ELECTRON MICROSCOPIC STUDIES OF HUMAN GLOMERULONEPHRITIS WITH FERRITIN-CONJUGATED ANTIBODY

Localization of Antigen-Antibody Complexes in Glomerular Structures of Patients with Acute Glomerulonephritis*

By GIUSEPPE A. ANDRES,‡ M.D., LIDIA ACCINNI,‡,§ Ph.D., KONRAD C. HSU,‖ Ph.D., JOHN B. ZABRISKIE,¶ M.D., and BEATRICE C. SEEGAL,‖ M.D.

(From the Istituto Patologia Medica, Università di Roma, Rome, Italy, the Department of Microbiology, Columbia University, New York, and The Rockefeller University)

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The hypothesis that an antigen-antibody reaction plays a role in the pathogenesis of acute and progressive human glomerulonephritis has been the subject of intensive study in recent years. It has been reported that human γ-globulin (1–4) and complement (5, 6) are selectively deposited in the glomerular structures. Their presence in these areas is suggestive of an antigen-antibody reaction; the nature of the antigen is not yet established. It has been shown, however, that fluorescein-labeled antibody to Group A, Type 12 streptococcus is bound in the glomeruli of more than half of the renal biopsies obtained from patients with acute glomerulonephritis (3, 6). Furthermore, preliminary studies with ferritin-labeled antibodies have exhibited areas within the glomerulus where human γ-globulin, complement, and streptococcal products are localized (6, 7).

The purpose of this paper is to report the histologic and immunologic findings following electron microscopic examination of renal biopsies from 4 patients with severe acute glomerulonephritis. Employing the immunoferritin technique, the location of human 7S γ-globulin, β_{1C} (C'3), and antigen(s) of Group

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‡ Present address, Istituto Patologia Medica, Università di Roma, Rome, Italy.

§ Fellow Consiglio Nazionale delle Ricerche.

‖ Present address, Department of Microbiology, Columbia University, New York.

¶ Present address, The Rockefeller University.

A, Type 12 streptococcus in the glomerular lesions has been determined. The possible relationship of these data to the pathogenesis of the disease is discussed.

Materials and Methods

Patient Material.—The data reported are derived from a study of renal tissue taken at first biopsy from 4 patients, 11 to 25 days after the onset of symptoms and signs of severe, acute glomerulonephritis. The clinical course in each patient was as follows.

Patient Ma, a 32-yr-old housewife with no previous history of renal disease, was admitted to the hospital[1] 8 July 1963, with the diagnosis of acute glomerulonephritis. 1 month prior to admission the patient had tonsillitis with fever and was treated with penicillin for 4 days with apparent improvement of the pharyngitis. 10 days before admission fever recurred and generalized edema, hematuria, hypertension, and nitrogen retention were found. On admission signs of severe renal failure were evident with extensive edema, oliguria, and hypertension of 160/100. Laboratory tests revealed marked hematuria, numerous cell casts and a blood urea nitrogen (BUN) of 280 mg %. The antistreptolysin O (ASO) titer on admission was 100 Todd units and 2 wk later rose to 250 units. The lupus erythematosus (LE) cell test was negative. The first renal biopsy was performed 5 days after admission. The light microscopy sections showed widespread lesions of acute inter-, intra-, and extracapillary glomerulonephritis. In spite of treatment with steroids and hemodialysis, renal failure was progressive. A second biopsy taken 25 days after admission showed further extension of the acute glomerular lesions with obliteration of the capillaries and early hyalinization. The patient died 2 wk later. In addition to the renal damage noted above a generalized arteritis also was found at necropsy, similar to that described by Fordham and his associates (8).

Patient Pg, a 21-yr-old man, was admitted 11 October 1963, with the referral diagnosis of acute glomerulonephritis. The past history revealed that the patient had had repeated bouts of tonsillitis and that a tonsillectomy had been performed 13 yr previously. There was, however, no evidence of prior renal disease. On September 20th the patient developed an acute pharyngitis associated with high fever, was treated with a tetracycline preparation for 1 day and the symptoms temporarily subsided. He remained in good health until October 2nd when hematuria, generalized edema, oliguria, and hypertension were noted. In addition to the physical findings, urinalysis on admission showed proteinuria, red blood cells, and cellular casts. The BUN was 255 mg %, serum potassium was 6.5 meq/l and the ASO titer was 1250 Todd units. The LE cell test was negative. The first kidney biopsy was performed on October 14 and showed acute proliferative inter- and intracapillary glomerulonephritis.

The patient received steroids and antibiotics without apparent improvement. 2 February 1964, a second renal biopsy showed formation of glomerular crescents and progressive hyalinization. The patient was discharged shortly after the second biopsy and his present clinical status is unknown.

Patient Ci, a 14-yr-old girl, was admitted to the hospital 3 November 1963, with the diagnosis of severe acute glomerulonephritis and renal failure. 17 days before admission the patient, as well as two siblings, had tonsillitis with high fever. Two injections of penicillin were given and the symptoms subsided, only to return 6 days later. No medication was given at this time and 1 wk later hematuria and generalized edema were noted. On admission only 50 to 100 ml of urine was voided in 24 hr, the BUN was 280 mg %, the serum potassium 8.5 meq/l and the ASO 833 Todd units. The LE cell test was negative. Treatment was started with 60 mg of prednisone, daily, and peritoneal dialysis was performed. The first renal biopsy was taken on November 23rd. Sections of the biopsy showed inter-, intra-, and extracapillary proliferative glomerulonephritis.

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[1] Istituto Patologia Medica Università, Policlinico, Roma.

1 month after admission the BUN was 150 mg %. Prednisone was reduced gradually to 10 mg daily, but signs of severe renal failure immediately reappeared. The blood pressure rose to 250/150 and the patient experienced two episodes of severe pulmonary edema. She was again placed on high doses of steroids and periodic peritoneal dialysis was performed. Over the next 3 months the BUN and blood pressure returned to normal, and 6 months after admission all urinary abnormalities had disappeared. A second renal biopsy was performed 9 June 1964. Thickening of the capillary walls and early hyalinization were present in about 50% of the glomeruli.

Follow-up examination in April, 1965, revealed that the patient was in apparent good health and the blood pressure, BUN, and urinalysis were within normal limits.

Patient Mt, a 16-yr-old boy was admitted to the hospital 16 March 1964, with the diagnosis of acute glomerulonephritis. There was no history of previous renal disease. 10 days before admission the patient noted the onset of malaise, headache, sore throat, and slight fever, followed 4 days later by hematuria. At this time his physician found oliguria, generalized edema, and hypertension (190/110). On admission to the hospital the urine contained protein, red blood cells, and casts; the BUN was 31 mg %, the serum potassium 4.48 meq/l and the ASO titer 1250 Todd units. The LE cell test was negative. The first kidney biopsy was performed 5 days after admission. Light microscopy showed acute proliferative inter- and intracapillary glomerulonephritis.

The patient was treated with penicillin for the preceding streptococcal infection. His condition did not improve and after 20 days in the hospital he was started on oral cortisone. 10 days later a progressive improvement in urinary findings was noted. Examination of the tissue obtained with a second biopsy (15 May 1964) revealed moderate signs of acute proliferative glomerulonephritis. The patient was discharged at the end of June with mild proteinuria and hematuria; subsequently physical examinations and urinalyses became normal.

Preparation of Antisera for Fluorescein and Ferritin Labeling.—The anti-7S γ-globulin sera were kindly supplied by Dr. Arthur J. L. Strauss (9). Antisera to β_{1C} were obtained through the courtesy of Dr. H. G. Kunkel (5).

Antisera to Group A streptococci, Types 12, 14, and 49, were produced in rabbits using the immunization schedule designed for the preparation of type-specific antisera. The organisms from an overnight culture in 500 ml of Todd-Hewitt broth were centrifuged, resuspended in a small volume of saline, and killed by heating at 56°C for 30 min. The volume was brought to 60 ml, and the vaccine stored in the refrigerator during the immunization procedure. The rabbits were immunized by the intravenous injection of 0.5 to 1.0 ml doses on 3 successive days of each of several weeks. Test bleedings were assayed with crude M protein extracts (10) and sera with high precipitating activity were used for conjugation. The antisera were not absorbed with other Group A strains.

Dr. Ruegsegger of Lederle Laboratories, Pearl River, New York, provided antisera to Pneumococcus Type II. The antivaccinia sera were prepared by Dr. Margaret Holden (11). The technique employed for fluorescein-labeling of the globulins prepared from the antisera is essentially that in general use and has been previously described (12). The method for conjugating antisera globulins with ferritin has been previously described (13, 14). The sera referred to above were all labeled with fluorescein and ferritin; however, labeling of antisera to Types 14 and 49 Group A streptococci with ferritin was not completed in time for these experiments, hence only the results with fluorescence microscopy are reported for these latter antisera.

