ELECTRON MICROSCOPY OF SERUM SICKNESS NEPHRITIS*, ‡

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PLATES 65 TO 70

(Received for publication, June 30, 1958)

The morphology of antigen-antibody reactions in tissues has been amply documented by studies with the light microscope. At the cytological level, however, the intracellular changes elicited by antigen-antibody union have been poorly defined and vaguely located. As an experimental model to study this problem, serum sickness nephritis was selected first, because there was already a fairly large accumulation of data on the electron microscopic anatomy of the kidney which could be used for comparison (1–17), and second, because specific antigen has been shown to be present in the glomerulus by the fluorescent microscopic technique (18). Serum sickness offered the possibility of studying the cellular events in the development of renal lesions.

Materials and Methods

Six albino rabbits, weighing between 2.5 and 3.0 kg., were used. Two were controls; four were injected intravenously with 250 mg. of bovine serum albumin (BSA)[1] per kg. of body weight. The BSA was purified, crystallized, and tagged lightly with I^{131}. The rate of antigen elimination was followed by determining the disappearance of serum radioactivity. Immune elimination occurred in all four animals 11 days after the injection of antigen; two were killed 1 day later and two were killed 2 days later; *i.e.*, 12 and 13 days after antigen administration. It has been shown that during the period of immune elimination of antigen, the glomerular lesions were most extensive and severe (18), while prior to this period glomerular lesions could not be found by light microscopy. After antigen was completely lost from the circulation, glomerular lesions were diminished in number and severity. Fluorescence microscopy showed antigen only in those glomeruli which were altered.

RESULTS

The structure of the normal glomerulus is briefly reviewed here to point out certain salient features for comparison with glomeruli of experimental rabbits.

The glomerulus (Figs. 1 and 3) is constructed of a network of patent capillaries which communicate with one another by short, narrow channels. Endothelial cells are commonly

* This work was supported by a grant from the United States Public Health Service. No. E-1229(C).

‡ This is reprint No. 169 from the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh.

[1] Purchased from Armour Laboratories, Chicago, lot No. P76908.

found in these channels or in the adjacent capillary lumens. The endothelial cytoplasm beyond the nuclear zone usually is attenuated to an electron-dense membrane, averaging 350 A in thickness, lining the inner surface of the basement membrane and perforated by pores ranging from 250 to 500 A in diameter. The basement membrane is composed of an amorphous material which in cross-section is more dense centrally than at its edges. Its width, averaging 1200 A, is remarkably constant throughout the glomerular capillaries. In the short communicating channels, where it tends to vary in width, it is generally thicker than elsewhere and forms an intraluminal mesh. The basement membrane is studded on its external surface by electron-dense epithelial foot processes which are regularly spaced. Over some relatively small areas of capillary wall, however, it is covered with thin sheets of epithelial cytoplasm instead of foot processes. The capillary wall, then, consists of three membranes; an inner porous endothelial lining, a basement membrane, and an outer covering of foot processes with spaces between them. Epithelial and endothelial cells are about equal in number.

In serum sickness nephritis, the renal lesion appeared to be confined to the glomerulus. By light microscopy a majority of the glomeruli were affected; a small number, however, were unchanged, and rarely only a single tuft in a glomerulus showed cellular increase while the remainder was unaltered.

The most striking and extensive change was the swelling and proliferation of the glomerular endothelial cells (Figs. 2 and 4). In severely affected glomeruli, the capillaries were enlarged and their lumens completely obliterated as a result of the endothelial increase. In glomeruli less severely involved the lumen was marked by a red cell intimately encircled by the swollen and proliferated endothelial cells or by a slit-like space. Endothelial cytoplasm was not applied as a thin lining membrane, but instead, free of pores, filled out the interior of the capillary. In some areas, the cytoplasm blended with the basement membrane so that no demarcation was possible between these two elements. Many cells contained a fairly extensive and well developed endoplasmic reticulum as shown in Fig. 4. Polymorphonuclear leucocytes were not seen in the glomeruli, but rarely, a cell resembling a lymphocyte or a macrophage was observed among the swollen endothelial elements.

The thickness, structure, and texture of the basement membrane were for the most part unaffected. There were, however, focal thickenings and excrescences and deposits of an electron-dense material which blended without demarcation into basement membrane of apparently normal appearance (Figs. 5 and 6). These deposits were most often seen on the luminal side, but were observed twice on the extracapillary side of the vessels (Fig. 2).

On the epithelial side of the vessels, foot processes were diminished in number and replaced by thin sheets of epithelial cytoplasm. When foot processes remained, they tended to be irregular in shape and less electron dense than normal. The nuclei, mitochondria, Golgi apparatus, and other cytoplasmic elements of epithelial cells did not appear to be altered.

