7) of rat as well as other factors influence the apparent symptoms and pathologic response of the animals, many interpretations particularly of the histologic alterations have been advanced. These experiments were designed to study the histopathologic course of this experimental renal disease for a period of 10 weeks with comparable light and electron microscopic observations.

Method

Twenty nine male Sprague-Dawley rats weighing 100 to 200 gm. were given potent nephrotoxic serum, 1 ml./gram body weight. This dosage is known by bioassay to produce severe immediate disease. The method for preparing nephrotoxic serum has been previously described (4). 1 ml. was given intravenously in a single injection, the remaining portion of doses larger than 1 ml. was given subcutaneously at the same time. Animals were sacrificed at 1, 3, 6, 12, 24, 36, 48, and 72 hours and 1, 2, 3, 4, and 10 weeks after injection. One kidney of each animal was prepared by osmic fixation according to the method described by Pease (8). At the time of sacrifice the animal was anesthetized with nembutal and one kidney exteriorized posteriorly with as little disturbance of the circulation as possible. 1 per cent osmic acid freshly buffered to pH 7.4 with sodium acetate–veronal was dripped on this decapsulated kidney at the rate of 1 drop per minute for 40 minutes. 1 to 2 mm. cubes were cut from the cortex and placed in freshly buffered 1 per cent osmic for 30 minutes. The tissue was dehydrated by passage

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through 80 per cent and absolute ethyl alcohol, placed in equal parts of absolute alcohol–methacrylate solution, then embedded in methyl butyl methacrylate solution. This was hardened by heating to 50°C. Sections were cut at 0.04 μ with a glass knife in a modified rotary microtome. The sections were floated onto copper screens that had a coating of collodion reinforced with a layer of vaporized carbon. These were examined on an RCA EM-2 electron microscope.

The other kidney of each animal was fixed in 4 per cent formol, embedded in paraffin, and sectioned at 4 to 5 μ for light microscopic study. The following stains were used: colloidal iron and periodic acid–Schiff stain for mucopolysaccharides (9, 10), trichrome stain (11), fibrin stain (12), and Gomori's silver stain (13).

RESULTS

Light Microscopic Observations in Diseased Rats.—By light microscopy the most prominent change in the first 24 hours of disease was the infiltration of the glomerular capillaries by polymorphonuclear leucocytes. This neutrophilic infiltration was particularly marked from the 3rd to the 6th hour (Fig. 2). At the 6th hour swelling of the epithelial cells and slight increase in the blue staining material surfacing the glomerular capillary were present. Rinehart (10) considers this material to be acid mucopolysaccharide. By 72 hours, swelling of endothelial and epithelial cells could be seen and an increase in the amount of periodic acid–Schiff-positive material within the glomerular capillary lumina as well as thickening of the periodic acid–Schiff-positive basement membrane. In 1 week, almost all the Schiff-positive material seemed to be part of the greatly thickened capillary wall (Fig. 3). Approximately 50 per cent of the tissue examined within the first 2 weeks of disease had fibrin-positive staining thrombi within the glomerular capillary lumina comparable to the capillary lesions of the generalized Shwartzman reaction (Fig. 4). After a few days the thrombi no longer stained positive for fibrin and became indistinguishable within the pathologic lesion of the capillary. 50 to 60 per cent of these animals had exudation into the subcapsular space with splitting of Bowman's capsule and proliferation of the epithelial cells. In a few animals, material staining positive for fibrin was seen in this exudation for the entire period of observation. This indicates continued activity of the process (Fig. 5). However, the majority of animals showed organization of the exudative material with obliteration of glomeruli in this chronic stage of disease.

Electron Microscopic Observations in Normal Rats.—The resolving power of the electron microscope allows the filtering surface of the glomerulus to be separated into three layers. On the intracapillary surface there is a complete covering which is uniformly porous and appears to be a continuation of the cytoplasm of the endothelial cells. Outside of this is the basement membrane, a continuous, homogenous layer which does not appear porous and seemingly has no intimate cellular connection. The extracapillary surface is covered with foot-like projections which come from long processes of the epithelial cells. There is a clear space between each foot process. Epithelial cells appear more

numerous than endothelial cells. Between the endothelial lining and basement membrane and the basement membrane and the foot processes there is a clear or non-staining layer the nature of which has not been determined. Bowman's capsule appears laminated and thinly lined by flat epithelial cells (Figs. 6 and 7).