Preparation and Examination of Tissues.—The method of obtaining renal biopsies and of exposing pieces of tissue to ferritin-labeled antibody for electron microscopic studies has been reported (6). Briefly, bits of the renal biopsy were fixed for 1 hour at 0°C in 5% formalin buffered with phosphates at pH 7.2. The fragments were then washed and cut in the cold

room into very small portions under the dissecting microscope. These were then immersed in the ferritin-conjugated antibodies to human 7S γ-globulin, β_{1C}, and Type 12 streptococcus for 20 min at room temperature, washed three times in buffer, fixed in osmium, and embedded in Araldite. Other bits of tissue were exposed to ferritin-labeled antisera to pneumococcus Type II, vaccinia virus, and purified ferritin alone, in order to check the specificity of ferritin binding in the tissues.

Normal renal tissues and tissues from a variety of kidneys affected with diseases other than acute glomerulonephritis were prepared and examined using the same ferritin-labeled antisera.

Another fragment of each biopsy was frozen, cut, and stained with fluorescein-labeled antibodies for examination in ultraviolet light (6). The remainder of the tissue was processed for routine histologic examination or fixed in osmium tetroxide (15) and embedded in Araldite for electron microscopy. Semithin or thin sections were stained with PAS, periodic acid silver-methenamine (PASM) (16), Prussian blue, or lead hydroxide and examined with the light or electron microscope.

RESULTS

Histopathology of Glomerular Structures

The glomerular changes found in the 4 patients reported here were essentially similar and exhibited all the lesions that are characteristic of poststreptococcal acute proliferative glomerulonephritis (17). However, certain differences among the renal lesions were apparent: the amount of glomerular damage was more extensive in patients Ma and Ci and very early crescent formation was seen. In addition, light microscopy studies of sections of patient Ma taken at necropsy revealed the presence of generalized vascular lesions (8). Subendothelial deposits were observed by electron microscopy in the renal arteriolar walls of patients Pg and Mt.

Several authors have previously described the ultrastructure of the glomerulus in acute human glomerulonephritis (18, 19) and the natural progression of the disease (20–22). A few morphologic observations useful in interpreting the immunologic findings to be presented here are mentioned in order to orient the reader. These were determined on sections of the renal tissues directly fixed in osmium for morphologic examination. Succeeding electron micrographs from the immunologic studies will illustrate these findings. Obliteration of many of the capillary lumina is due to mesangial or endothelial proliferation and to the presence of polymorphonuclear leukocytes which contain phagocytized foreign material. In the lumina of several capillaries and beneath the thin layer of the endothelial cytoplasm foreign precipitates are present. The proliferating mesangial and endothelial cells lose their cohesion and the resulting intercellular spaces join the capillary lumen with the basement membrane. These canals contain foreign material. The endothelial cells show micropinocytic vesicles and absorption droplets. Foreign material, which forms deposits of variable electron opacity, accumulates mostly in the mesangial and in the subendothelial areas. On the epithelial side of the basement membrane the morpho-

logic lesions are represented by focal subepithelial deposits and by partial fusion of the foot processes. In the Bowman's space aggregates morphologically similar to those observed in the capillary lumen are also present.

Immunologic Study of Glomerular Structures

Portions of renal tissues were treated with a series of fluorescein- or ferritin-conjugated antibodies. Fluorescein-labeled antibodies to 7S γ-globulin, complement (β_{1C}, a part of C'3) and Group A, Type 12 streptococcus were specifically bound in the glomeruli of these patients. Control antisera, including the two for Group A streptococci of Types 14 and 49 did not stain the glomeruli (6).

The ferritin-conjugated antibodies to γ-globulin, C'3, and Type 12 streptococcus also were specifically bound in the glomerular structures of the biopsies. Electron microscopic studies showed the presence of the ferritin conjugates in certain areas of all glomeruli, which were identical for each of the 3 labeled antisera. The findings are presented in a sequence which attempts to represent the natural evolution of the pathologic process.

Localization of Ferritin-Labeled Antibody to 7S γ-Globulin.—Fig. 1 shows a section of a glomerulus from patient Mt which has been treated with ferritin-labeled antibody to human 7S γ-globulin. Ferritin is bound in aggregates of electron-opaque material present in the capillary lumen (see inset a). A gap in the endothelial cytoplasm which seems to allow direct penetration of material from the capillary lumen into the subendothelial spaces is illustrated and here subendothelial deposits also contain ferritin granules. On the mesangial side of the basement membrane ferritin-labeled antibody is bound in two large intercellular spaces connected by a small canal. Heavy concentration of 7S γ-globulin in the mesangial area is shown in Fig. 2. This section, taken from the biopsy material of patient Ma, illustrates the binding of the ferritin-labeled antibody in the electron-opaque material situated between the mesangial cells. Inset a, Fig. 2, shows a frozen section from the kidney of the same patient, Ma, stained with fluorescein-labeled antibody to human γ-globulin. The marked fluorescence of the glomerular structures indicates the presence of great excess of γ-globulin.

Localization of Ferritin-Labeled Antibody to β_{1C}.—Fig. 3 is a montage of electron micrographs obtained from patient Ci's renal tissues which had been treated with antibody to β_{1C}. The montage gives an overall view of the capillary wall. Despite imperfect preservation, the figure shows proliferating cells which almost completely obliterate the capillary lumen. Between the cells there is a system of canals which contain foreign material of variable electron opacity. In the left part of the montage the basement membrane with subendothelial and subepithelial deposits is visible. Examination at higher magnification (boxed areas c to f) shows that the conjugated antibody is bound to electron-opaque material in pinocytic vesicles and intracellular granules of endothelial

cells (Fig. 3, inset a). Ferritin is also localized in the intricate system of intercellular canals. Inset b of Fig. 3 shows a granule or a cross-section of a canal in which the ferritin-antibody complex is bound. Figs. 4 and 5 (boxed areas e and f of Fig. 3) both illustrate the localization of antibody to β_{1C}(C'3) in the canals between cells. In Fig. 4, *V* marks an area, probably a cross-section of a canal which communicates with other intercellular spaces at another level; ferritin is not seen in this area. In Fig. 5 it is seen that ferritin granules occur also in a subendothelial deposit, in the basement membrane, and in a focal subepithelial deposit. Fig. 6, a second montage of electron micrographs from the same tissue, includes both capillary lumen and capsular space. It is notable from an immunologic point of view in that the position of the antigen-binding antibody to β_{1C} is further identified in electron-opaque material aggregated in Bowman's space between the foot processes (inset c). Boxed area a, which is placed in Fig. 2, shows localization of ferritin in a subendothelial space and includes an area of greater electron opacity which is not tagged by ferritin, indicating the absence of β_{1C} in this denser deposit. The presence of β_{1C} at the luminal side of the glomerular capillary wall is demonstrated in inset c of Fig. 1 where ferritin tagging is seen in the foreign material which accumulates beneath the endothelial cytoplasm. A gap in the endothelium appears to permit access of the material from the capillary lumen. The similarity of the position of β_{1C} to that demonstrated for 7S γ-globulin is readily apparent.

Foreign electron-opaque material phagocytized by polymorphonuclear leukocytes may not bind ferritin-conjugated antibodies. Fig. 7 shows that ferritin-conjugated β_{1C} does not localize in the phagocytized foreign material, whereas it is present in other areas.

Localization of Ferritin-Labeled Antibody to T12 Antigen.—Antibody to Group A, Type 12 streptococcus localized in the same areas where γ-globulin and β_{1C} were found. Fig. 8 illustrates part of a glomerular capillary wall from patient Ci. The tissue was treated with ferritin-labeled antibody to Type 12 streptococcus. The basement membrane, lying between an endothelial cell and part of a foot process, has bound the ferritin-labeled antibody. The focal subepithelial deposit of greater electron opacity does not contain ferritin. Inset a shows localization of the same antiserum, labeled with fluorescein, in another glomerulus of the patient. In another section of this patient's glomerular tissue (Fig. 9) ferritin-labeled streptococcal antibody is also localized in the foreign electron-opaque material in the mesangial region.

Localization of Ferritin-Labeled Antibodies in Wall of Arterioles.—Inset a in Fig. 10 shows an arteriole found in the renal medulla of patient Pg. Deposits of foreign material are present between the endothelium and the tunica media. This piece of tissue had been treated with ferritin-labeled antibody to Type 12 streptococcus. The labeled antibody is found in the subendothelial deposits and between the smooth muscle cells up to the membrana elastica externa (Fig. 10).

In patient Mt, foreign material seen in the basement membrane of a medullary arteriole also was tagged by ferritin-labeled antibody to human γ-globulin.