DISCUSSION

The most prominent changes in the glomeruli of serum sickness disease were marked endothelial cell increase and swelling, most likely the result of injury

due to antigen-antibody complexes. Recently, soluble antigen-antibody complexes have been shown to be capable of producing cutaneous, vascular, and glomerular lesions (19–22). Since by fluorescence microscopy antigen was found in the glomeruli only during the period of rapid elimination, and since antigen-antibody complexes circulated during this time, it was assumed that the complexes produced the endothelial alterations. There were, however, sufficient alterations of the basement membrane and of the epithelial foot processes to indicate that the primary site of injury might have been in either of these loci, and that the endothelial changes were secondary. From a comparison of the fluorescent and electron micrographs, the deposition of antigen would seem to be in the increased endothelial cells. It would be hazardous, however, to conclude that antigen was not present on the basement membrane or at the point of contact between epithelial cytoplasm and basement membrane.

Despite examination of many glomeruli from each experimental rabbit and of several partly altered glomeruli, the initial locus of injury was not observed in this series of animals. It would be of significant interest to determine at what site on or in the cell the antigen-antibody reaction elicited injury.

The morphology of serum sickness nephritis resembled in some respects the lesions described for acute human glomerulonephritis (23, 24), and for nephrotoxic nephritis (25–27). In both of these latter entities, there has been described endothelial proliferation, loss of foot processes, and in some instances thickening of the basement membrane. At present, one cannot conclude that the structural changes in all three morbid processes are the same, or that they occur in the same sequence. Too few cases of acute glomerulonephritis have been studied by electron microscopy and the sequence of events in nephrotoxic nephritis has not been fully explored to make valid comparisons between these three diseases. It would be extremely interesting, however, if the anatomical lesions were similar, since the etiologies of at least two of these processes, nephrotoxic and serum sickness nephritis, are different. In nephrotoxic nephritis, the kidney is antigen, more specifically glomerular capillary basement membrane and exogenous antibody induces immediate injury (28–30). In serum sickness nephritis, the antigen is exogenous and the host antibody reacts with the antigen to produce an antigen-antibody complex that localizes in the glomerulus. Further study by both electron and fluorescent microscopy may reveal more precisely the locus of injury in these morbid processes and pinpoint the site of antigen-antibody reaction.

SUMMARY

The renal lesions of serum sickness were studied with the electron microscope. The most prominent change was a marked swelling and proliferation of glomerular endothelial cells causing obliteration of the capillary lumen. The basement membrane also showed focal thickenings and excrescences. Deposits of electron-dense material blended into the basement membrane. On the

extracapillary side epithelial foot processes were reduced in number and replaced by broad sheets of cytoplasm which were closely applied to the basement membrane. From a comparison of electron and fluorescent microscopic studies of the glomerulus in serum sickness, it would seem that antigen-antibody complexes initiated injury in endothelial cells, although the possibility of the primary reaction occurring on basement membrane cannot be excluded.

BIBLIOGRAPHY

1. Pease, D. C., and Baker, R. F., Electron microscopy of the kidney, *Am. J. Anat.*, 1950, **87**, 349.
2. Dalton, A. J., Structural details of some of the epithelial cell types in the kidney of the mouse as revealed by the electron microscope, *J. Nat. Cancer Inst.*, 1951, **11**, 1163.
3. Oberling, C., Gautier, A., and Bernhard, W., La Structure de capillaires glomerulaires vue au microscope electronique, *Presse Med.*, 1951, **59**, 938.
4. Hall, B. V., Studies of normal glomerular structure by electron microscopy, *Proc. 5th Ann. Conf. Nephrotic Syndrome*, 1953, 1–39.
5. Rinehart, J. F., Farquhar, M. G., Jung, H. C., and Abul-Haj, S. K., The normal glomerulus and its basic reactions in disease, *Am. J. Path.*, 1953, **29**, 21.
6. Hall, B. V., Further studies of normal renal glomerular structure, *Proc. 6th Ann. Conf. Nephrotic Syndrome*, 1954, 1–39.
7. Pease, D. C., Further studies of the kidney cortex by electron microscopy, *Anat. Rec.*, 1954, **18**, 339.
8. Reid, R. T. W., Observations on the structure of the renal glomerulus of the mouse revealed by the electron microscope. *Australian J. Biol. and Med. Sc.* 1954, **32**, 235.
9. Bargmann, W., Knoop, A., and Schiebler, T. H., Histologische, Cytochemische, und Elektronmikroskopische Intersuchungen an Nephron (mit Beruchsichtigung der Mitochondrien), *Z. Zellforsch. u. mik. Anat.*, 1955, **42**, 386.
10. Mueller, C. B., Mason, A. D., Jr., and Stout, D. G., Anatomy of the Glomerulus, *Am. J. Med.*, 1955, **18**, 267.
11. Pease, D. C., and Boher, R. F., Electron microscopy of the vascular bed of the kidney cortex, *Anat. Rec.*, 1955, **121**, 701.
12. Policard, A., Collet, A., Giltaire-Ralyee, L., Recherches au microscope electronique sur la structure du glomerule renal des mammiferes, *Arch. Anat. Micr. et Morph. Exp.* 1955, **44**, 1.
13. Rhodin, J., Electron microscopy of the glomerular capillary wall. *Exp. Cell Research*, 1955, **8**, 512.
14. Rinehart, J. F., Fine structure of renal glomerulus as revealed by electron microscopy, *Arch. Path.* 1955, **59**, 439.
15. Sakaguchi, H. Fine structure of the renal glomerulus, *Keio J. Med.*, 1955, **4**, 103.
16. Yamada, E., The fine structure of the renal glomerulus of the mouse, *J. Biophys. and Biochem. Cytol.*, 1955, **1**, 551.
17. Bergstrand, A., Electron microscopic investigations of the renal glomeruli, *Lab. Inv.*, 1957, **6**, 191.

