Electron Microscopy of Nephropathia Epidemica

Renal Tubular Basement Membrane

Y. Collan, MD, J. Lähdevirta, MD, and E. J. Jokinen, MD

Tubular basement membranes in kidney biopsies from 18 patients with nephropathia epidemica were studied by electron microscopy. Both in the cortex and in the medulla there was splitting of the basement membrane. Thickened basement membrane around occasional tubules contained membrane vesicles, usually empty but also with a core and a diameter of approximately 180 nm. Membranous convoluted structures and light finely fibrillar areas in the basement membranes were seen. Splitting of the basement membrane was most prominent in the medulla, and the membrane was filled with round to oval particles 55 to 470 nm in diameter. Of the possible mechanisms of damage at the basement membrane level in this disease, the findings suggest liberation of antigen from the tubular cells and reaction of circulating antibodies with the antigen in the basement membrane. (Am J Pathol 92:167–172, 1978)

NEPHROPATHIA EPIDEMICA (NE) is a Scandinavian and north European variety of the hemorrhagic fever with renal syndrome or Korean hemorrhagic fever (KHF).[1,2] The disease was first described in 1934,[3,4] and since then the clinical findings and epidemiologic features have been thoroughly described.[2,5] The data suggest that the disease is caused by a virus; virologic studies further support this assumption.[6] The causative antigen in KHF has been isolated [7] and serologic findings have suggested a close antigenic relationship between the agents causing KHF and those causing NE.[8] Our immunohistochemical study of the glomeruli showed that circulating immune complexes are involved and also demonstrated tissue-bound immunoglobulins in tubular basement membranes in NE.[9,10] We have made an electron microscopic study of the changes in the tubular basement membranes.

Materials and Methods

The material consisted of 20 percutaneous renal biopsy specimens taken during and after the acute stage of the disease in 18 patients [11] from the epidemic area in Finland. Diagnosis was based on the typical clinical picture of the disease.[2] Five biopsy specimens were taken 3 to 9 days, 8 specimens were taken 10 to 19 days, 5 specimens were taken 20 to 35 days, and 2 specimens were taken 3.5 and 6.5 months after the onset of fever which characterizes the clinical onset of the disease (the day of onset was counted as the first day).

From the Department of Pathology, the Third Department of Medicine, and the Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland.

Accepted for publication March 13, 1978.

Address reprint requests to Y. Collan, MD, Department of Pathology, University of Helsinki, Haartmaninк. 3, SF-00290 Helsinki 29, Finland.

0002-9440/78/0710-0167$01.00

There were 4 females and 14 males. The mean age of the patients was 35.1 years (range, 15 to 63 years).

Peak serum creatinine value during the disease varied between 112 and 910 μmole/liter. The biopsy specimens were fixed in 3% phosphate-buffered glutaraldehyde (pH 7.3) for 3 hours, transferrred to 0.2 M phosphate-buffered sucrose solution, and kept there for 1 to 6 days in 4 C. After this the specimen was postfixed in 1% phosphate-buffered osmium tetroxide for 1 hour, dehydrated, and embedded in Epon. Ultrathin sections were cut with glass knives and stained with 1% uranyl acetate in 50% ethanol and with lead citrate. Semithin 1-μ thick sections were also cut and stained with 1% methylene blue in 1% sodium borate solution. Electron microscopic sections were studied with a Hitachi HS 7S electron microscope.

Results

In the cortex, most samples showed splitting of the basement membrane in places, around both proximal and distal tubules (Figure 1). There was thickening of the basement membrane with vesicular or tubular membranous structures embedded in it (Figures 2 and 3). Vesicles with a diameter of 180 nm with a darker inner core (diameter 135 nm) were seen (Figure 4). Occasionally the thickened basement membrane around the distal tubules showed lighter finely fibrillar areas (Figure 5) which could also include small membranous vesicles.

In the medulla, the basement membrane around the loops of Henle showed areas filled with round or oval particles (Figures 6 and 7), with diameters varying between 55 and 470 nm. Splitting of the basement membrane was prominent at these places.

Discussion

Ultrastructural changes in kidney tubules in human disease have not been clearly defined. This is mostly due to the prominence of artifactual changes in tubular cells in kidney biopsy specimens. Glomeruli are better preserved and have served as a source of ultrastructural data, contributing to the new classification of glomerular disease.[12] The fact that the basement membranes of the tubules show less artifactual changes than the tubular cells has encouraged us to carry out this study.