Controls.—Control experiments (see Materials and Methods) were performed in order to test the specificity of the binding of ferritin-labeled antibody. No binding was observed. Two examples of these experiments are illustrated in Figs. 11 and 12. In the first, a section of kidney from patient Ci was treated with ferritin-labeled antiserum to vaccinia virus. Only a few ferritin granules are seen on the epithelial side of the basement membrane, shown in higher magnification in inset a. Inset b is a higher magnification of an electron-opaque aggregate in the capillary lumen. Only a rare ferritin granule is seen. The second example (Fig. 12) shows a portion of a capillary wall from a normal human kidney treated with ferritin-labeled antibody to human γ-globulin. Minimal localization of ferritin molecules is evident. Inset a shows a higher magnification of one portion of the basement membrane. Evidence for the specificity of the binding of ferritin-labeled antibody is further strengthened by the fact that specific antibodies do not localize in the cellular cytoplasm or in the nuclei of any of the renal tissues and fail to localize in certain electron-opaque subendothelial deposits which probably contain fibrinogen products.

DISCUSSION

The histologic changes observed by light and electron microscopy in the 4 cases of severe acute glomerulonephritis are characterized by proliferation of mesangial and endothelial cells, loss of cellular cohesion, infiltration of polymorphonuclear leukocytes, and increased permeability of the glomerular capillary wall. All these findings contributed to the severe inflammatory reaction noted in these kidney sections.

Preliminary studies of the current biopsy material, utilizing the fluorescent antibody technique, show that the glomerular structures contain increased concentrations of human γ-globulin, a fraction of human complement, and Group A, Type 12 streptococcal antigens (6). Simultaneous studies with ferritin-labeled antibody to the same three antigens were briefly reported (7) for 2 of these patients. These reactants were seen in electron-opaque foreign material noted in the glomerular structures. The findings support the hypothesis that these foreign deposits contain antigen-antibody complexes.

Farquhar and her associates (18) first described the presence of foreign electron-opaque material in areas of the glomerulus in renal disease. The presence of such deposits, not only in the glomeruli but also in the walls of arterioles, as reported here, has important implications in the understanding of the pathogenesis of human glomerulonephritis (18–23). A review of the immunologic observations involving the foreign electron-opaque material permits a tentative hypothesis based on the sequence of events in the 4 cases of severe acute glomerulonephritis.

1. Human γ-globulin, complement, and products of Group A streptococci circulate

in the blood stream and can be demonstrated by means of ferritin-labeled specific antibodies in lumina of capillaries and in arteriolar walls in renal tissue. They are presumed to represent antigen-antibody complexes.

2. In response to deposition of the antigen-antibody complexes and to activity of polymorphonuclear leukocytes in the glomerular vascular system there is an acute inflammatory process characterized by active proliferation of mesangial and endothelial cells and by swelling of their cytoplasm. The proliferating cells lose their cohesion and intercellular canals are found which link the capillary lumen with the mesangial area or with the endothelial side of the basement membrane.

3. There is selective localization and concentration of the foreign electron-opaque material with formation of deposits in the mesangial area or in the glomerular capillary wall. This reaction is probably due to the anatomic and physiologic properties of the glomerular unit: (*a*) Most likely the mesangial deposits are a result of increased phagocytic activity of the mesangial cells. This response has been demonstrated by functional studies showing that injected tracers accumulate in the cytoplasm of these cells which are found within the intercapillary space (25). Furthermore, evidence for the phagocytic activity of the intracapillary cells in experimental glomerulonephritis has also been reported (26). In addition the intricate intercellular spaces of the axial capillary region are widened and allow penetration of the antigen-antibody complexes from the capillary lumen to the deep mesangial area where deposits are located. (*b*) Selective filtration, a property of the basement membrane, could account for the subendothelial deposition of foreign complexes. (*c*) The "focal" subepithelial deposits may result from aggregation of the complexes during transport from the capillary lumen to Bowman's space during which there may be a decreased solubility of these complexes (27, 28). Only 50% of the "focal" subepithelial deposits are tagged by ferritin-conjugated antibodies to 7S γ-globulin or β_{1C} whereas antibodies to Type 12 streptococci have not been found in these areas. Whether this is due to lack of specific antigen or is a consequence of difficult penetration of ferritin conjugates is not known.

4. Foreign precipitates of greater electron opacity among the deposits do not bind the ferritin-labeled antibodies. Presumably these precipitates contain fibrinogen products or other serum proteins (26).

5. The position of the deposits is important since they appear to be inaccessible to the phagocytic activity of the leukocytes which usually transport foreign material from sites of lesions (29). It is thus possible to imagine that the renal damage once induced could continue for long periods without further antigenic stimulation.

6. A large number of polymorphonuclear leukocytes appear in the capillary lumina as a result of the chemotactic attraction of the antigen-antibody complexes (30). Ferritin-conjugated antibody usually is not bound to the immune precipitates which have been phagocytized by polymorphonuclear leukocytes (31). One possible explanation for this behavior is that these phagocytized precipitates have been digested by leukocytic granules (lysosomes) (32) and antigenic determinants have been destroyed. The immunoferritin technique gives further evidence that during intracellular transport a digestion of the antigen-antibody complexes may take place. Indeed, whereas endothelial absorption droplets close to the lumen (probably phagosomes) contain ferritin-conjugated antibodies, other endothelial or epithelial droplets (probably digestive vacuoles) are not tagged by the ferritin conjugates.

7. The morphologic signs of increased permeability of the glomerular capillary walls may be the result of the release of histamine-like substances secondary to antigen-antibody complement union (33, 34).

All the glomerular changes described in the present study on human acute glomerulonephritis have their counterpart in experimental glomerular disease induced by antigen-antibody complexes. The possibility of inducing acute glomerulonephritis by injecting complexes into normal animals was first suggested by McCluskey, Benacerraf, and associates (35, 36). Serum sickness disease with marked antibody response may follow one-shot (37) or daily injections of a number of antigens (24). The glomerular localization of these antigen-antibody complexes produces proliferation of endothelial and mesangial cells, the accumulation of complexes on both sides of the basement membrane and in the intercapillary area, infiltration of polymorphonuclear leukocytes, and increased vascular permeability. In addition, other reports indicate that circulating complexes may initiate intravascular coagulation (38) and lead to the deposition of fibrin mixed with immune complexes within glomeruli (39). Following repeated injections of bovine serum albumin, studies with ferritin-conjugated antisera (40) show that antigen-antibody complexes are present in the capillary lumen, and that the antigen is localized in these immune complexes. Recent studies (41–43) indicate that the complexes penetrate the capillary walls through gaps which form between adjacent endothelial cells and the cellular damage seems to involve primarily the desmosomes with loss of cellular cohesion (44). These vascular changes are reminiscent of inflammation elicited by mild mechanical trauma (45), histamine, or serotonin (46).

A significant characteristic of the glomerular lesions which occurs in serum sickness disease (24) or after the injection of heterologous complexes (47) is their association with lesions in other organs. Here the number of circulating complexes is probably much greater since they tend to localize in many other organs in addition to the renal glomerulus (23). In contrast, Dixon's work (24) shows that following daily prolonged exposure to a low level of complexes the kidney appears to be the only organ affected. Thus, the dosage and actual duration of exposure probably are responsible for the various manifestations seen in the different forms of experimentally induced renal disease.

In the patients with severe acute glomerulonephritis, illustrated here, an unusually large amount of foreign material tagged by ferritin-conjugated antibody was present probably accounting for deposits observed not only in the glomeruli but also in arterioles of the medulla in two instances. The clinical picture and histologic appearance of this form of human severe glomerulonephritis appear to be similar to that observed in severe serum sickness (23, 40). In other less severe and more frequent forms of human glomerulonephritis, at present under study, only a small amount of foreign material tagged by ferritin-conjugated anti-

bodies is present in glomerular structures and never in the arteries of the renal medulla. These more benign forms of human glomerulonephritis may result from a low level of circulating complexes which localize in glomerular capillaries (usually in glomerular basement membranes) owing to a rich renal blood flow and the normal filtering function of this organ.

Recent reports of the role of Group A streptococci in experimentally induced glomerulonephritis lend further support to the concept that circulating streptococcal products may play a major role in the pathogenesis of this disease. It has been reported that nephritogenic strains of streptococci grown in diffusion chambers in the peritoneal cavity are capable of producing renal lesions in rats (48). Furthermore, Kantor's studies (49) have demonstrated that streptococcal M protein associated with a fibrinogen complex is present within the glomerular structures in rats following the injection of M protein extract.

The Type 12 streptococcal antigens which are presumed to form a part of the circulating antigen-antibody complexes in these 4 cases of severe acute glomerulonephritis have not yet been identified. Work in progress indicates that the antigen probably resides in the cell wall fraction of the streptococcus. Its mode of transport to the glomerulus is apparently as antigen in an antigen-antibody complex, although an additional serum protein carrier, such as fibrinogen, may be present.

Current studies are directed toward 3 goals: the identification of the streptococcal antigens in the renal tissues of patients with acute glomerulonephritis; the determination of serum proteins other than γ-globulin present in excess in the glomerular structures; and the specific antibody or antibodies present in the localized γ-globulin of these patients.

