Virchows Arch. A Path. Anat. and Histol. 369, 269—282 (1976)

Accelerated Serum Sickness in the Rabbit

II. Glomerular Ultrastructural Lesions in Transient Proliferative and Progressive Disorganizing Glomerulonephritis

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Received October 24, 1975

Summary. Electron microscopic analysis was performed on the development of irreversible glomerular distortion in experimental serum sickness nephritis in the rabbit. In the animals showing transient albuminuria, glomerular hypercellularity was due to the accumulation of monocytes and polymorphonuclear leukocytes and was seen to recover to nearly normal glomerulus. In this condition the glomerular structure was observed to be well preserved throughout the inflammation.

In contrast, in the animals showing persistent proteinnuria, a disorganizing process was found in their glomeruli. Mesangial disintegration resulted in collapsed scarring or circumferential mesangial interposition of the glomeruli. Extracapillary exudation, sometimes with the rupture of the glomerular basement membrane, was often associated with granulomatous glomerular lesions or crescent formation. The results showed that the structural disintegration is a fundamental event in the development of progressive glomerulonephritis.

Key words: Serum sickness — Glomerulonephritis — Hypercellularity — Mesangiolysis.

Introduction

Experimental acute serum sickness nephritis in the rabbit has been known as an appropriate animal model for poststreptococcal glomerulonephritis (PSGN) in man (Fish *et al.*, 1966; Arakawa and Kimmelstiel, 1970). Most cases of PSGN recover completely in both children and adults, but there are some which show a rapidly progressive course into uremia. Acute serum sickness nephritis initiated by a single intravenous injection with a large amount of antigen into the rabbit usually shows a transient clinical course and an almost complete recovery. As reported in the previous report, however, a procedure which provides for an increase in the amount of antigen-antibody complexes in the circulation also increases the incidence of glomerulonephritis and evokes disorganizing irreversible glomerulonephritis in some rabbits (Kobayashi and Shigematsu, 1973). Analysis of the disease process resulting in this irreversible glomerulonephritis will contribute to the understanding of the fundamental histological basis for rapidly progressive glomerular damages in human PSGN.

Mesangial disintegration and lysis or rupture of the glomerular basement membrane (GBM) will be shown as the fundamental tissue manifestations of the development of intracapillary or extracapillary glomerulonephritis.

Materials and Methods

The tissues studied with the electron microscope were taken from rabbits treated by Kobayashi and Shigematsu (1973). In their previous report the details of the immunologic

procedures, preparations of reagents and manipulation of rabbits have been recorded, and in this report only those details that are pertinent to our observations will be presented. Female rabbits weighing 2.5–3.5 kg were used. There were three groups divided as follows:

Group I: Fifteen rabbits received 3 mg of bovine serum albumin (BSA, The Armour Laboratories) with Freund's complete adjuvant in the hind pads and subcutaneously, and intravenous injections of 250 mg/kg of BSA 4 days later. Fourteen rabbits showed transient proteinuria around 12 days after the treatment and then recovered within the succeeding 7 days. The renal specimens from these rabbits were used for the analysis of transient glomerular injuries.

Group II: Twelve rabbits were treated similarly to Group I but were given additional intravenous injections of 250 mg/kg of BSA 7 days after the first intravenous injection. Six of the rabbits showed heavy proteinuria of up to 3,000 mg/day with persistent glomerular injury; their renal tissues were used for the study of glomerular distortion and disorganization.

Group III: Four rabbits received a mixture of physiological saline (0.5 ml) and Freund's complete adjuvant in the hind pads and subcutaneously, and intravenous injections of 2 cc of physiological saline, 4 and again 7 days later. They showed no renal abnormality. From each group one to four succeeding renal biopsies were performed in certain animals from 15 to 90 experimental days. All rabbits were killed within 11 months. Small pieces of renal cortex were fixed in 2.5% glutaraldehyde for 1.5 hr and postfixed in 1% osmium tetroxide for 90 min. After dehydration with graded ethanol, they were embedded in Epon 812. They were observed with a Hitachi 11DS electron microscope. Other tissue blocks were prepared for light and fluorescent microscopy.

Results

Transient Proliferative Glomerulonephritis

Development and resolution of transient proliferative glomerulonephritis was analyzed in all animals during the time of albuminuria and in the stage of clinical recovery in Groups I and II. The proliferative glomerular change at the onset of proteinuria was seen mainly due to the accumulation of phagocytes—monocytes and polymorphonuclear leukocytes—in the capillary lumen (Fig. 1). Monocytes were characterized by the presence of lysosomal granules, prominent cytoplasmic processes and vacuoles. They frequently enlarged themselves in the capillary lumen, resulting in its occulusion, but there was no formation of junctional complexes between the endothelial cells. It was not a rare occasion that they transformed into larger epithelioid cells connected to each other with interdigitations. Some phagocytes were further seen emigrating in the mesangial matrix and thus the expansion of the glomerular tufts resulted in the simplification and lobulation of the glomerulus (Fig. 1). Subendothelial spaces were edematous and swollen with proteinaceous amorphous materials. Local exfoliation of endothelial cells was accompanied by the direct contact of monocytes with the inner surface of the GBM. Formation of fibrin and fibrinoid materials were found in the capillary lumen and to a lesser degree in the mesangial matrix. Some of the monocytes were seen to have participated in the removal of these clotting materials (Fig. 1, inset). Subepithelial deposits (humps) were observed on rare occasions. Podocytes showed local fusions of foot processes. The mesangial cells were swollen and displayed extended cytoplasms. There was local increase of cellularity in the mesangial and endothelial cells, but this was not observed as a major factor contributing to glomerular hypercellularity.