Ultrastructure of immune complex deposits in the glomeruli was reviewed by Churg and Grishman.[13] In chronic glomerulonephritis, immune complex deposits in the tubular basement membrane have corresponding appearance. In nephropathia epidemica there were no dark homogeneous or finely granular deposits in the tubular basement membrane, but deposits composed of vesicular or tubular membranous structures were seen. We noted that the sites positive in immunohistochemistry corresponded to the sites where the vesicular membranous structures were seen on electron microscopy. The lighter finely fibrillar areas inside the basement membranes might be former sites of deposits because such areas are also seen in the glomeruli after earlier immune complex deposition.[13]

It is probable that many characteristic features of the deposits are degenerative. Corresponding membranous debris is seen in obsolescent glomeruli.[14] Bariety and Callard, in their extensive study of glomerular ultrastructure, showed that the membranous convoluted structures in Figure 3 can be found in the glomeruli in numerous disease states.[15] The various kinds of small extracellular particles shown in Figures 4, 6, and 7 are probably related to the round extracellular particles described by Bariety et al [16] and are also degenerative. The diameter of these particles varied, and because they were never seen inside the cells we do not think that they are viral particles.

Our findings clarify the possible mechanisms of tubular cell damage discussed in connection with immunohistochemical findings in this disease.[10] In the early phases of the disease, viral antigen might pass into the primary filtrate and be absorbed into the tubular cells. On the other hand, it is possible, but not proved, that replication of the causative virus primarily occurs inside the cells of the kidney tubules. Finally, tubular cell necrosis and other damage occurs in NE, probably resulting in free autoantigens.

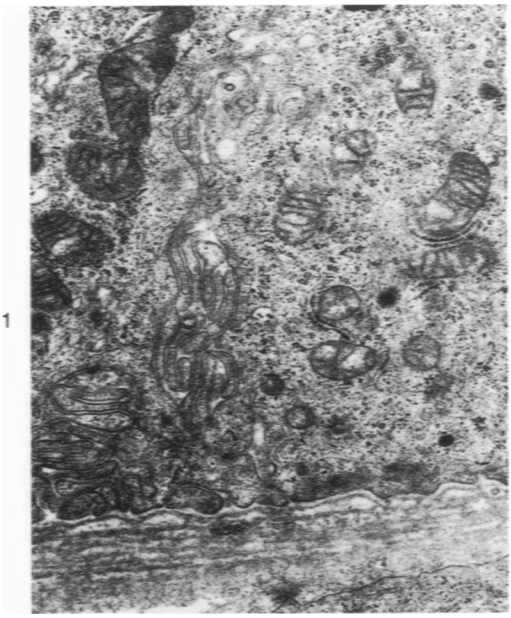
Antigenic material produced by any of these mechanisms, leaking out from the tubules and reacting with circulating antibodies, would give rise to positive immunohistochemical findings along the basement membranes of kidney tubules, a mechanism suggested by Klassen et al.[17] Activation of complement in this connection would result in cell damage and lead to accumulation of material from degraded cell membranes at the site of antigen–antibody reaction. The findings in the tubular basement membrane could also be explained by preformed antigen–antibody complexes which diffuse into the basement membranes from the capillaries.[18,19] This mechanism, however, appears less probable because dark deposits were not found in NE. Immune complex disease induced by autologous kidney extract can lead to findings similar to those found in NE.[20,21]

The change described in Figure 1, ie, splitting of the tubular basement membrane, has been extensive in certain experimental models with no apparent connection with immune complex diseases.[22] Such change has also been present in normal human kidney biopsy specimens.[23,24] In nephropathia epidemica this change also occurs with various kinds of membranous structures, suggesting degeneration (Figures 6 and 7). Extensive splitting of the basement membrane is possibly the result of enzymatic damage caused by the inflammatory processes occurring in the kidney in this disease.