SUMMARY AND CONCLUSIONS

1. Kidney biopsies from 4 cases of severe acute glomerulonephritis were obtained 11 to 25 days after the onset of clinical manifestations of the disease. These tissues were treated with ferritin-conjugated antibodies to 7S γ-globulin, β_{1C}, and Type 12 streptococcal products. Adjacent pieces of the biopsied material were treated with control ferritin-labeled antisera or with ferritin alone. As further controls, normal renal tissue and renal tissue from patients with other kidney diseases were treated with the same antisera.

The 3 antisera to 7S γ-globulin, β_{1C} and Type 12 streptococcus were specifically bound in electron-opaque foreign material in the following renal areas: (*a*) the lumen of glomerular capillaries; (*b*) medullary arteriolar walls (2 cases); (*c*) pinocytic vacuoles and absorption droplets of endothelial or mesangial cells; (*d*) canals between proliferating mesangial or endothelial cells which connect the capillary lumen with the deep mesangial region or with the endothelial side of the basement membrane; (*e*) basement membrane proper; (*f*) subendothelial and certain subepithelial deposits; and (*g*) Bowman's space.

2. None of the 3 ferritin-conjugated antisera listed above were bound to the nuclei of glomerular cells or to portions of the cytoplasm other than those specified.

3. Ferritin-conjugated antisera to pneumococcus Type II and vaccinia virus and ferritin alone were not bound to any structures in the glomerular tissue.

4. None of the ferritin-conjugated antisera bound to normal renal tissue or to kidney tissue from other renal disease.

5. The data obtained are compatible with the following working hypothesis:

Antigen-antibody aggregates of Type 12 streptococcal products, γ-globulin, and complement are present in the circulating blood of patients with severe acute glomerulonephritis. Large amounts of the complexes are caught in the filtering system of the glomeruli. The inflammatory reactions seen in the glomerular structures result from the presence of the immune complexes and of the polymorphonuclear leukocytes which conjointly may be responsible for the disease.

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EXPLANATION OF PLATES

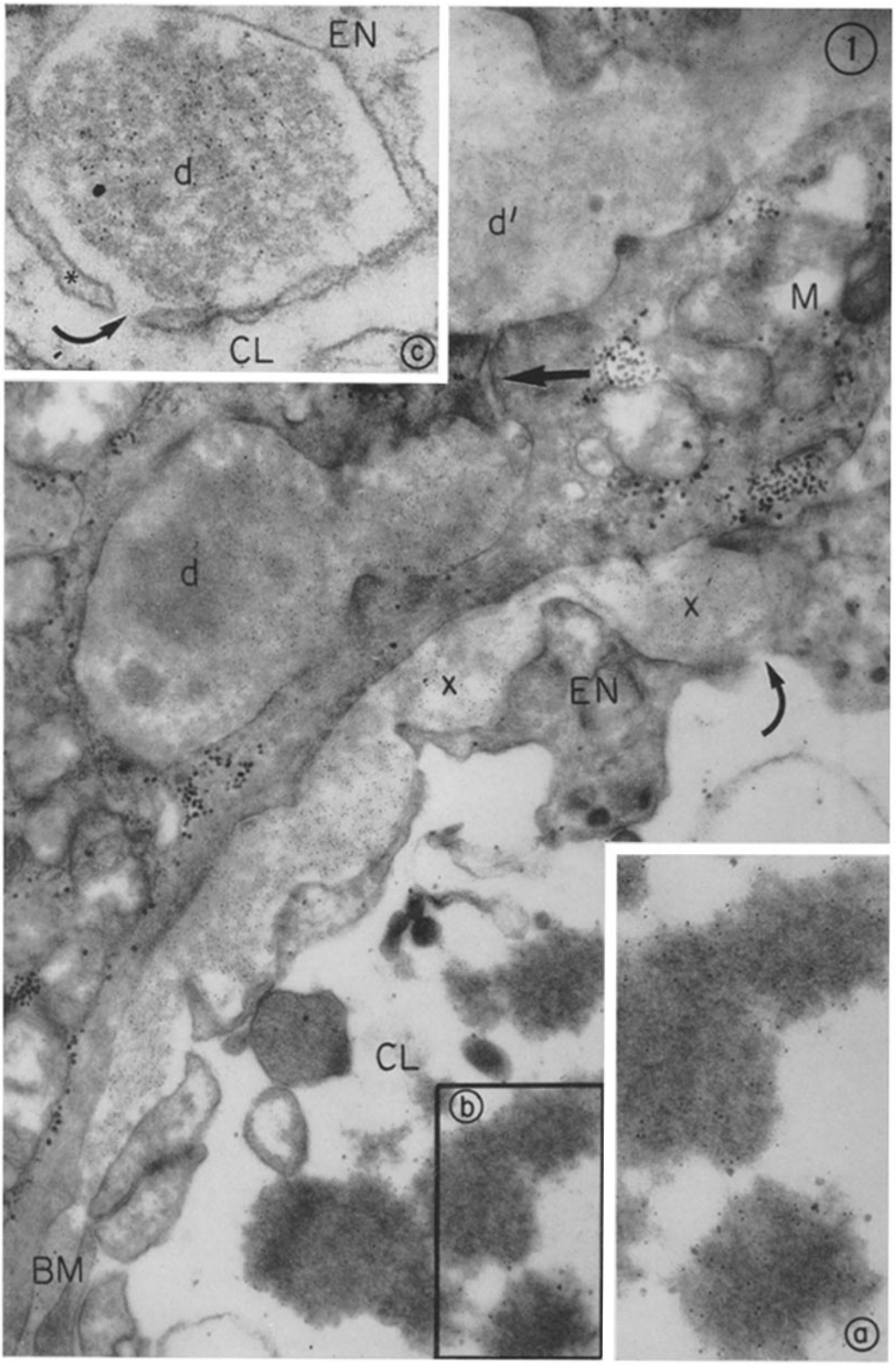
All illustrations are of renal tissue.

PLATE 51

FIG. 1. Patient Mt: glomerular capillary wall from renal tissue treated with ferritin-conjugated antibody to human 7S γ-globulin. In the picture the mesangial cytoplasm (*M*), the basement membrane (*BM*), the endothelial cytoplasm (*EN*) and the capillary lumen (*CL*) are visible. Ferritin-conjugated antibody localizes in the four aggregates of electron-opaque material present in the lumen. Ferritin is also localized in the subendothelial deposits (*X*). The curved arrow indicates a gap in the thin endothelial cytoplasm which seems to allow free penetration from the lumen into the subendothelial space. On the mesangial side large spaces (*d* and *d'*), connected by a small canal (straight arrow), contain foreign material tagged by ferritin granules. × 34,000.

Inset a at the lower right corner is a higher magnification of boxed area b showing parts of the luminal aggregates. × 63,000.

Inset c in the upper left corner of the picture shows part of a capillary wall from patient Ci stained with ferritin-conjugated antibody to β_{1C}. Ferritin is seen in the foreign material (*d*) which accumulates beneath the thin layer (*) of the endothelial cytoplasm (*EN*). The arrow indicates an endothelial gap which permits access from the lumen into the space (*d*). × 54,000.