The resolution of glomerular hypercellularity closely correlated with the disappearance of proteinuria. The phagocytes markedly decreased in numbers from the glomeruli. The glomerular capillary lumen as well as the mesangial matrix were

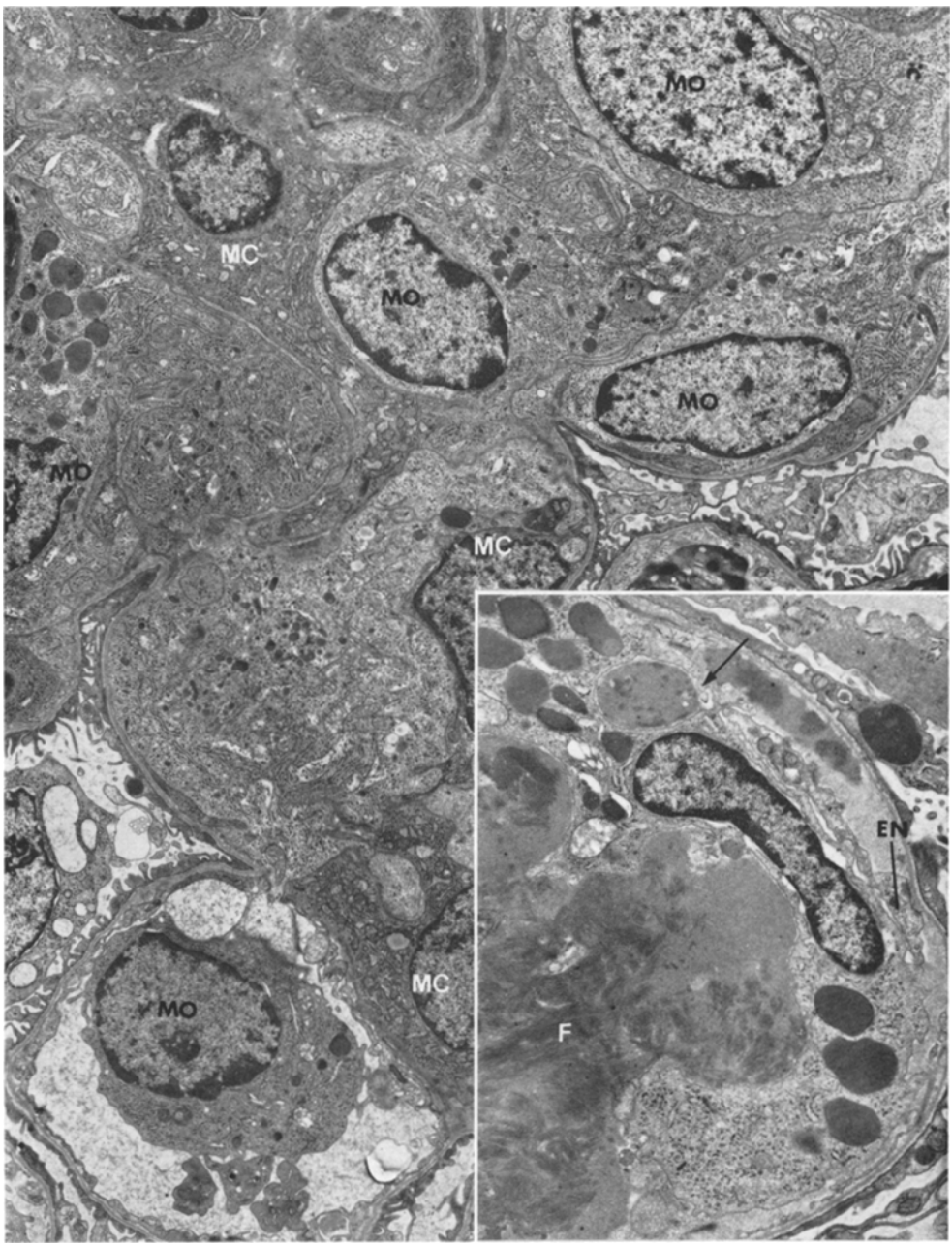


Fig. 1. Proliferative glomerulonephritis due to the accumulation of monocytes. Monocytes (*MO*) enlarge themselves in the cappillary lumen resulting in its occlusion. Mesangial cells (*MC*) can be distinguished from the monocytes by their higher electron density of the cytoplasm and the presence of peripheral filamentous zone. Group I-SS10B_1 (12 days after intravenous injection of BSA) ×4,100. (Inset) Phagocytic activity of monocyte. Endothelial sheath (*EN*) is detached from the glomerular basement membrane. Fibrinoid material is engulfed by the cytoplasmic process of the monocyte (arrow) with abundant lysosomal granules. Strands of fibrin (*F*) are seen in the capillary lumen. SS10B_1×7,600