Electron Microscopic Observations in Diseased Rats.—The glomerulus of rats with nephrotoxic serum disease showed changes when examined at 1 hour with the electron microscope. In tangential cuts in scattered areas the foot processes were of varying density. The less dense material between the foot processes gave the appearance of partially filling the space, leaving only a narrow channel or clear space between neighboring foot processes. A tangential cut on normal basement membrane has in one instance shown similar osmophilic material about the foot processes. However, the density of the material was much less than in sections from rats that had received the nephrotoxic serum. Whether this is an anatomical structure or an artifact of tangential cutting can only be determined by further observations. At higher magnification the channels appeared to communicate with the basement membrane (Fig. 8). Whether or not there was actual penetration of the basement membrane could not be determined. In several of the animals examined 3 hours after injections of nephrotoxic serum, platelets were seen within the glomerular capillary as well as in the capsular space (Fig. 9). Also at this time there seemed to be disruption of the entire capillary wall in some areas and fragmentation of Bowman's capsule. Increased cellularity in the capsular space and in Bowman's capsule became prominent. By the 6th hour more general thickening of the basement membrane became apparent (Fig. 10). This abnormality persisted throughout the 10 week period of observation. By 1 week, the disarray of the entire glomerular capillary, lumen and wall, as well as the capsular space became so gross that interpretation of these findings could not be attempted.

DISCUSSION

Light Microscopic Observations in Diseased Rats.—In general, previous investigators present similar findings in rats receiving large doses of potent nephrotoxic rabbit serum as those described in this paper. Masugi (14) first observed the occurrence of "fibrin thrombi" in the glomerular capillaries early in the course of this disease, thickening of the basement membrane and swelling of the endothelial and epithelial cells. Smadel's (15) and Smadel and Farr's (3) findings were similar as well as those of Heymann (2). Ehrich *et al.* (4) and other more recent investigators (5) have pointed out the efficacy of utilizing mucopolysaccharide stains in studying this glomerular lesion. With the Ritter and Oleson or the Rinehart and Abul-Haj colloidal iron and periodic acid–Schiff stain it is possible to illustrate that the epithelial cells surfacing the capillaries become more prominent first followed by thickening of the basement

membrane. In this condition in which the glomerular components are magnified by pathologic changes, there was no apparent substance derived from the epithelium crossing the basement membrane as described by Rinehart (16).

As indicated in previous communications (17, 18) it is believed that the capillary thrombosis which is frequently found in animals dying in the first 2 weeks of this disease is a non-specific reaction. It resembles the renal lesion of the generalized Shwartzman reaction. Evidence militating against the view that it represents a Shwartzman reaction is that the method for producing the generalized Shwartzman phenomenon has not yet proved successful in rats. In addition, the presence of platelets described by Stetson (19) to be an integral part of the thrombotic phenomenon of the localized Shwartzman reaction has been described to occur only occasionally in the generalized Shwartzman reaction described in reference 20. Although Weinreb *et al.* (5) write of "platelet thrombi" as being present in the renal capillaries of animals dying soon after large injections of nephrotoxic serum, there is no description of the presence of platelets. No platelets were seen by light microscopy in the thrombi in the kidneys of the animals in this study.

The chronic lesions of crescent formation and glomerular sclerosis observed in these animals are comparable to those described by other investigators. However, a larger percentage of animals in this study developed severe lesions than animals in those series previously reported. This is believed to be related to the dosage of potent serum and the frequency of occurrence of capillary thrombosis. The correlation between the thrombotic phenomenon which occurs early in the disease and the development of obliteration of glomeruli has previously been reported (21).

Electron Microscopic Observations in Normal Rats.—It is readily apparent that the great resolving power of the electron microscope augments artifacts as well as clarifies details of structure. However, new and improved techniques are resulting in the gradual development of a generally accepted electron microscopic picture of the rat glomerulus.