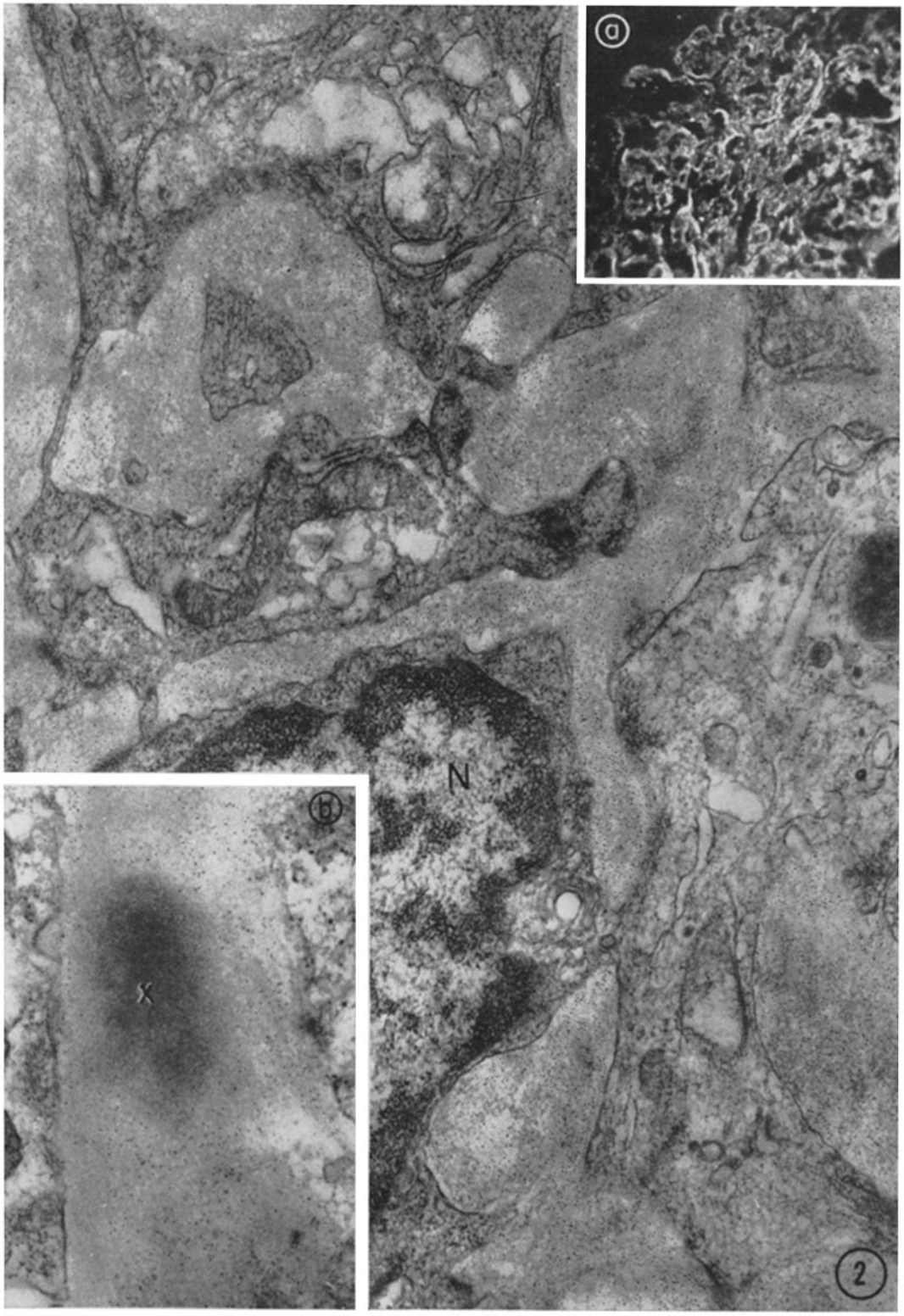
(Andres et al.: Human glomerulonephritis)

PLATE 52

FIG. 2. Patient Ma: foreign material in the mesangial area is tagged by ferritin-conjugated antibody to human 7S γ-globulin. A mesangial cell nucleus (*N*) is visible in the central part of the picture. × 38,000.

Inset a at the upper right shows a frozen section of patient Ma, stained with fluorescein-conjugated anti-7S γ-globulin. Fluorescence is localized in the glomerular structures. × 200.

Inset b in the lower left corner is a higher magnification of the boxed area a in Fig. 6. The more electron-opaque material (*X*) which may be formed by fibrinogen products is not tagged by the ferritin-conjugated antibody to β_{1C}. Ferritin instead is localized in the surrounding subendothelial space. × 48,000.



(Andres et al.: Human glomerulonephritis)

PLATE 53

FIG. 3. Patient Ci: montage of electron micrographs which shows a section through a glomerular capillary wall. The renal tissue was treated with ferritin-conjugated antibody to β_{1C}. The survey composition presents a view of the capillary lumen (*CL*) to the epithelial cytoplasm (*EP*). The capillary lumen is partially occupied by proliferating cells, four nuclei (*N*) of which are visible. In a cell (probably endothelial) one observes pinocytic vesicles (arrows) and a granule (probably a phagosome) containing electron-opaque material tagged by ferritin-conjugated antibody (see inset a, a higher magnification of boxed area C. × 48,000).

In inset b, × 39,000, a granule or a cross-section of an intercellular canal is represented in which ferritin-conjugated antibody is localized. Other granules (*V*), visible in the montage, do not appear to contain ferritin-conjugated antibody. An intricate system of intercellular canals seems to originate from the capillary lumen (curved arrow) and to lead to the endothelial side of the basement membrane (*BM*). The intercellular spaces contain foreign material of variable electron opacity in which ferritin-conjugated antibody localizes. The straight arrows indicate points where the proliferating cells still maintain a relative cohesion. A subepithelial (*d*) and a subendothelial (*d'*) deposit are visible in the left lower quadrant (see Fig. 5). × 21,000.

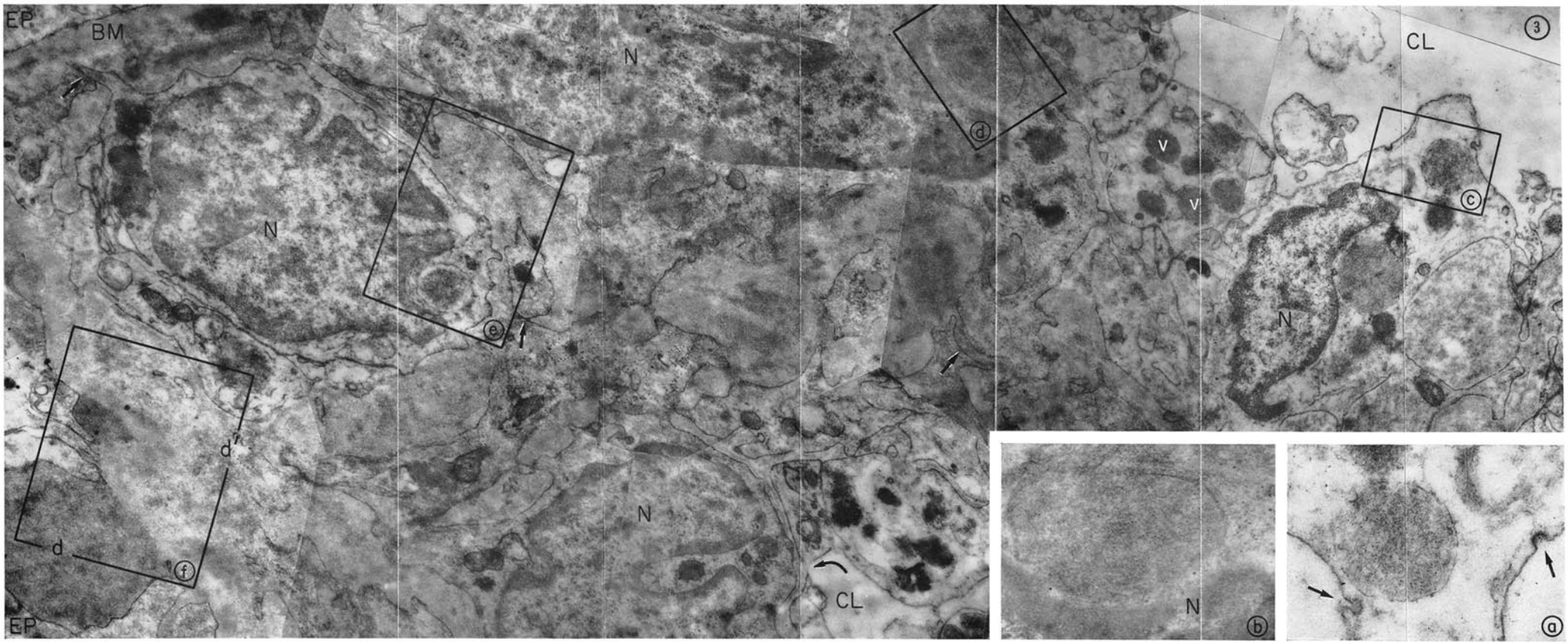
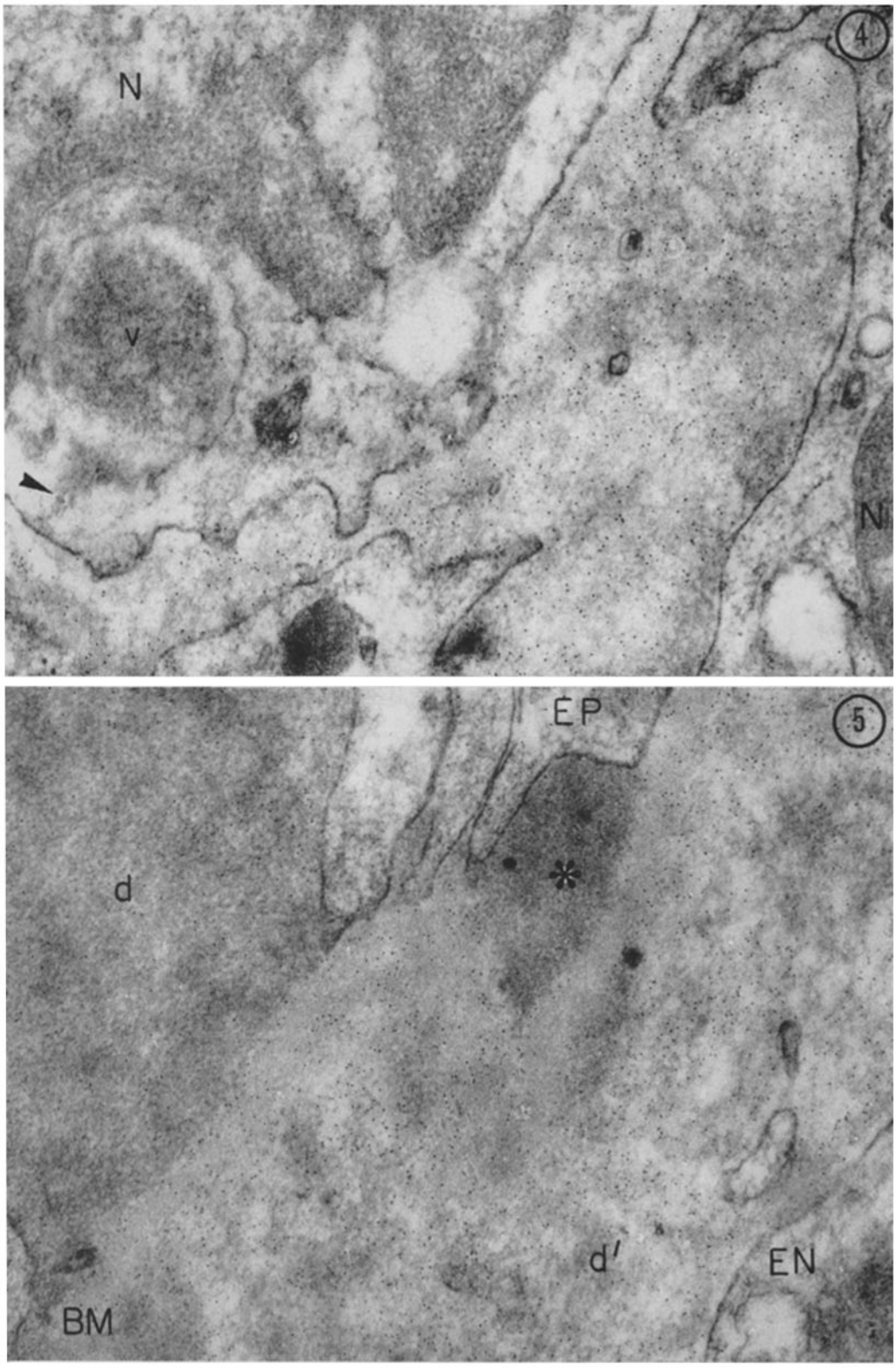