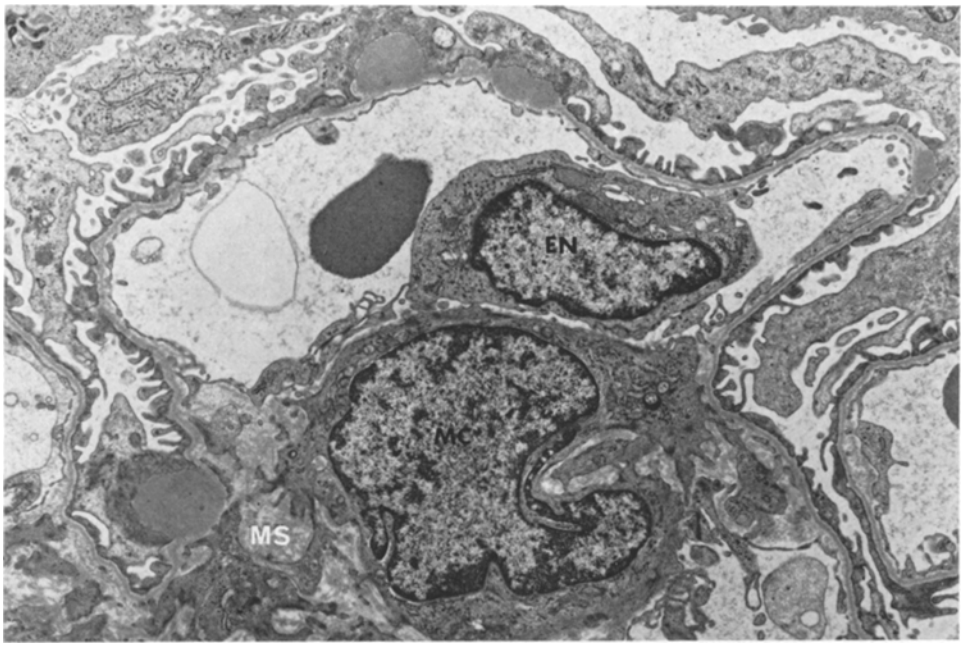


Fig. 2. Resolution of proliferative glomerulonephritis. Same case as Fig. 1. 19 days after intravenous injection of BSA. Though the mesangial matrix (*MS*) is still seen edematous, the capillary lumen is found to become patent. Numerous electron dense deposits are observed in the subepithelial space with the local fusion of the foot processes. SS10A ×6,000

well preserved after the disappearance of the phagocytes. Though the capillary lumen was seen to become patent, the swelling of the mesangium and subendothelial spaces due to the accumulation of proteinaceous materials could still be observed in some segments (Fig. 2). The subepithelial deposits with fusion of foot processes were seen to be prominent in some animals (Fig. 2). An increase of mesangial matrix and cells was noted to have persisted in focal and local distribution.

Progressive Disorganizing Glomerulonephritis

Unhealable glomerular lesions were observed in the animals of Group II which showed persistent proteinuria. The glomerular disorganization was seen to consist intracapillary and/or extracapillary extension of the glomerular alteration.

(a) Intracapillary disorganization: Though the accumulation of phagocytes was a prominent feature here, as it was in *transient proliferative glomerulonephritis*, definite damage to the axial region was observed to be an added feature of this type of disorganization. In local and segmental glomerular tufts, the subendothelial swelling following the repletion of proteinaceous material was so extensive as to lift up the whole endothelial sheath from the GBM. In some capillary lumen of these segments, the endothelial sheath was totally absent and the inner surface of the GBM as well as the mesangial matrix were directly

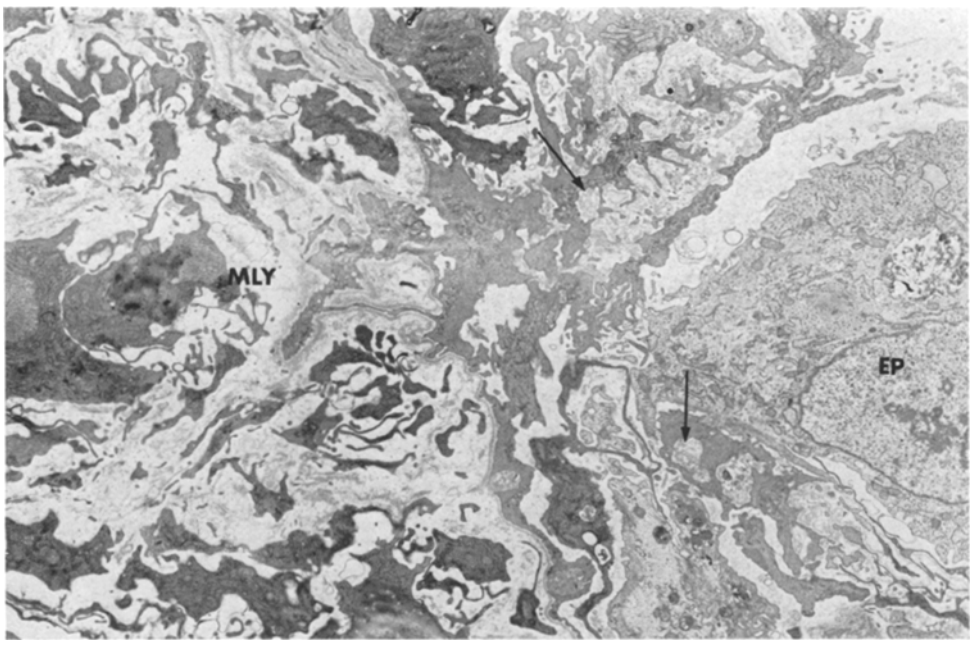
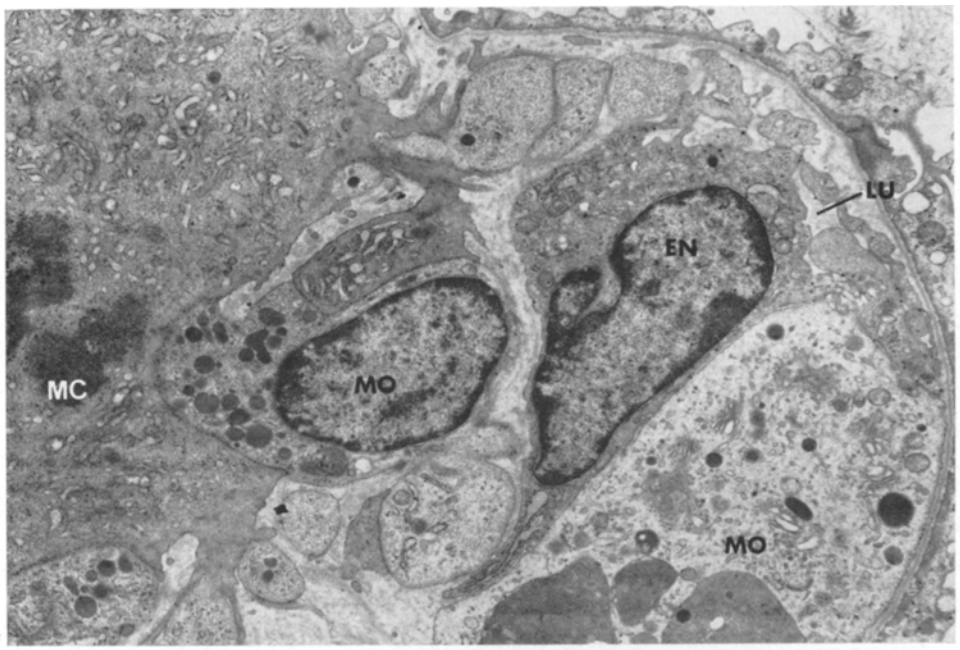