In the first detailed description of electron microscopy of the kidney in 1950, Pease and Baker (22) described the basement membrane as being incompletely covered by the endothelial and epithelial cells and considered the foot processes of the epithelial cells to be integral parts of the basement membrane. In 1951, Dalton (23) described in mouse kidney three complete layers of the capillary wall including an innermost layer of "fine striations consisting of alternating zones of greater and lesser density." Rinehart's (16) findings agree for the most part with those of Dalton. However, he described the endothelial layer as an extension of mucoid substance from the epithelial cell which pierced the basement membrane at regular intervals. Hall (24), like Dalton, described three layers of the capillary wall. However, he considered the basement membrane, called lamina densa by him, to be porous. Reid (25) failed

to see penetrations in the basement membrane and considered it a homogenous layer with neither pores nor ridges. He described "closely packed vacuoles" lining the basement membrane and thought that this represented degenerative changes. Most recently, Mueller (26) has described the anatomy of the glomerulus in dog and man. The findings described in this paper in rats are for the most part in complete agreement with those of Mueller. However, he questioned the reality of the porous endothelial lining and considered the possibility that there "exist thin layers of apposed plasma membranes covering the apertures." The question of the nature of the mesangium which is dealt with in such excellent detail by Mueller, unfortunately is not further elucidated by this nephrotoxic disease as the doses used in these experiments did not produce proliferative changes.

Electron Microscopic Observations in Diseased Rats.—Various interpretations have been given as to the functional significance of the structure of glomerular capillary wall. Hall (27) speaks of "protoplasmic pulsations" in the arm-like processes of the epithelial cells which "could actively facilitate filtration." Reid (25), likewise, comments that the epithelial "ridges may possibly have a more active role connected with the production of glomerular filtrate." Mueller (26) calls attention to the change of infantile epithelium to that of epithelial cells of adults "whereby filtration may occur without having to pass through the epthelial cells itself."

The present observations on pathologic tissue suggest interesting evidence for speculation regarding the relationship of structure and function. Azotemia and anuria occurring immediately after injection of nephrotoxic serum and lasting for 1 to 2 hours have been repeatedly observed. The injection of nephrotoxic serum has been shown to produce immediate blanching of the kidney in the recipient animal (28). This vasoconstriction has been interpreted as causal in producing the anuria and azotemia (1). The electron microscopic findings indicate that already by 1 hour after injection of potent serum the space between the foot processes may be obstructed by osmophilic material. This damage to the filtering surface may also contribute to the manifest findings of renal shutdown. However, the azotemia and this histologic change subside and after the 6th hour proteinuria becomes progressively more prominent. At this time thickening of the basement membrane becomes readily apparent by electron microscopy. This thickening of the basement membrane coincides with the proteinuria. The presence of platelets in several sections of tissue examined by electron microscopy at 1 and 3 hours after injection of nephrotoxic serum is interesting in light of the finding of glomerular capillary thrombi by light microscopy.

SUMMARY

Nephrotoxic serum disease in rats has been studied by light and electron microscopy from 1 hour to 10 weeks after production of the disease.

By light microscopy leucocytic infiltration of the glomerular capillary was observed between the 3rd and 6th hour. At 6 hours an increase in colloidal iron-positive material was observed coating the extraluminal surface of the capillaries. Also at this time swelling of the endothelial cells becomes prominent. By 72 hours, thickening of the basement membrane was observed. Glomerular capillary thrombi were observed in approximately half the tissue examined in the first 2 weeks of disease. 50 per cent of the animals showed severe chronic lesions, exudation into the capsular space, crescent formation, and obliteration of glomeruli.

At 1 hour electron microscopic pictures showed that osmophilic material may line the foot processes of the epithelial cells and obliterate all but narrow channels of the space between the feet. By 6 hours thickening of the basement membrane was prominent. This change persisted throughout 10 weeks of observation. The tissue from animals which had severe chronic alterations by light microscopy revealed changes which could not be interpreted at this time.