18. Dixon, F. J., Vazquez, J. J., Weigle, W. O., and Cochrane, C. G., Pathogenesis of Serum Sickness, *A.M.A. Arch. Path.*, 1958, **65,** 18.
19. Germuth, F. G., Jr., and McKinnon, G. E., Studies of the biological properties of antigen-antibody complexes in sensitized normal guinea pigs, *Bull. Johns Hopkins Hosp.*, 1957, **101,** 13.
20. Germuth, F. G., Jr., and Pollack, A. D., The production of lesions of "serum sickness" in normal animals by the passive transfer of antibody in the presence of antigen, *Bull. Johns Hopkins Hosp.*, 1958, **102,** 245.
21. Cochrane, C. G., and Weigle, W. O., The cutaneous reaction to soluble antigen-antibody complexes. A comparison with the Arthus phenomenon, *Fed. Proc.*, 1958, **17,** 507.
22. Ishizaka, K., and Campbell, D. H., Biological activity of soluble antigen-antibody complexes. 1. Skin-reactive properties, *Proc. Soc. Exp. Biol. and Med.*, 1958, **97,** 635.
23. Farquhar, M. G., Vernier, R. L., and Good, R. A., An electron microscope study of the glomerulus in nephrosis, glomerulonephritis, and lupus erythematosus, *J. Exp. Med.*, 1957, **106,** 649.
24. Farquhar, M. G., Vernier, R. L., and Good, R. A., The application of electron microscopy in pathology. Study of renal biopsy tissues, *Schweiz. Med. Woch.*, 1957, **87,** 17.
25. Piel, C. F., Dong, L., Modern, F. W. S., and Goodman, J. R., The glomerulus in experimental renal disease in rats as observed by light and electron microscopy, *J. Exp. Med.*, 1955, **102,** 573.
26. Reid, R. T. W., Electron microscopy of glomeruli in nephrotoxic serum nephritis, *Australian J. Exp. Biol. and Med. Sc.*, 1956, **34,** 143
27. Simer, P. H., Glomerulus in nephritic mice of the NH strain, *Anat. Rec.*, 1954 **118,** 409, (abstract).
28. Pressman, D., The zone of localization of antibodies. IV. J. Immunol., 1949, **63,** 375.
29. Krakower, C. A., and Greenspon, S. A., Localization of the nephrotoxic antigen within the isolated renal glomerulus, *A.M.A. Arch. Path.*, 1951, **51,** 629.
30. Cruikshank, B., and Hill, A. G. S., The histochemical demonstration of a connective-tissue antigen in the rat, *J. Path. and Bact.*, 1953, **66,** 283.