Fig. 3. Mesangiolysis. The glomerular tuft shows a globular appearance due to severe mesangial disintegration (*MLY*). Portions of degenerated cell cytoplasm are seen. Neither capillary lumen nor mesangial matrix can be distinguished. Amorphous and fine granular substances are surrounded by epithelial cells (arrows). Group II-SS72B_2 (14 days after the second injection of BSA). ×4,900

exposed to the lumen. As a result of the erosion of the luminal side of the mesangial matrix, the mesangial cell cytoplasm was often seen to be free in the blood stream. Sometimes the mesangial matrix was so widely altered by the progressive soaking in the plasma constituents as to change into a kind of blood cavity without matrix. Thus the mesangiolytic change reconstructed the glomerular tufts into baloon-like globular structure containing floating degenerated cells (Fig. 3). In some other segments, formations of fibrin and fibrinoid materials were detected in the mesangial matrix, a situation which was often associated with fibrin thrombi in the capillary lumen. Meanwhile, some part of the mesangial disintegration was seen to continue with a scarring or sclerosing process. Some of the mesangial cells showed a proliferation (Fig. 4) and some others protruded their cytoplasmic processes toward the edematous subendothelial space of the peripheral capillary loop (Fig. 5). As early as one week after the onset of proteinuria, partial or complete circumferential mesangial interposition was observed in local segmental distribution. On occasion avascular glomerular tufts were observed, which were composed only of atrophied podocytes and mesangial cells (Fig. 6). The matrix was thin and accompanied by granular deposits and cell debri. These collapsed and obsolescent glomerular tufts were seen among the tufts showing moderate or slight mesangial scarring.



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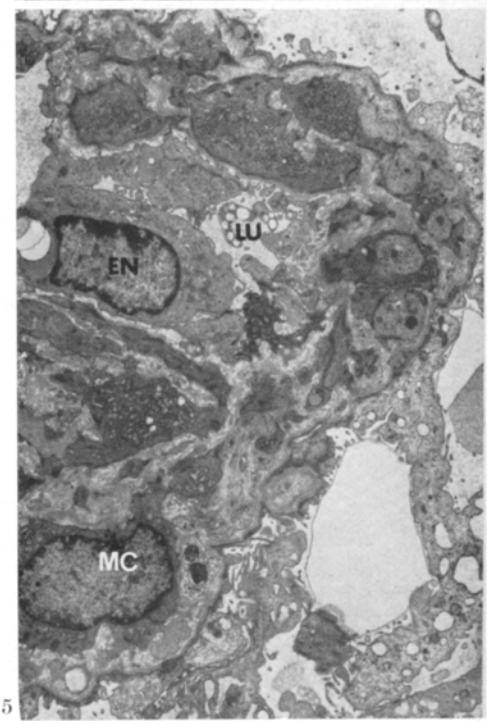

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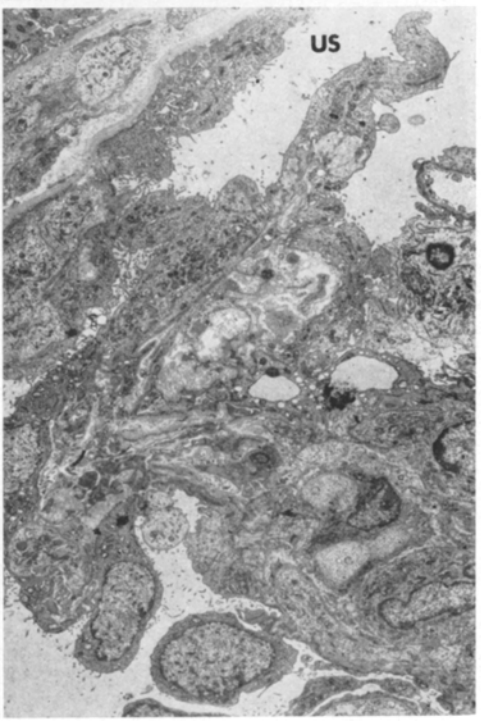

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Fig. 4. Mesangial cell proliferation. Cell division of mesangial cell is seen in the edematous mesangial matrix. Note the highly narrowed capillary lumen (*LU*) due to the subendothelial swelling and emigration of monocytes. Group II-SS9B$_2$. ×5,100