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EXPLANATION OF PLATES

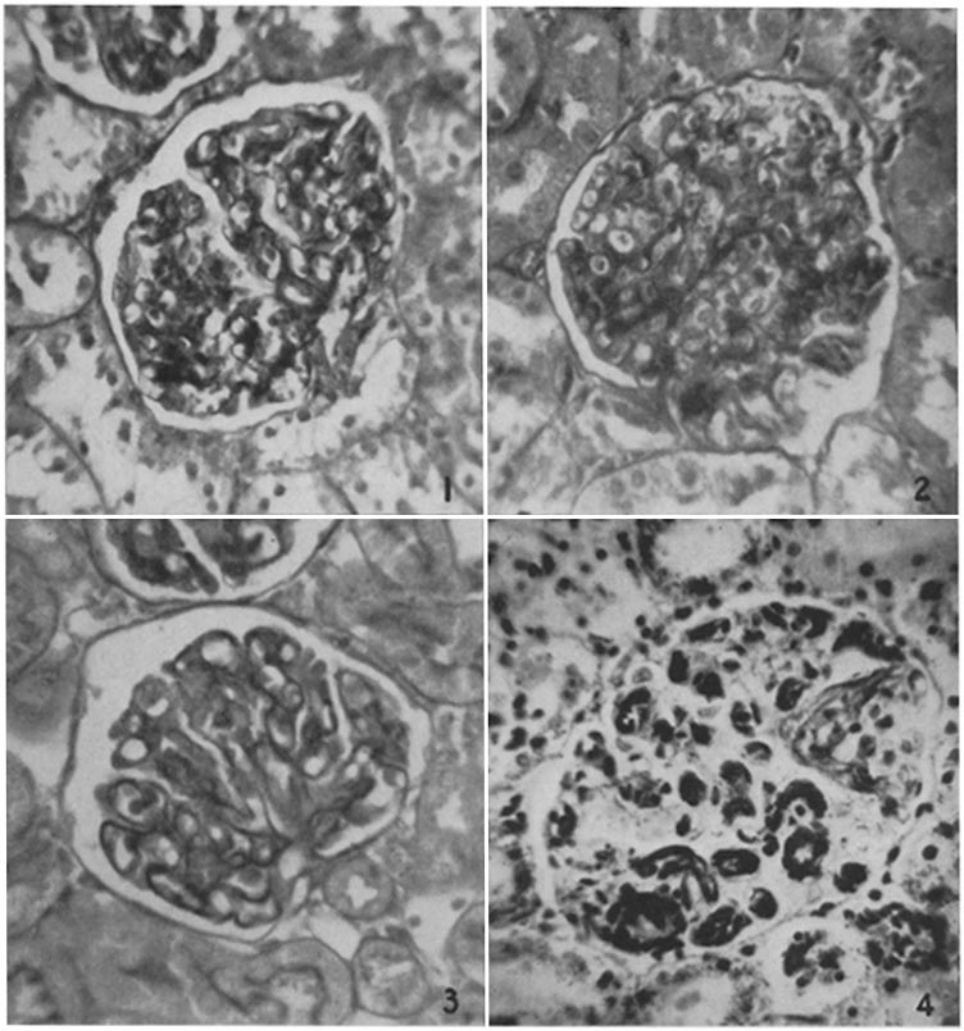
PLATE 63

FIG. 1. Normal rat glomerulus visualized by light microscopy. Colloidal iron periodic acid–Schiff stain. Approximately × 320.

FIG. 2. Rat glomerulus 3 hours after injection of nephrotoxic serum. The capillaries are filled with polymorphonuclear leucocytes. Colloidal iron periodic acid–Schiff stain. Approximately × 320.

FIG. 3. Rat glomerulus 7 days after injection of nephrotoxic serum. The markedly thickened glomerular capillary wall is periodic acid–Schiff positive. Approximately × 320.

FIG. 4. Rat glomerulus 3 days after injection of nephrotoxic serum. The glomerular capillaries are filled with thrombi which stain positive with fibrin stain. Approximately × 320.