EP
BM
N
N
N
CL
EP
d
d'
d
e
f
V
V
CL
3
N
c
N
b
a

PLATE 54

FIG. 4. Patient Ci: higher magnification of boxed area e in Fig. 3. Ferritin-conjugated antibody is localized in the material contained in the intercellular space. *V* probably labels a cross section of a canal which communicates (arrow) with other intercellular spaces at a different level. Ferritin does not localize in this area. × 43,000.

FIG. 5. Patient Ci: the picture represents a higher magnification of boxed area f of Fig. 3. Ferritin-conjugated antibody is localized in the focal subepithelial deposit (*d*), in the basement membrane (*BM*) and in the subendothelial deposit (*d'*). Only a few ferritin granules are visible in the electron-opaque subepithelial deposit indicated with the asterisk. × 38,000.



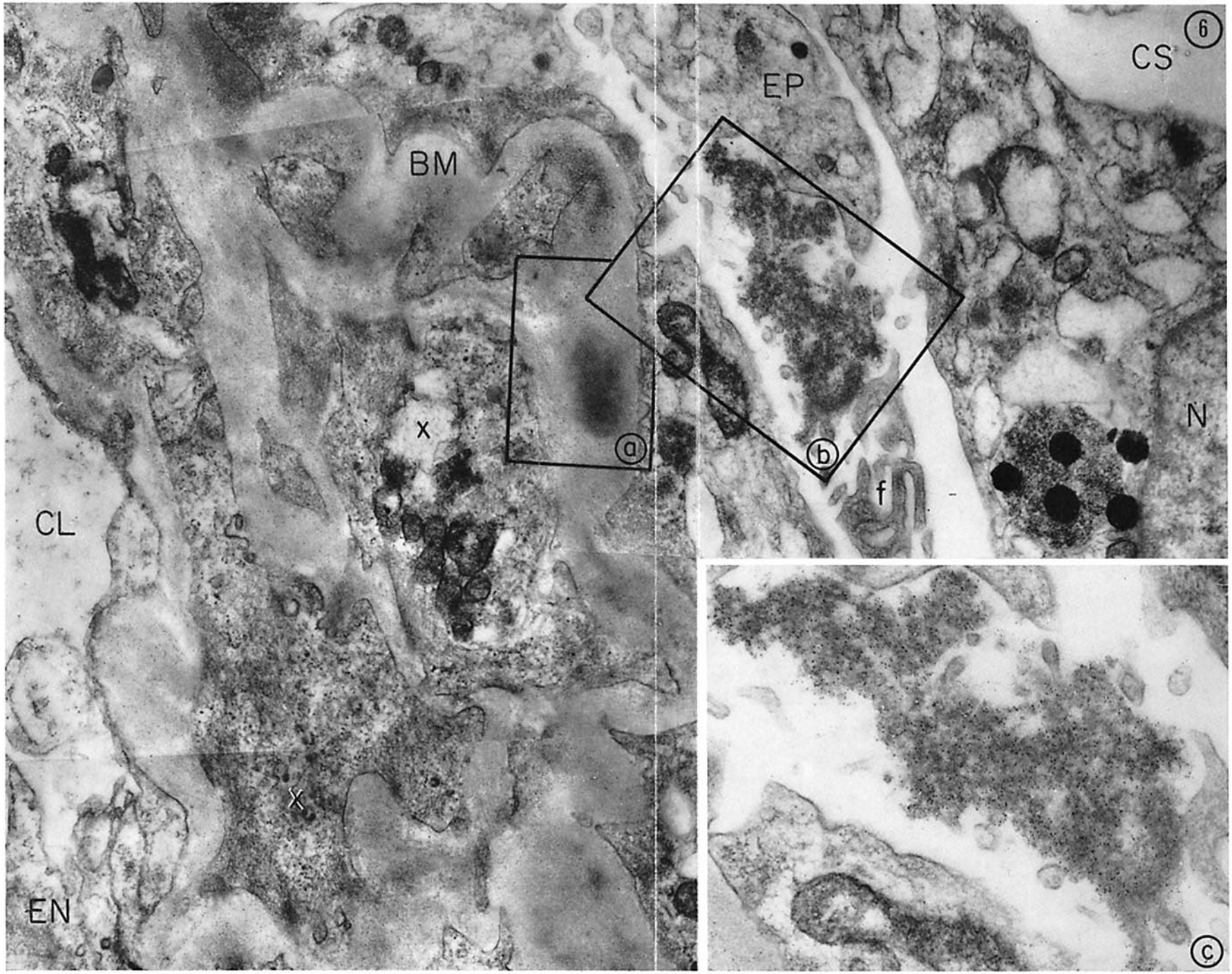
(Andres et al.: Human glomerulonephritis)

PLATE 55

FIG. 6. Patient Ci: a montage of electron micrographs showing a part of a glomerular capillary wall. The section was treated with ferritin-labeled antibody to β_{1C}. The capillary lumen (*CL*) is located on the left and Bowman's space (*CS*) is on the right side of the picture. In this section the basement membrane (*BM*) is undulating and appears to be cut at different levels. Rims of cellular cytoplasm (*X*) appear to be trapped between the folds of the basement membrane. Ferritin-conjugated antibody to β_{1C} localizes in the basement membrane (*BM*). In Bowman's space, between the foot processes (*f*) and part of the epithelial cytoplasm (*EP*), aggregates of electron-opaque material are tagged by ferritin-conjugated antibody. On the right of the photographic field part of the nucleus (*N*) and of the cytoplasm of an epithelial cell are visible. × 24,000.

The boxed area a is illustrated at higher magnification in Fig. 2, inset b. Ferritin-conjugated antibody does not localize in the more electron-opaque material which probably contains fibrinogen products. × 48,000.

Inset c, lower right corner of Fig. 6, shows a higher magnification of the aggregates of electron-opaque material between the foot processes. × 62,000.