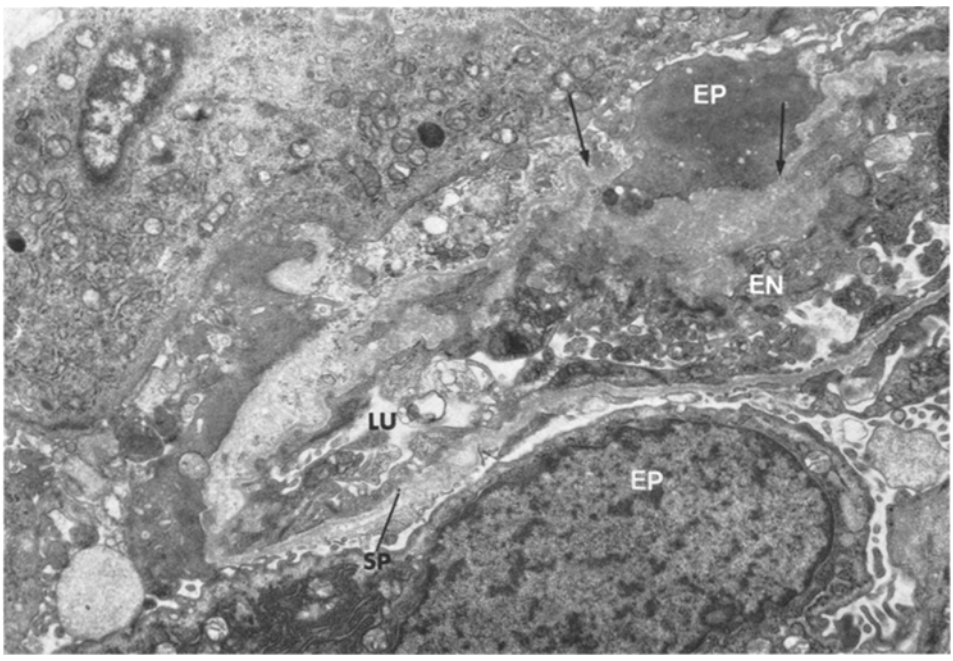

Fig. 7. GBM alteration. Lamina densa is unclear between the arrows, where the subendothelial accumulation of electron dense materials and fusion of foot processes with increased basal density are observed. Rarefaction or splitting (*SP*) is seen in the left lower part of the capillary loop. Deposition of materials with less electron density and granular or vesicular appearance is observed in the subepithelial aspect of the GBM. Group II-SS47B_2 (19 days after the second injection ot BSA). ×9,600

(b) Extracapillary disorganization: Varing degrees of damage were observed in the subepithelial aspect of GBM. Rarefraction or splitting was the common feature, and the density of lamina densa was seen to be highly decreased in some parts of the GBM (Fig. 7). Podocytes showed fusion of foot processes and increased basal density with an accumulation of fibrillar structure in the cytoplasm (Fig. 7). Fusions of the GBM with the capsular basement membrane were observed in some peripheral capillary loops. Subepithelial electron dense deposits were not prominent. Extravasation of fibrin or fibrinoid materials into the urinary space was commonly found in the capillary loops with fibrin formation where the GBM was seen loosened and decreased in electron density (Fig. 8). Podocytes were lifted up or exfoliated from the GBM due to the massive sub-

Fig. 5. Circumferential mesangial interposition. Mesangial cell protrudes its cytoplasmic processes toward the peripheral part of the capillary loop, accompanying the production of the matrix. Group II-SS9B_2. ×3,600

Fig. 6. Avascular tuft. Podocytes cover the winkling GBM and matrix devoid of vascular structure. Group II-SS47B_1 (5 days after the second injection of BSA). ×2,000