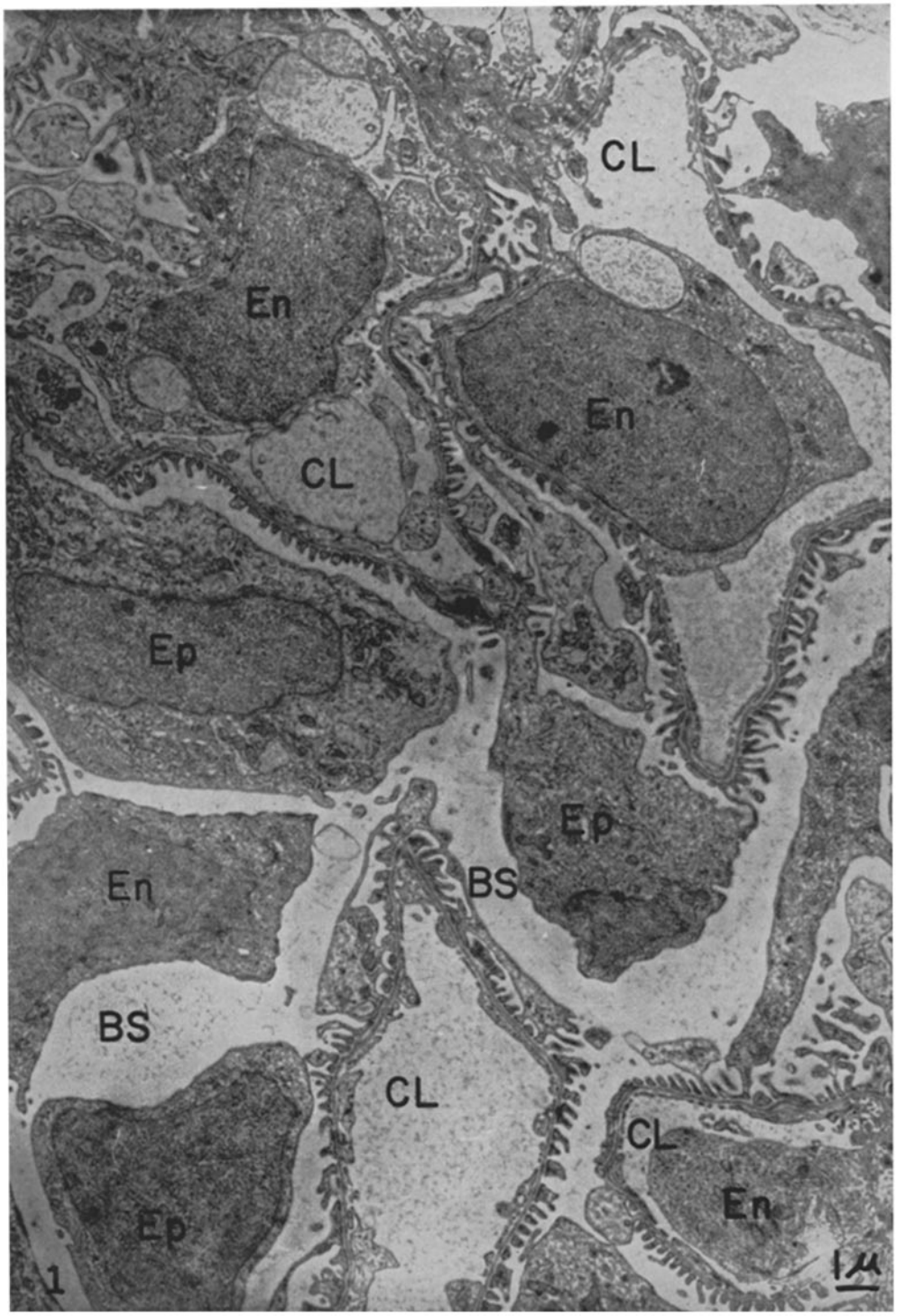
EXPLANATION OF PLATES

Abbreviations for Figures

BM	Basement membrane	*dep*	Deposit of electron dense material
BS	Bowman's space	*er*	Endoplasmic reticulum
CL	Capillary lumen	*fp*	Foot processes
En	Endothelium	*g*	Granules
Ep	Epithelium	*m*	Mitochondria
Rbc	Red blood cell		

PLATE 65

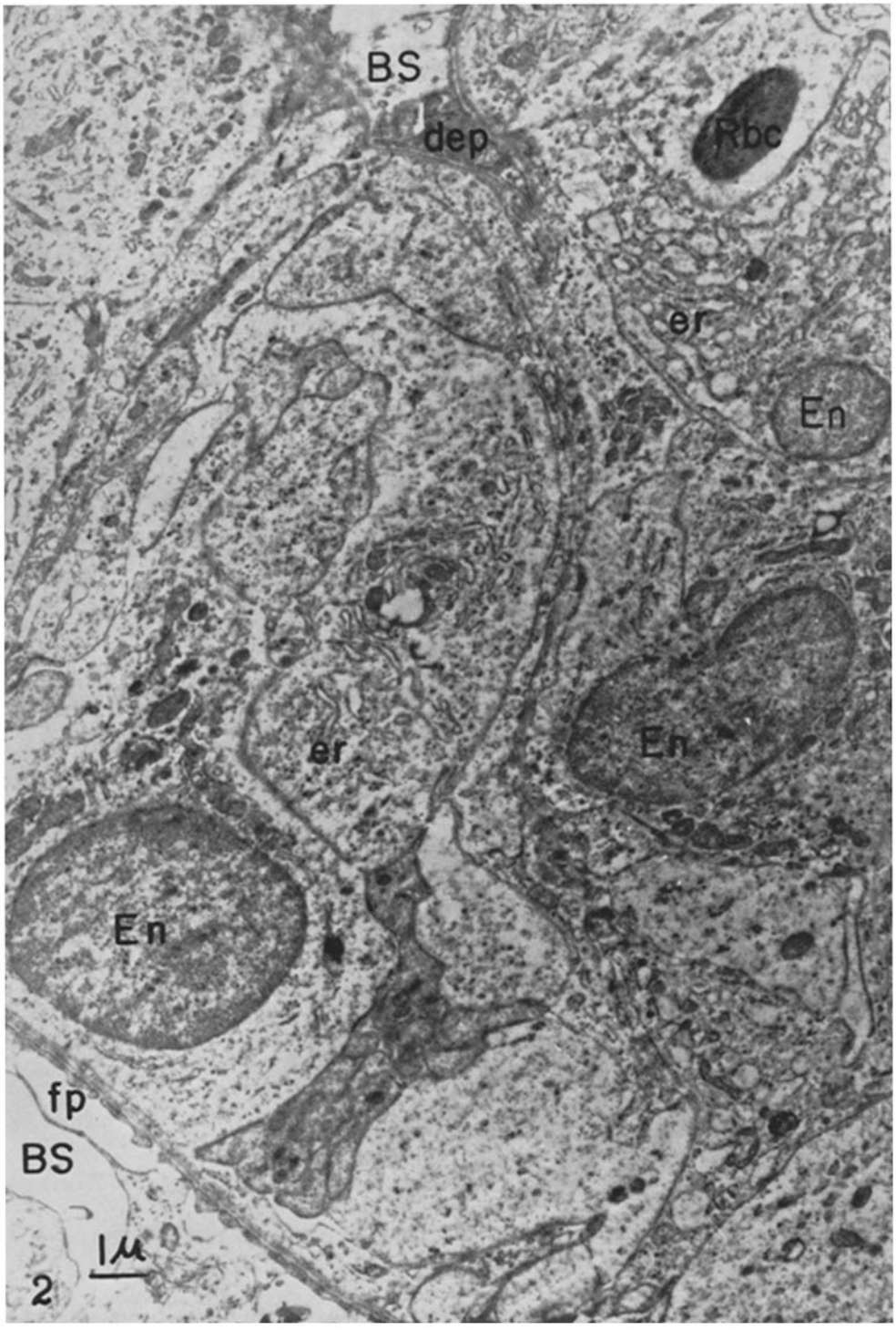
FIG. 1. Glomerulus from a control rabbit. The capillary loops are patent. The foot processes of epithelial cells are arranged in an orderly fashion and show their characteristic shape and electron density. The basement membrane is fairly constant in thickness and exhibits a central dense zone flanked by two lighter areas. A thin perforated electron-dense endothelial lining is visible over many portions of the basement membrane. Note especially the size of the capillary lumens and the number of endothelial nuclei. × 8500.