References

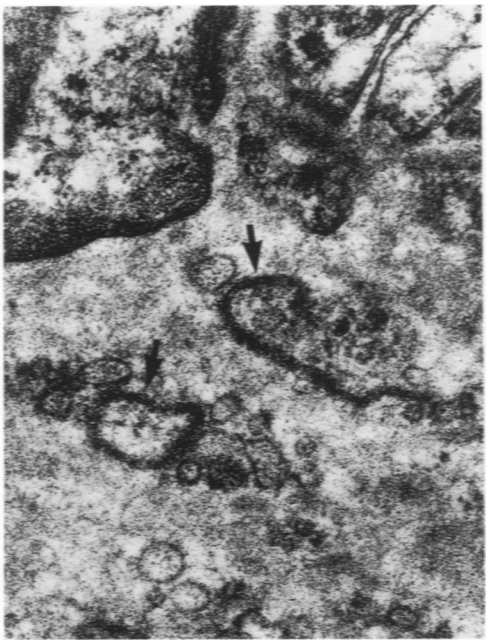
1. Gajdusek DC: Virus hemorrhagic fevers: Special reference to hemorrhagic fever with renal syndrome (epidemic hemorrhagic fever). J Pediatr 60:841–857, 1962

2. Lähdevirta J: Nephropathia epidemica in Finland: A clinical, histological and epidemiological study. Ann Clin Res 3(Suppl 8):1–154, 1971
3. Zetterholm S: Akuta nefriter simulerande akuta bukfall. Läkartidningen 31:425–429, 1934
4. Myhrman G: En njursjukdom med egenartad symptombild. Nord Med Tidsskr 7:793–794, 1934
5. Nyström K: Incidence and prevalence of endemic benign (epidemic) nephropathy in AC County, Sweden, in relation to population density and prevalence of small rodents. Acta Med Scand [Suppl] 609:1–92, 1977
6. Oker-Blom N, von Bonsdorff C-H, Brummer-Korvenkontio M, Hovi T, Penttinen K, Saikku P, Vaheri A, Lähdevirta J: Nephropathia epidemica in Finland. Second International Symposium on Arctic Arbo-Viruses and Third International Conference on Comparative Virology. Mont Gabriel, Canada, 1977. Academic Press (In press)
7. Lee HW, Lee PW, Johnson KM: Isolation of the etiologic agent of Korean hemorrhagic fever. J Infect Dis 137:298–308, 1978
8. Lee HW, Lähdevirta J: Personal communication, 1977
9. Jokinen EJ, Collan Y, Lähdevirta J: Renal immune complexes in epidemic nephropathy. Lancet 1:1012–1013, 1977
10. Jokinen EJ, Lähdevirta J, Collan Y: Nephropathia epidemica: Immunohistochemical study of pathogenesis. Clin Nephrol 9:1–5, 1978
11. Collan Y, Lähdevirta J, Jokinen EJ: Electron microscopy of nephropathia epidemica: Glomerular changes. Virchows Arch [Pathol Anat] 377:129–144, 1978
12. Churg J, Grishman E: Ultrastructure of glomerular disease: A review. Kidney Int 7:254–270, 1975
13. Churg J, Grishman E: Ultrastructure of immune deposits in renal glomeruli. Ann Intern Med 76:479–486, 1972
14. Nagle RB, Kohnen PW, Bulger RE, Striker GE, Benditt EP: Ultrastructure of human renal obsolescent glomeruli. Lab Invest 21:519–526, 1969
15. Bariety J, Callard P: Striated membranous structures in renal glomerular tufts: An electron microscopy study of 340 human renal biopsies. Lab Invest 32:636–641, 1975
16. Bariety J, Callard P, Appay MD, Grossetete J, Mandet C: Ultrastructural study of some frequent and poorly known intraglomerular structures. Adv Nephrol 3:153–172, 1974
17. Klassen J, McCluskey RT, Milgrom F: Nonglomerular renal disease produced in rabbits by immunization with homologous kidney. Am J Pathol 63:333–358, 1971
18. Brentjens JR, O'Connell DW, Pawlowski IB, Andres GA: Extra-glomerular lesions associated with deposition of circulating antigen–antibody complexes in kidneys of rabbits with chronic serum sickness. Clin Immunol Immunopathol 3:112–126, 1974
19. Brentjens JR, Sepulveda M, Baliah T, Bentzel C, Erlanger BF, Elwood C, Montes M, Hsu KC, Andres GA: Interstitial immune complex nephritis in patients with systemic lupus erythematosus. Kidney Int 7:342–350, 1975
20. Laguens R, Segal A: Experimental autologous immune-complex nephritis: An electron microscope and immunohistochemical study. Exp Mol Pathol 11:89–98, 1969
21. Sugisaki T, Klassen J, Milgrom F, Andres GA, McCluskey RT: Immunopathologic study of an autoimmune tubular and interstitial renal disease in brown Norway rats. Lab Invest 28:658–671, 1973
22. Madrazo AA, Churg J: Radiation nephritis: Chronic changes following moderate doses of radiation. Lab Invest 34:283–290, 1976
23. Tisher CC, Bulger RE, Trump BF: Human renal ultrastructure. I. Proximal tubule of healthy individuals. Lab Invest 15:1357–1394, 1966
24. Tisher CC, Bulger RE, Trump BF: Human renal ultrastructure. III. The distal tubule in healthy individuals. Lab Invest 18:655–668, 1968