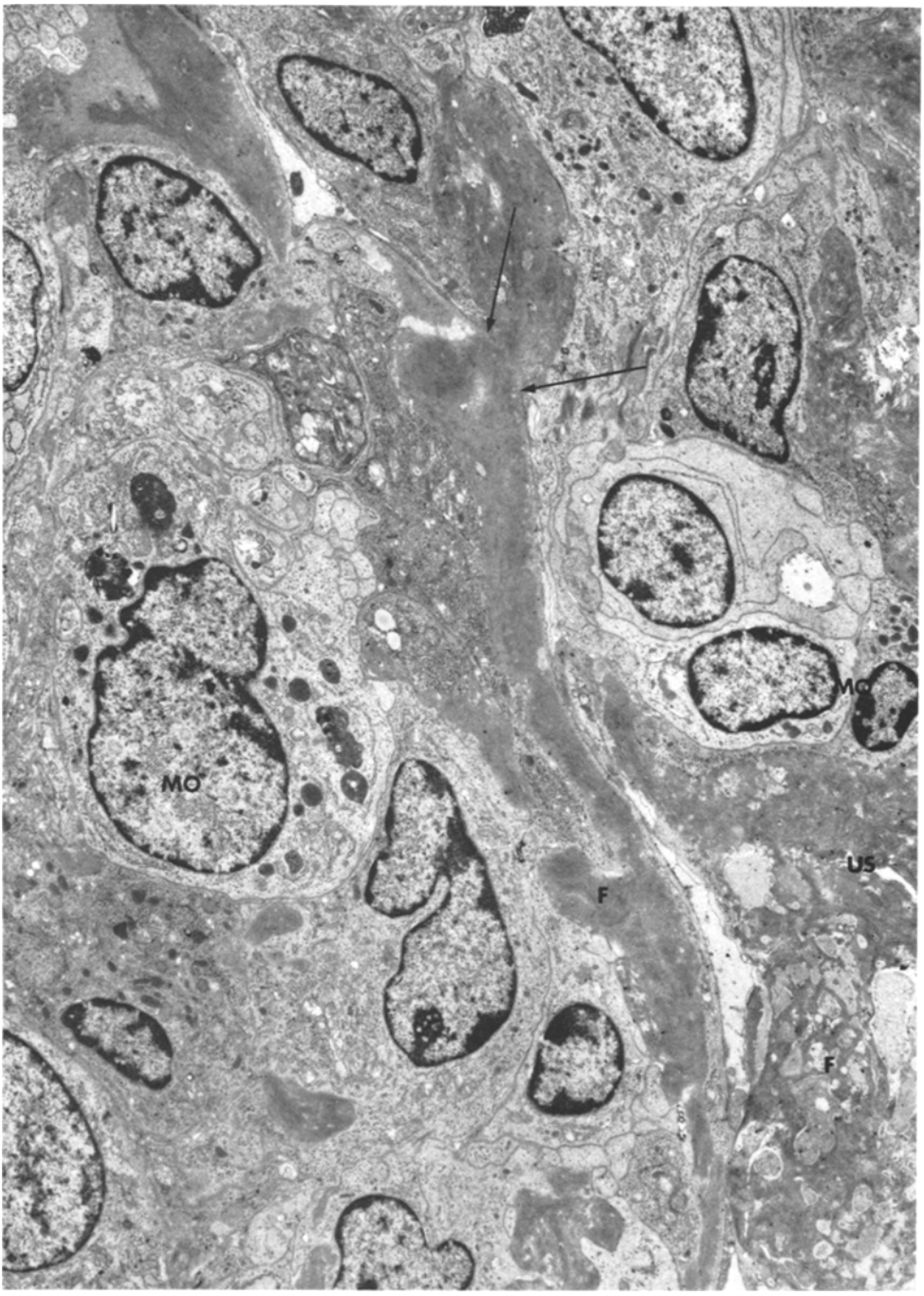

Fig. 8. Efflux of inflammatory products into urinary space. Prominent intracapillary fibrin formation (*F*) is seen accompanied by the infiltration of monocytes. Extravasation of fibrinoid materials is seen between the arrows. In the urinary space (*US*) the fibrionid materials are intermingled with cell debris and wandering monocytes. Group II-SS54B_1 (4 days after the second injection of BSA). ×5,000

epithelial accumulation of fibrinoid materials. Degenerated and necrotic epithelial cells were intermingled with fibrin and extravasated phagocytes in the Bowman's space. A small interruption and further a rupture or break of the GBM was observed to be closely related to the presence of inflammatory products in the urinary space. Through the gap of the GBM an efflux of intracapillary elements was observed, which included inflammatory cells (Fig. 9) and products as well as mesangial components. This resulted in the further opening of the gap. It was usually difficult to find any ruptured edges of the GBM, because granulomatous lesions consisting of podocytes and extravasated elements were seen to develop soon after the rupture of the GBM (Fig. 10). In these lesions a matrix similar to the mesangium was produced accompanied by scattered strands of collagen fibers. The discrimination of cell types in the matrix was difficult except for the podocytes covering the granulomatous lesion. Some resembled mesangial cells while others were like fibroblasts and wandering monocytes. There was no capillary development in the granulomatous lesion. The proliferation of epithelial cells (podocytes and capsular epithelial cells) was observed to be in intimate contact with the fibrin network in the Bowman's space. Epithelial cells were seen to proliferate and extend their cytoplasm to cover the fibrin mass, and a basement membrane-like structure was seen beneath the cytoplasm. The proliferation of epithelial cells often coexisted with the granulomatous lesion in the Bowman's space.

Discussion

The clinical onset and recovery of glomerulonephritis clearly correlate with the inflammation and repair of the glomerulus. The resolution of proliferative glomerulonephritis in serum sickness has been shown to be mainly due to the disappearance of phagocytes from the glomerulus. Though there is a moderate local increase of intrinsic glomerular cells, they do not result in the glomerular disorganization, thereby losing glomerular function. The persistence of subepithelial humps seemingly does not correlate with the progression of glomerular damage (Arakawa and Kimmelstiel, 1970). The accumulation of monocytes appears to participate in the removement of the inflammatory products including injured cells and tissue debris formed in the glomeruli. In fact, there apparently exists evidence that there is a prominent phagocytic activity in monocytes appearing in the glomerular capillary lumen when carbon particles are injected at the onset of glomerulonephritis (Sano, 1975). In contrast, there is no apparent phagocytic activity in the endothelial nor in the epithelial cells. This suggests that the glomeruli can recover to nearly normal structure if the hypercellularity is mainly composed of the phagocytes. A similar sequence of events has been observed in the second phase of rabbit Masugi nephritis where monocytes are seen to increase in the glomerular capillary lumen following the combination of host antibody to the heterologous globulin (duck nephrotoxin) fixed along the GBM, and disappear in accordance with repairment of the glomeruli (Kondo and Shigematsu, 1972). At least a part of the transient and recoverable glomerular hypercellularity was shown to result from the accumulation of monocytes, a situation which appears to agree with the suggestion of Strunk and his associates (1964) that blood monocytes participate in human proliferative glomerulonephritis.