(Feldman: Serum sickness nephritis)

PLATE 66

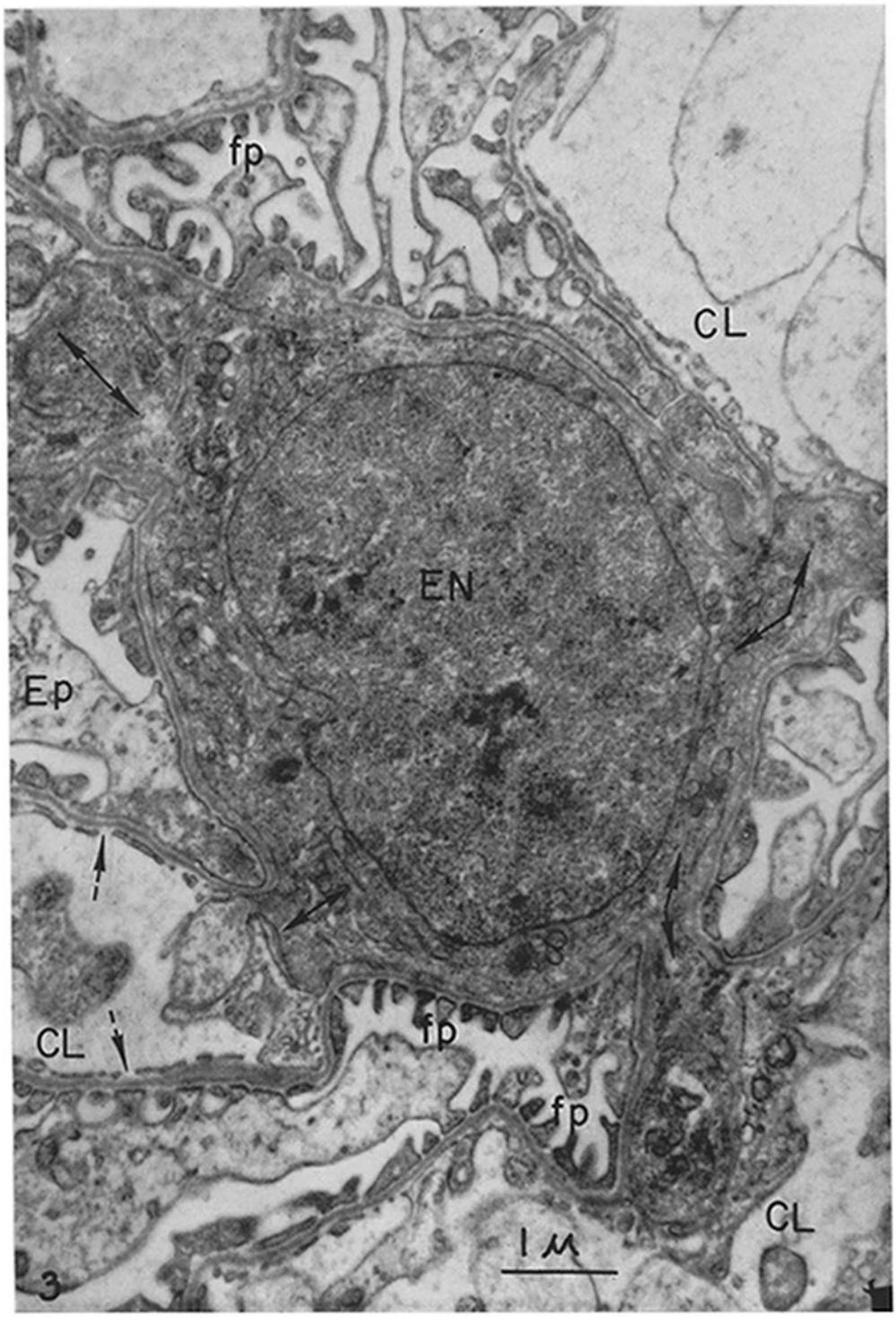
FIG. 2. Glomerulus from rabbit with serum sickness nephritis. Approximately the same magnification as in Fig. 1. The capillary loop is enlarged and the number of endothelial nuclei is increased. The lumen is almost obliterated by cytoplasm of endothelial cells. A portion of a red blood cell marks the site of the lumen. A deposit of electron-dense amorphous material (*dep*) is seen contiguous with basement membrane from the extracapillary side. Endoplasmic reticulum (*er*) is prominent in several cells and appears swollen. Foot processes are flattened and fused. × 9000.