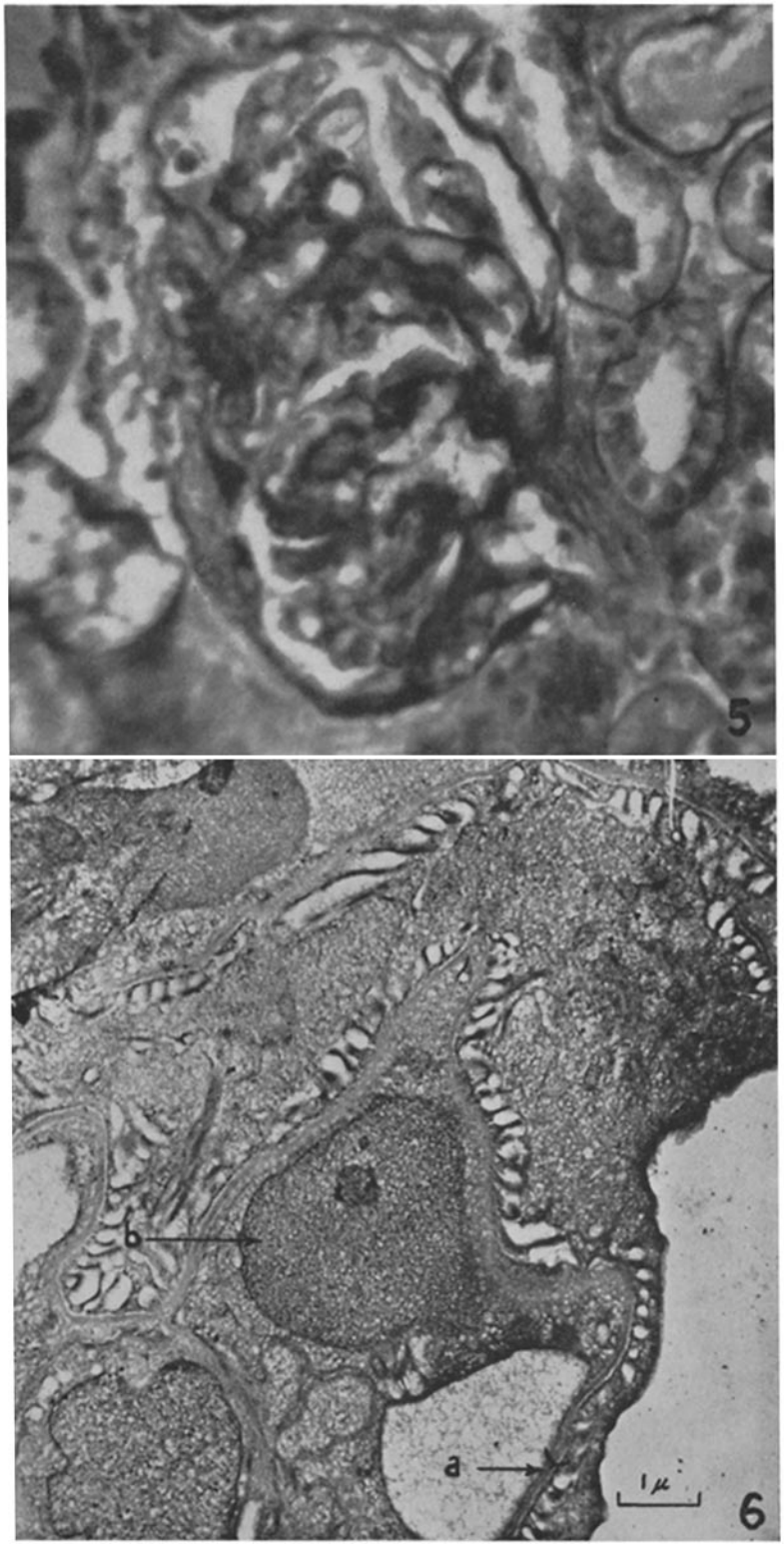


(Piel *et al.*: The glomerulus in experimental renal disease)

PLATE 64

FIG. 5. Rat glomerulus 4 weeks after injection of nephrotoxic serum. The subcapsular exudation on the left side of the glomerulus stains positive for fibrin indicating continued activity. Approximately × 450.

FIG. 6. Normal rat glomerulus visualized by electron microscopy. Within the capillary lumen can be seen the nucleus of an endothelial cell (*b*). The capillary wall is composed of three layers (*a*): on the intraluminal side, the porous layer called lamina fenestrata by Hall; the middle layer which is the basement membrane; the outer surface which is covered by foot-like projections from the arm-like processes of the epithelial cells. There is no epithelial cell in this figure. Reduced from a magnification of 23,760 to 10,525.