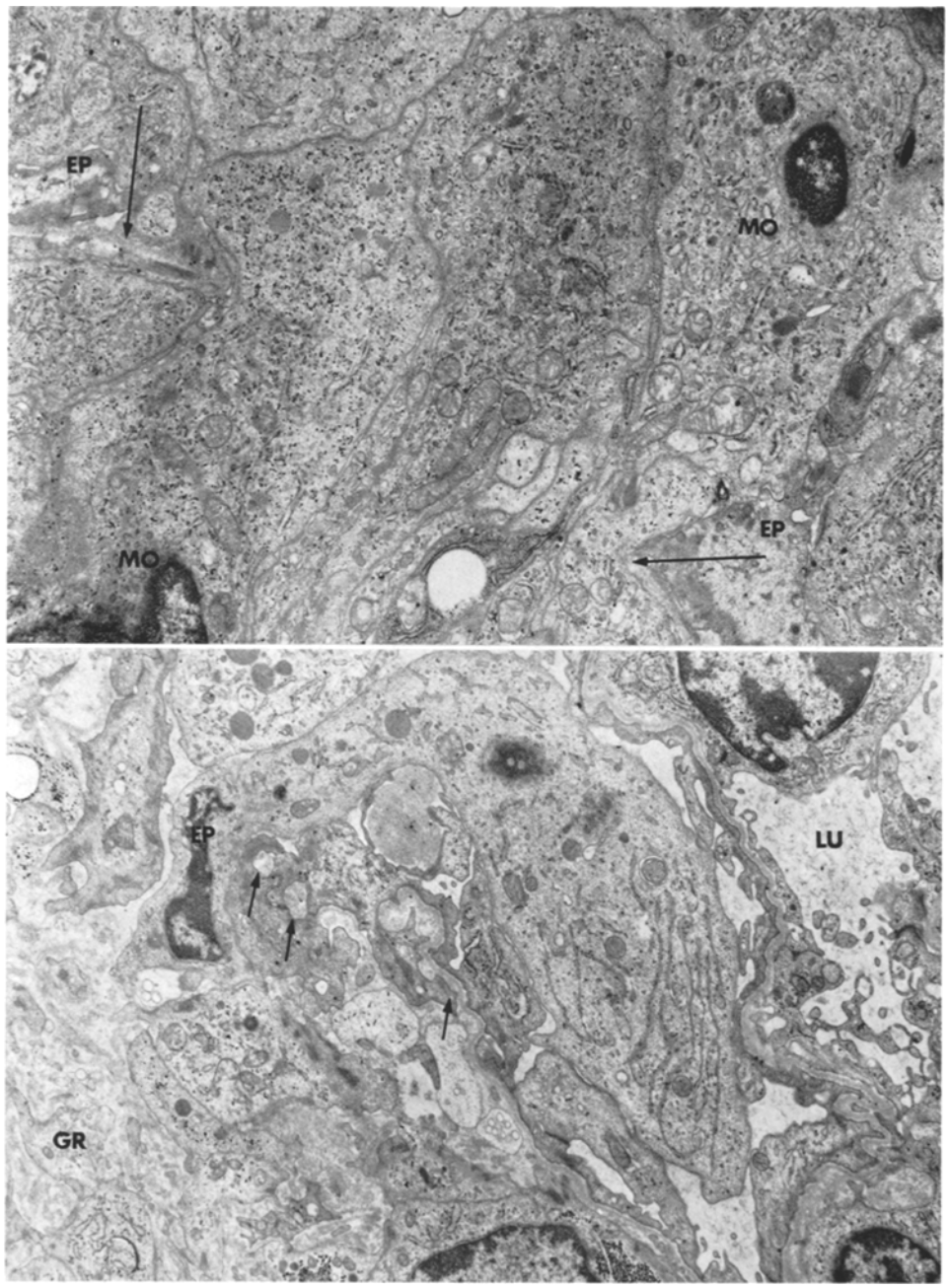

Fig. 9. Rupture of GBM. The GBM is interrupted between the arrows and monocytes are seen to emigrate into the urinary space. Group II-SS101B_1 (7 days after the second injection of BSA). ×9,400

Fig. 10. Rupture of GBM. The edge of ruptured GBM and nodules consisting of basement membrane like materials are covered by the epithelial cell (arrows). The epithelial cytoplasm is seen to make contact with the matrix of the granulomatous lesion in the left part of the figure (*GR*). Group II-SS47B_1. ×7,500

Thus the disappearance of phagocytes from the glomerulus is considered to provide one of the best explanations for the resolution of proliferative glomerulonephritis (Jones, 1951; Shigematsu *et al.*, 1973).

Collapse of the glomerular tufts as well as circumferential mesangial interposition (Arakawa and Kimmelstiel, 1969) are both histological manifestations deprived of the function of glomerular filtration. These glomerular distortions seem to have resulted from mesangial disintegration. Following the edematous swelling of the subendothelial space, the mesangial matrix is seen to lose its electron density and shows a widening because of the repletion of proteinaceous material including fibrinoid substance and an emigration of wandering phagocytes. The capillary lumens become obscure because the endothelial cells are often denuded from the capillary wall and the mesangial cells are separated from the mesangial matrix. The extreme disintegration of mesangial architecture quite resembles the mesangiolysis seen in Habu-snake poisoning, though there are no prominent tissue and cell reactions in the latter condition (Suzuki *et al.*, 1963). When the axial damage is moderate and the capillary lumen is preserved, succeeding events seem to be the proliferation and enlargement of mesangial cells with the increase of the mesangial matrix along the inner side of the GBM. This results in partial or complete circumferential mesangial interposition. When the mesangial damage is so severe as to destroy the capillary structure, the lesion changes into collapsed glomerular tufts with sclerosis and hyalinization of the matrix.