(Feldman: Serum sickness nephritis)

PLATE 67

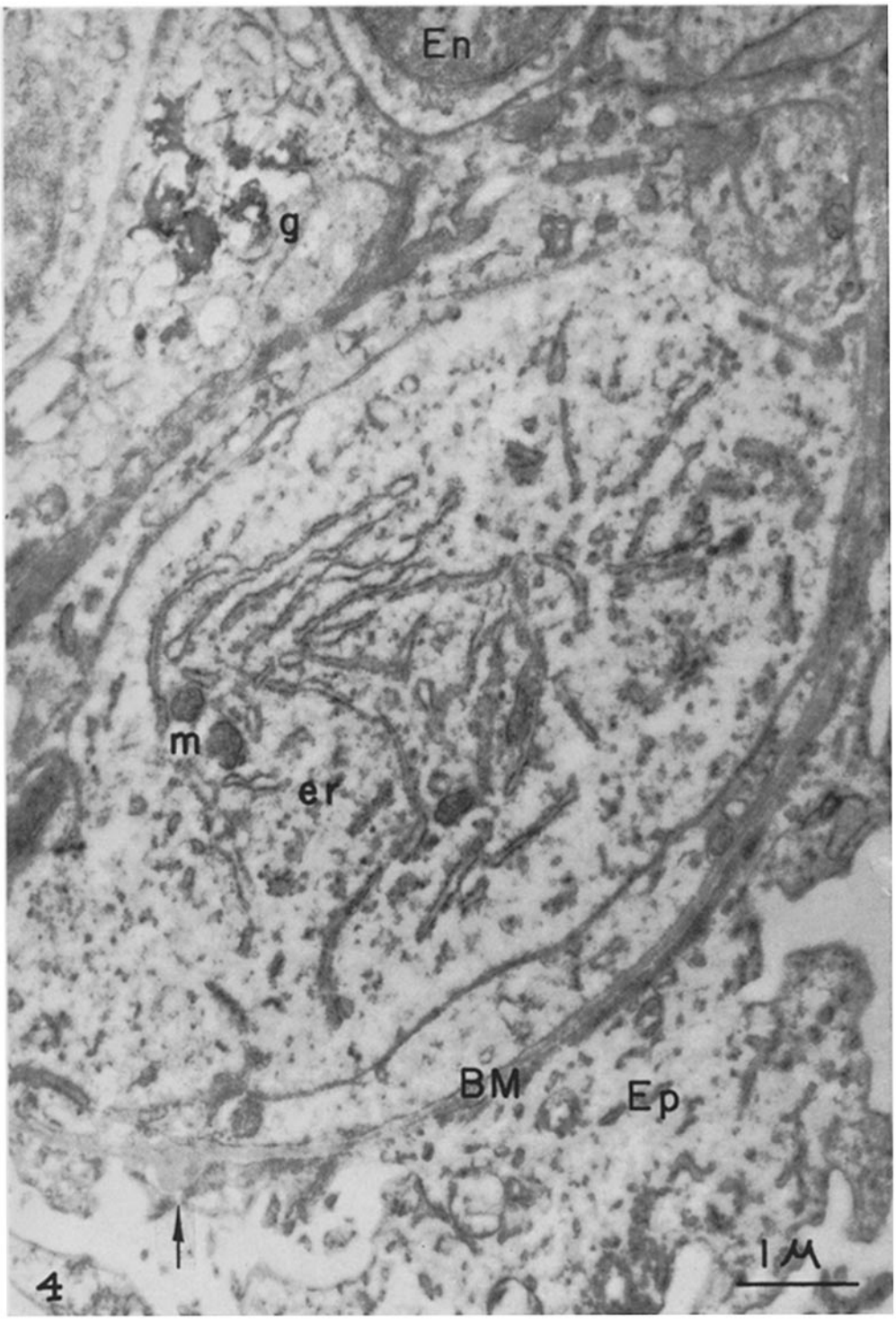
FIG. 3. Glomerulus from a control rabbit. An endothelial cell occupies a short communicating channel between four capillary loops (solid arrows). The basement membrane in this area shows several irregular thickenings. The perforated endothelial lining is well illustrated (dotted arrows). Foot processes are fairly regularly spaced over the basement membrane. At their point of contact with the basement membrane, they are somewhat flared and reveal a dense line separating the cytoplasm from the light zone of the basement membrane. Note the density of the endothelial cytoplasm. × 18,000.