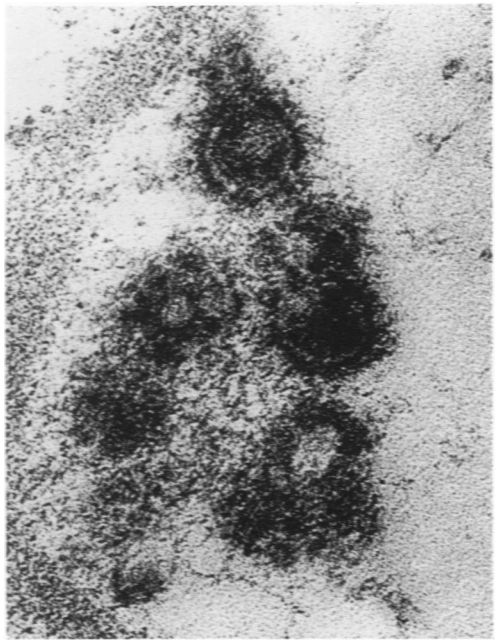


1

2

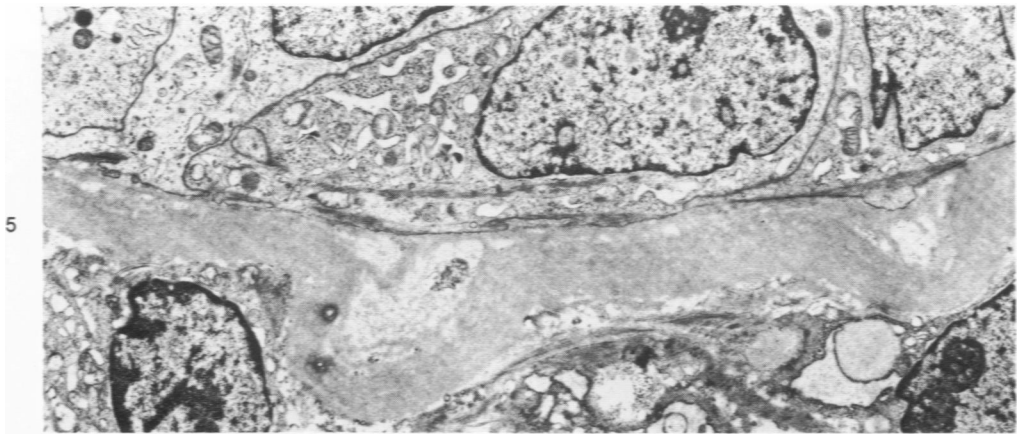


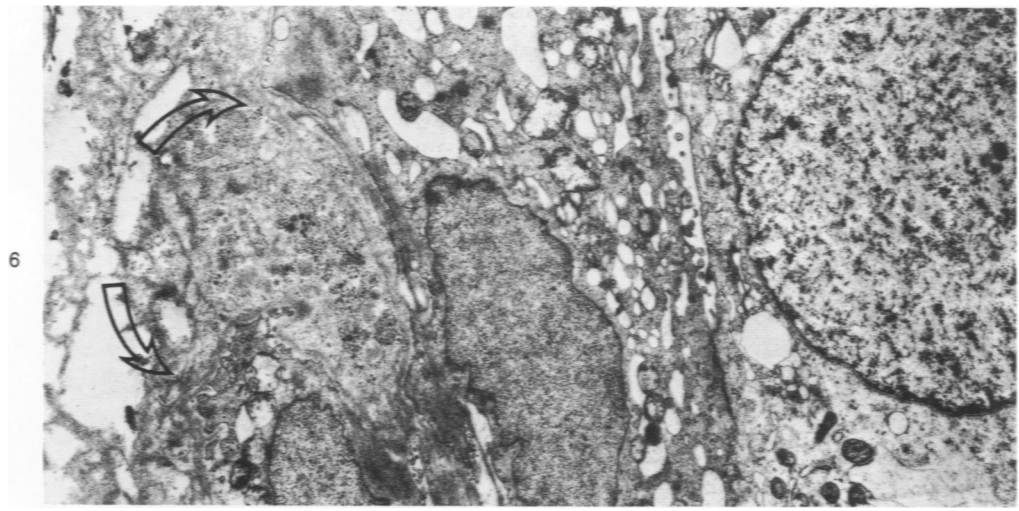
3



4

Figure 1—Nephropathia epidemica, 3 days after the onset of fever. Part of a distal tubule with interdigitating lateral cell membranes at the basement membrane. Note apparent splitting of the basement membrane with repeating light and dark layers. (× 12,500) **Figure 2**—Nephropathia epidemica, 3 days after the onset of fever. Prominently thickened basement membrane of a distal tubule with membranous vesicular and tubular structures within the membrane. (× 11,000) **Figure 3**—Nephropathia epidemica, 3 days after the onset of fever. Membranous convoluted structures (*arrows*) inside the thickened basement membrane. There are also small membrane vesicles inside the basement membrane. (× 35,000) **Figure 4**—Nephropathia epidemica, 17 days after the onset of