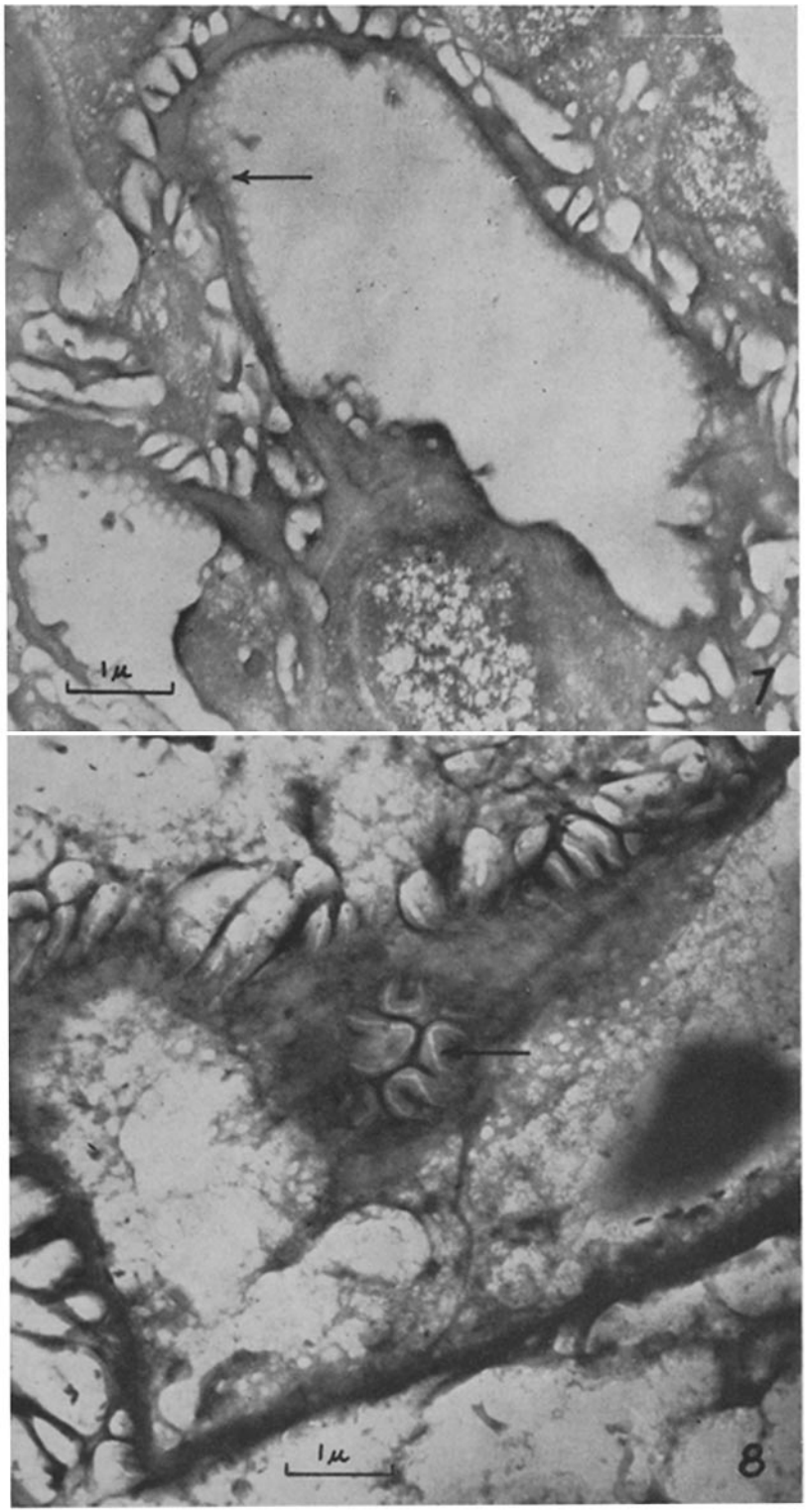


(Piel *et al.*: The glomerulus in experimental renal disease)

PLATE 65

FIG. 7. This is an electron microscopic picture of a glomerulus from a normal rat which shows the inner porous layer of the capillary wall (arrow). Reduced from magnification of 36,050 to 14,420.

FIG. 8. Glomerular capillary wall of a rat observed by electron microscopy one hour after injection of nephrotoxic serum. Osmophilic material fills the clear spaces between neighboring foot processes leaving narrow channels (arrow). Reduced from magnification of 36,050 to 14,160.