(Feldman: Serum sickness nephritis)

PLATE 68

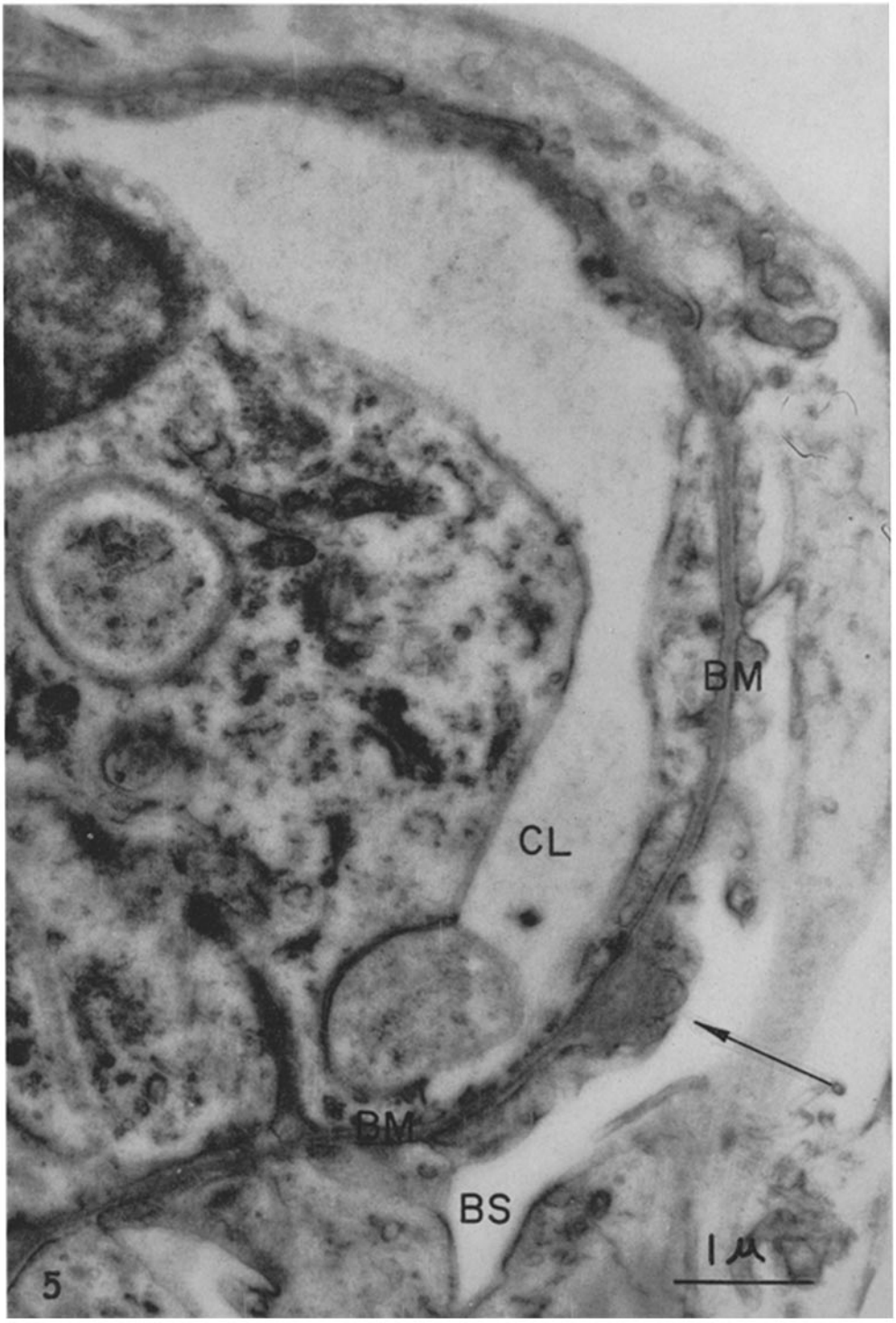
FIG. 4. Glomerular capillary loop in serum sickness nephritis. The lumen is completely filled with cytoplasm in which a fairly well developed endoplasmic reticulum (*er*) is present. Close to the nucleus is a cluster of small granules (*g*). Foot processes are replaced by sheets of epithelial cytoplasm. At arrow there is an irregular thickening of the basement membrane. Compare density of cytoplasm with that seen in Fig. 3. × 19,000.