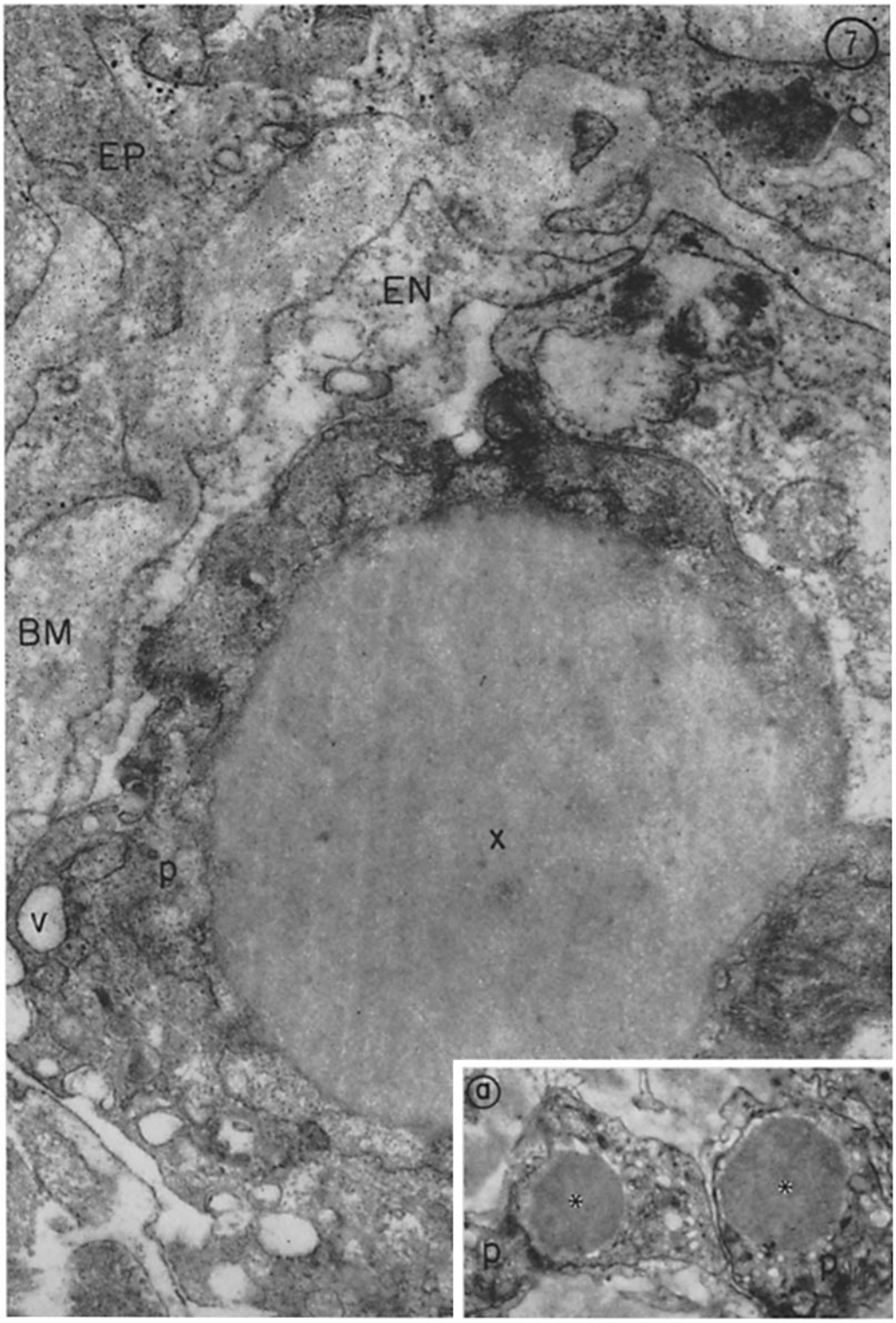


(Andres et al.: Human glomerulonephritis)

PLATE 56

FIG. 7. Patient Ci: the picture shows that the foreign electron-opaque material (*X*) contained in a polymorphonuclear leukocyte (*P*) does not bind the ferritin-conjugated antibody to β_{1C}. Ferritin granules are localized in the basement membrane (*BM*) and in spaces between the epithelial cytoplasm (*EP*). In the cytoplasm of the polymorphonuclear leukocyte *V* indicates empty vesicles which probably result from degranulation of the leukocyte during phagocytosis. × 39,000.

In inset a two polymorphonuclear leukocytes (*P*) containing aggregates of foreign material (*) are compressed and distorted between strands of basement membrane–like material. × 7,000.



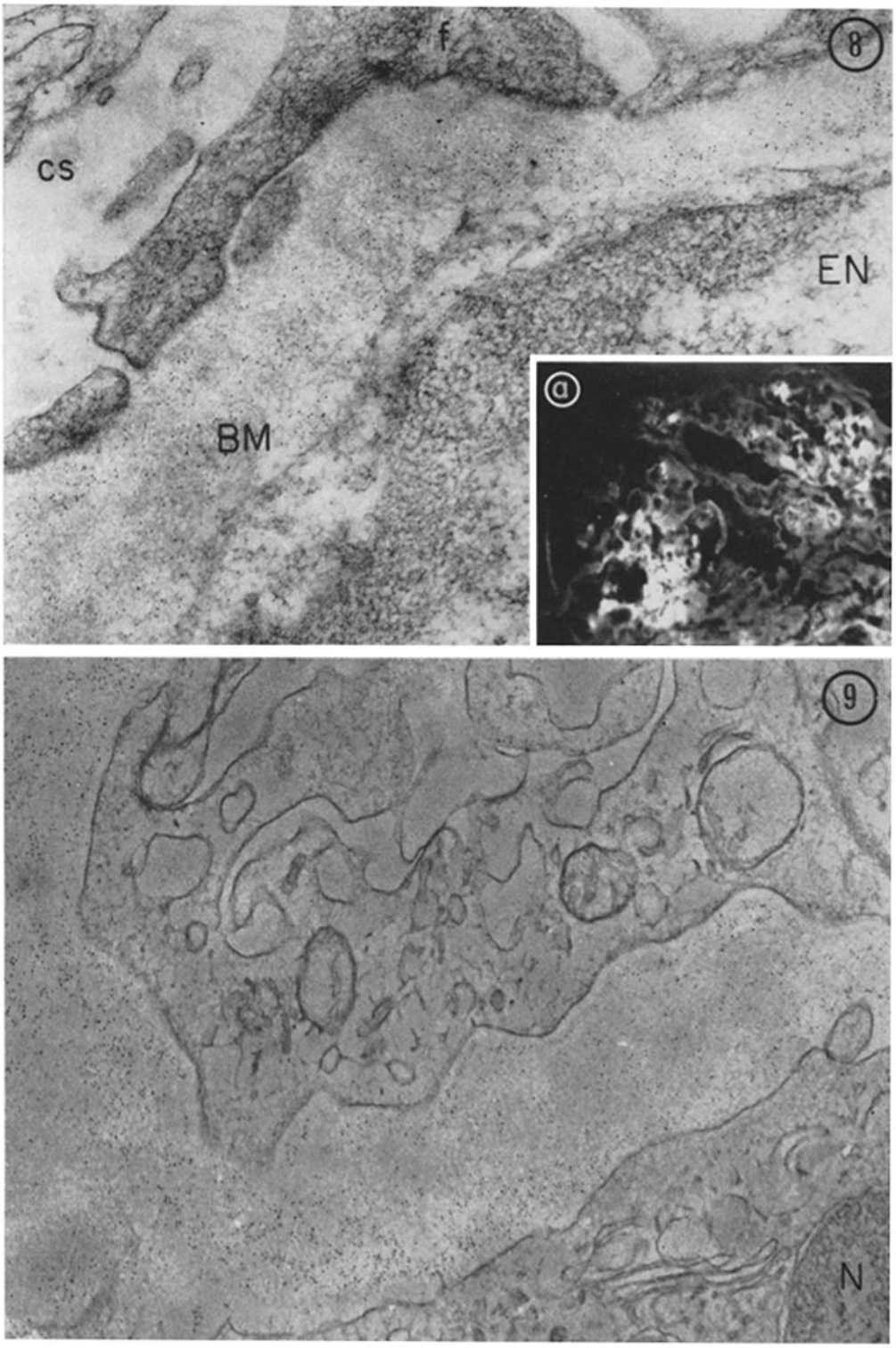
(Andres et al.: Human glomerulonephritis)

PLATE 57

FIG. 8. Patient Ci: the picture shows part of a glomerular capillary wall. Ferritin-conjugated antibody to products of the streptococcus Group A, Type 12 are localized in glomerular basement membrane (*BM*). The small focal subepithelial deposit does not contain ferritin. *EN* indicates part of an endothelial nucleus; *f*, part of an epithelial foot process; and *cs*, the capsular space. × 39,000.

Inset a illustrates the localization of fluorescein-conjugated antistreptococcal serum in the glomerular structures. × 200.

FIG. 9. Patient Ci: the mesangial area from a section exposed to ferritin-conjugated antibody to products of the Group A, Type 12 streptococcus. Tagging of foreign material between cells is illustrated. *N* indicates part of the nucleus of a mesangial cell. × 33,000.