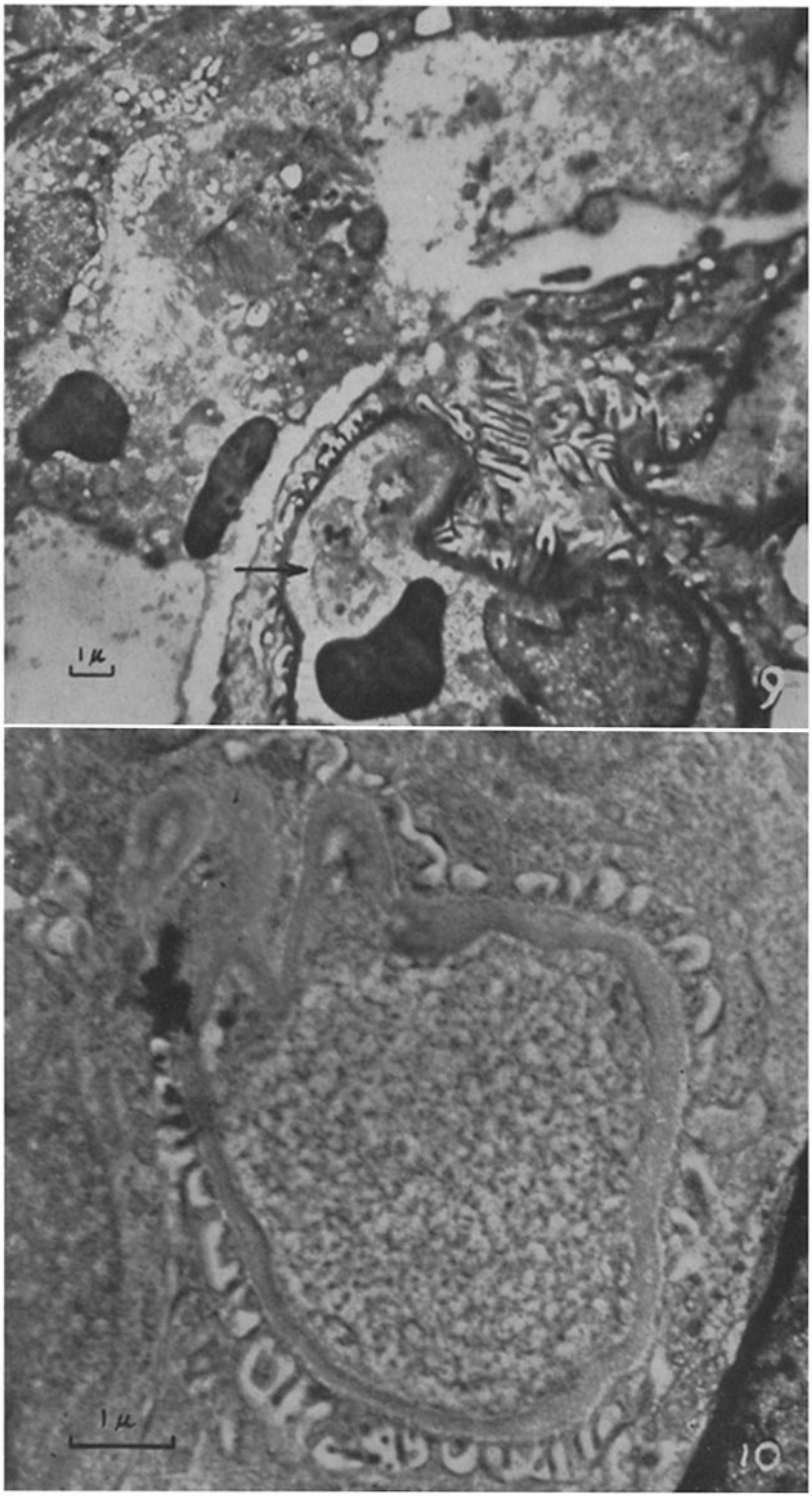


(Piel *et al.*: The glomerulus in experimental renal disease)

PLATE 66

FIG. 9. Electron microscopic picture of a rat glomerulus observed 1 hour after injection of nephrotoxic serum. Three platelets (arrow) are seen within the lumen of the capillary. The dark mass to the left of the platelets is a red cell. Two other red cells are seen in the subcapsular space. Reduced from magnification of 13,020 to 5,300.

FIG. 10. This electron microscopic photograph of a cross section of a glomerular capillary shows markedly thickened basement membrane. This is from a rat three weeks after injection of nephrotoxic serum. Reduced from magnification of 33,475 to 13,390.



(Piel *et al.*: The glomerulus in experimental renal disease)