(Feldman: Serum sickness nephritis)

PLATE 69

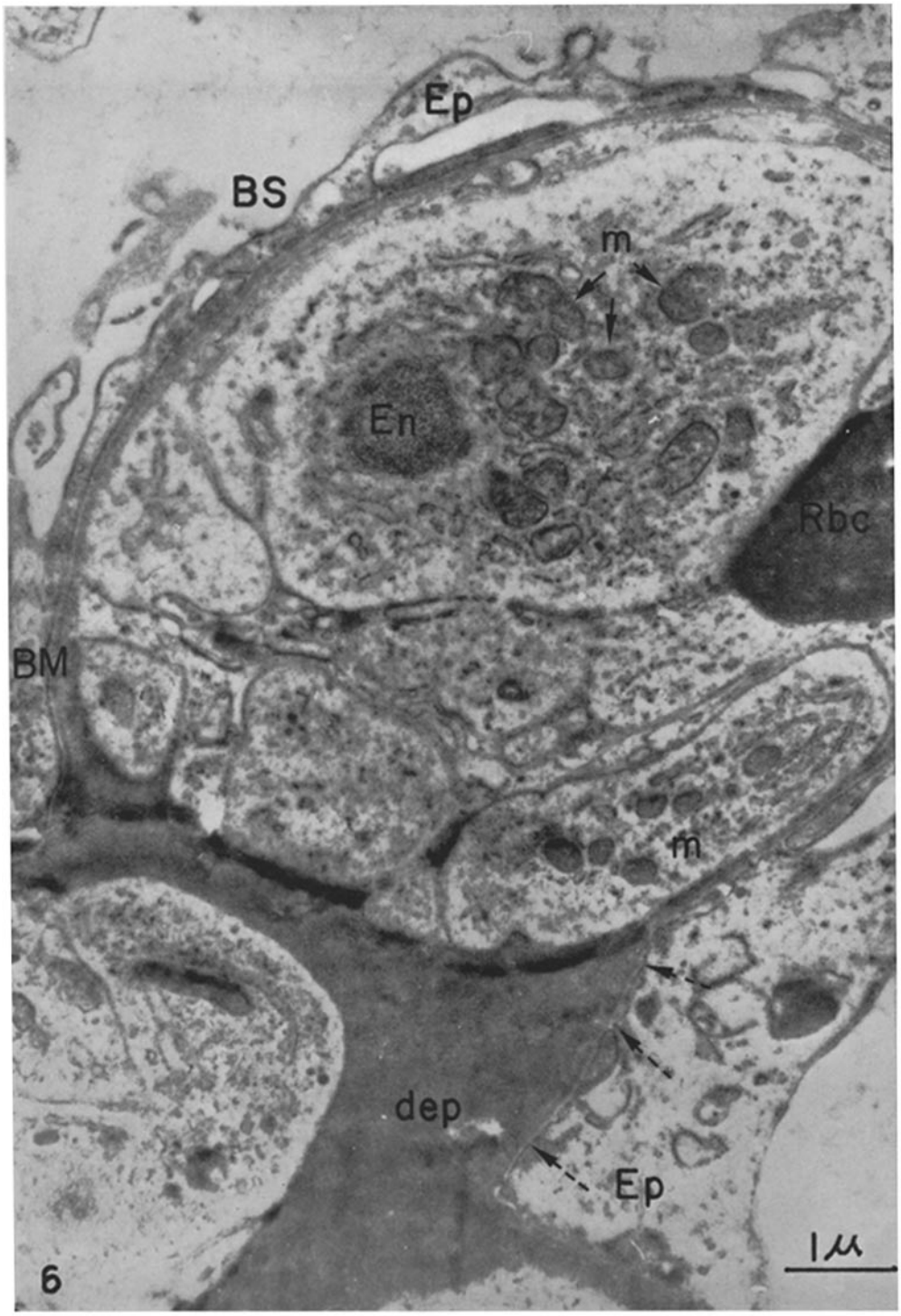
FIG. 5. A segment of a capillary loop in serum sickness nephritis. An excrescence of amorphous material blends with apparently normal basement membrane (arrow). Swollen endothelial cytoplasm, without pores, is applied closely to the basement membrane. × 21,500.



(Feldman: Serum sickness nephritis)

PLATE 70

FIG. 6. A capillary loop in serum sickness nephritis. An intraluminal deposit of finely granular electron dense material (*dep*) blends into basement membrane of normal appearance. Dotted arrows point to line of separation between epithelial cytoplasm and deposited material. A part of a red blood cell is surrounded by swollen endothelial cytoplasm. Foot processes are replaced by a sheet of epithelial cytoplasm. × 17,500.



(Feldman: Serum sickness nephritis)