Though the increasing amount of antigen-antibody complexes in the circulation correlates with the higher incidence of disorganizing glomerulonephritis, the mesangial disintegration does not always accompany the localization of antigen-antibody complexes as observed by flourescent microscopy (Kobayashi and Shigematsu, 1973). Electron microscopy revealed no deposition of electron dense materials in the axial region in addition to only a scanty presence of subepitherial humps when the mesangial disintegration was fully developed. It seems likely that the development of mesangial disintegration is not evoked directly by the localization of antigen-antibody complexes in the axial region. Supporting evidence has been reported in progressive Masugi nephritis in rabbits and rats where similar mesangial disintegration does not accompany the localization of immunoglobulin in the axial region (Kondo *et al.*, 1972; Shigematsu and Kobayashi, 1973).

Intravascular fibrin formation and its deposition in the loosened mesangial matrix are often observed in the glomerular tufts undergoing mesangial disintegration. Fibrin and its derivatives seem to play an important role in the disorganization process of glomeruli. Vassalli and his associates (1963) noticed that intravascular coagulation could induce glomerular injuries equivalent to different stages of glomerulonephritis. Further, some investigators observed that supression of the coagulation process resulted not only in the prevention of fibrin deposits, but also in marked diminution or suppression of Masugi nephritis (Vassalli *et al.*, 1964) or serum sickness nephritis (Kobayashi, 1975). On the contrary, it has also been well known that massive intravascular coagulation resulted in the cortical necrosis without further extension of glomerular distortion, possibly because of the severe damage of fibrinolysis (Bergstein, *et al.*, 1974; Warren and Khan, 1974). Thus the coagulation process is, seemingly, not the

only participant in the mesangiolysis. The sever insudative events in the mesangial matrix accompanying the activation of the coagulation process may play an important role in the development of mesangiolysis. Though the other factors contibuting to the mesangial disintegration are obscure at present, attention should be paid to the phenomenon as one of the histological bases for the development of irreversible glomerulonephritis. In fact, severe mesangial damages including mesangiolysis have been observed in the glomeruli in some human progressive glomerulonephritis (Shigematsu *et al.*, 1974; Churg and Grishman, 1975).

Extracapillary extension of glomerular lesions seems to be intimately related to the damage of the GBM. GBM alterations have been considered to be induced by the participation of antigen-antibody complexes from the circulation. Though the deposition of electron dense deposits, particularly in the subepithelial spaces, is characteristic in recoverable glomerular lesion in transient serum sickness, immune deposits are uncommon in the progressive glomerular lesion. It is probable that the highly increased permeability fails to trap the immune deposits within the GBM. It has been reported that membranous glomerulonephritis with prominent depositions of immune deposits along the GBM is a characteristic feature of chronic serum sickness nephritis (Dixon, *et al.*, 1961; Germuth, *et al.*, 1972a; Kuriyama, 1973). In this situation the glomerular structure is well preserved and there is no significant proliferation of instrinsic glomerular cells. It has been postulated that the continuous and persistent glomerular deposition of circulating soluble antigen-antibody complexes is the main histologic basis for the development of membranous glomerulonephritis. The manner of contribution of antigen-antibody complexes in acute progressive serum sickness nephritis thus shows a definite contrast to that of chronic serum sickness nephritis, a fact which calls attention to the necessity of clarifying the differences of the characters of antigen-antibody complexes formed in both conditions. Low avidity and low precipitability of the antibody have so far been noted in the latter condition (Nakabayashi, 1974).

Lysosomal enzymes from polymorphonuclear leukocyts (PMNs) do not seem to be the main causative agents for the increased permeability in serum sickness nephritis because PMNs are not the main cell types in the glomerular hypercellularity, unlike in the first phase of Masugi nephritis (Cochrane *et al.*, 1965; Gang *et al.*, 1970). Although the direct contact of the PMNs with the inner surface of the GBM can be seen in focal and segmental distribution, the histologically severe GBM damage does not always correlate with the localization of PMNs.

A concept that soluble factors which are released from basophils when specific antigen binds to IgE antibody on their surfaces, cause clumping of platelets and release of their vasoactive amines, has been proposed by Cochrane (1971) as basic events of increased vascular permeability underlying serum sickness. The histological analysis has not been able to confirm this concept.

The fibrin in the urinary space extravasated through the injured GBM seems to be participating in the proliferation of epithelial cells so long as the fibrin and its derivatives persist to deposit in the Bowman's space. Though the massive formation of fibrin in the urinary space causes, initially, degenerative and ne-

crotic changes in the epithelial cells, follow-up study elucidates that epithelial cells grow among the spaces of fibrin network and extend their cytoplasm over the fibrin mass.

The rupture of the GBM results in the extracapillary involvement of the inflammation. The break of the GBM permits the efflux of intracapillary components including mesangial cells and matrix into the urinary space, resulting in the formation of granulomatous lesions with the proliferation of epithelial cells. It has been known that in anti-GBM antibody induced glomerulonephritis (Steblay nephritis) or in the second phase of Masugi nephritis, the rupture of the GBM is the critical factor for the development of extracapillary crescentforming glomerulonephritis (Germuth *et al.*, 1972b; Kondo *et al.*, 1972). It is noteworthy that a similar sequence of events could be taking place in serum sickness nephritis, though the underlying pathogenetic mechanism is quite different from the former two. There are some reports dealing with the rupture or break of the GBM in human progres ive glomerulonephritis (Stejskal *et al.*, 1973; Morita *et al.*, 1973). Thus the presence of the rupture of the GBM could be taken as one of the critical signs for the progressive and disorganizing nature of glomerulonephritis.

The authors wish to acknowledge members of Department of Pathology, Chiba University, for many helpful advices. They also thank Mr. A. Gerz for preparation of English manuscript.

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