fever. Round particles (diameter, approximately 180 nm), with a dark core (diameter, 135 nm) in the medulla in the immediate neighborhood of the tubular basement membrane. (× 73,000)





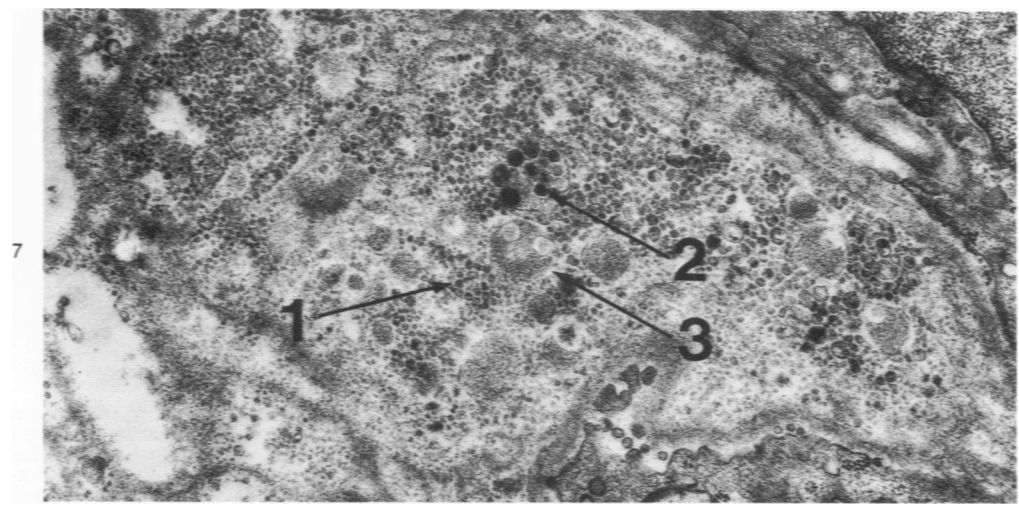


Figure 5—Nephropathia epidemica, 35 days after the onset of fever. Note light finely fibrillar areas inside the distinctly thickened tubular basement membrane. (× 5600) **Figure 6**—Nephropathia epidemica, 17 days after the onset of fever. Part of Henle's loop in the medulla. The basement membrane (*arrows*) is seen on the *left*. It shows splitting and a large collection of round particles. The epithelial cells show vacuolation of RER and SER lacunae. (× 5900) **Figure 7**—Higher magnification of Figure 6, showing various kinds of round particles in the tubular basement membrane. The diameters of various particles are as follows: *1*, 55 nm; *2*, 105 nm; *3*, 470 nm. (× 18,000)