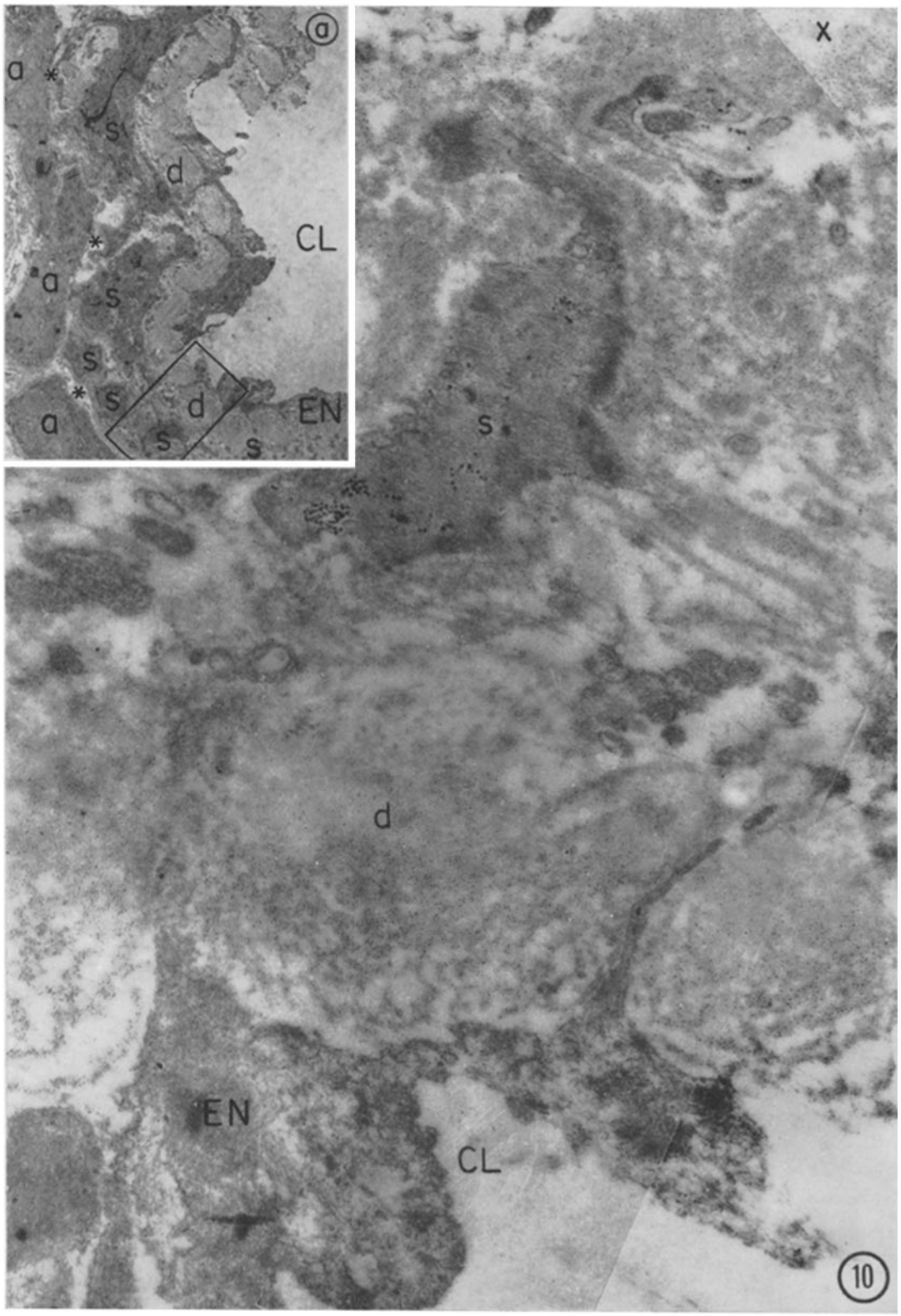
(Andres et al.: Human glomerulonephritis)

PLATE 58

FIG. 10. Patient Pg: a section of a small artery found in the renal medulla from a piece of tissue exposed to ferritin-conjugated antibody to products of the Group A, Type 12 streptococcus.

Inset a, at low magnification, shows that deposits of foreign material (*d*) are localized between the endothelium (*EN*) and the tunica media formed by smooth muscle cells (*s*). The asterisks indicate the membrana elastica externa and *a*, three cells of the tunica adventitia. The arterial lumen is labeled *CL*. × 3,000.

The higher magnification of the boxed area, indicated in inset a, shows ferritin-conjugated antibody to Group A, Type 12 streptococcus localized in subendothelial deposits (*d*). Foreign material tagged by ferritin granules penetrate also between the smooth muscle cells (*s*) up to the membrana elastica externa (*x*). *CL* indicates the capillary lumen. × 29,000.



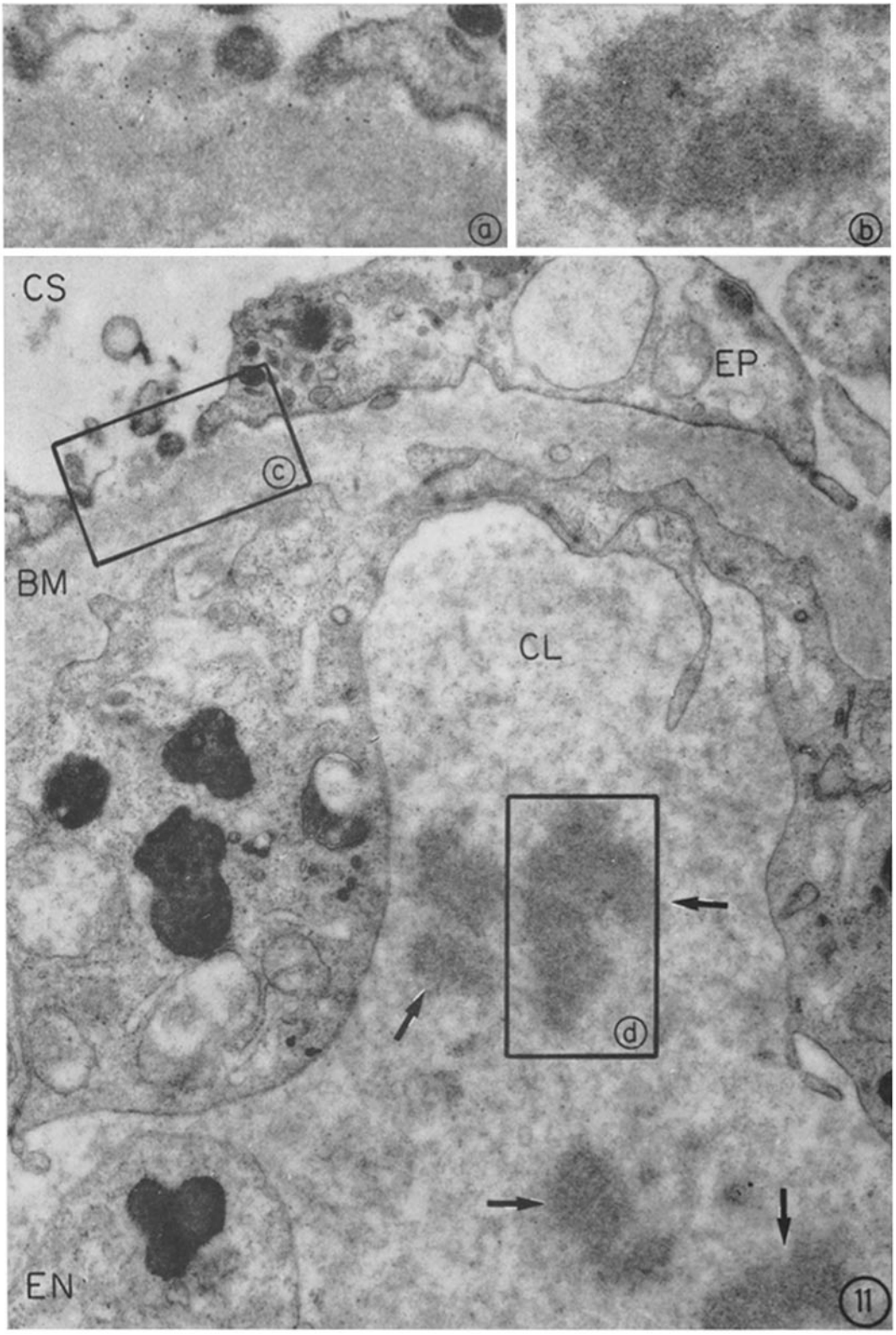
(Andres et al.: Human glomerulonephritis)

PLATE 59

FIG. 11. Patient Ci: renal tissue treated with ferritin-conjugated antibody to vaccinia virus. The picture shows part of a glomerular capillary containing aggregates of foreign material in the lumen. × 22,000.

Inset a, higher magnification of boxed area c. Only a few ferritin granules are localized on the epithelial side of the basement membrane (*BM*). × 55,000.

Inset b, aggregates (arrows) present in capillary lumen (*CL*) (boxed area d). Few, if any, ferritin particles are present. × 45,000.

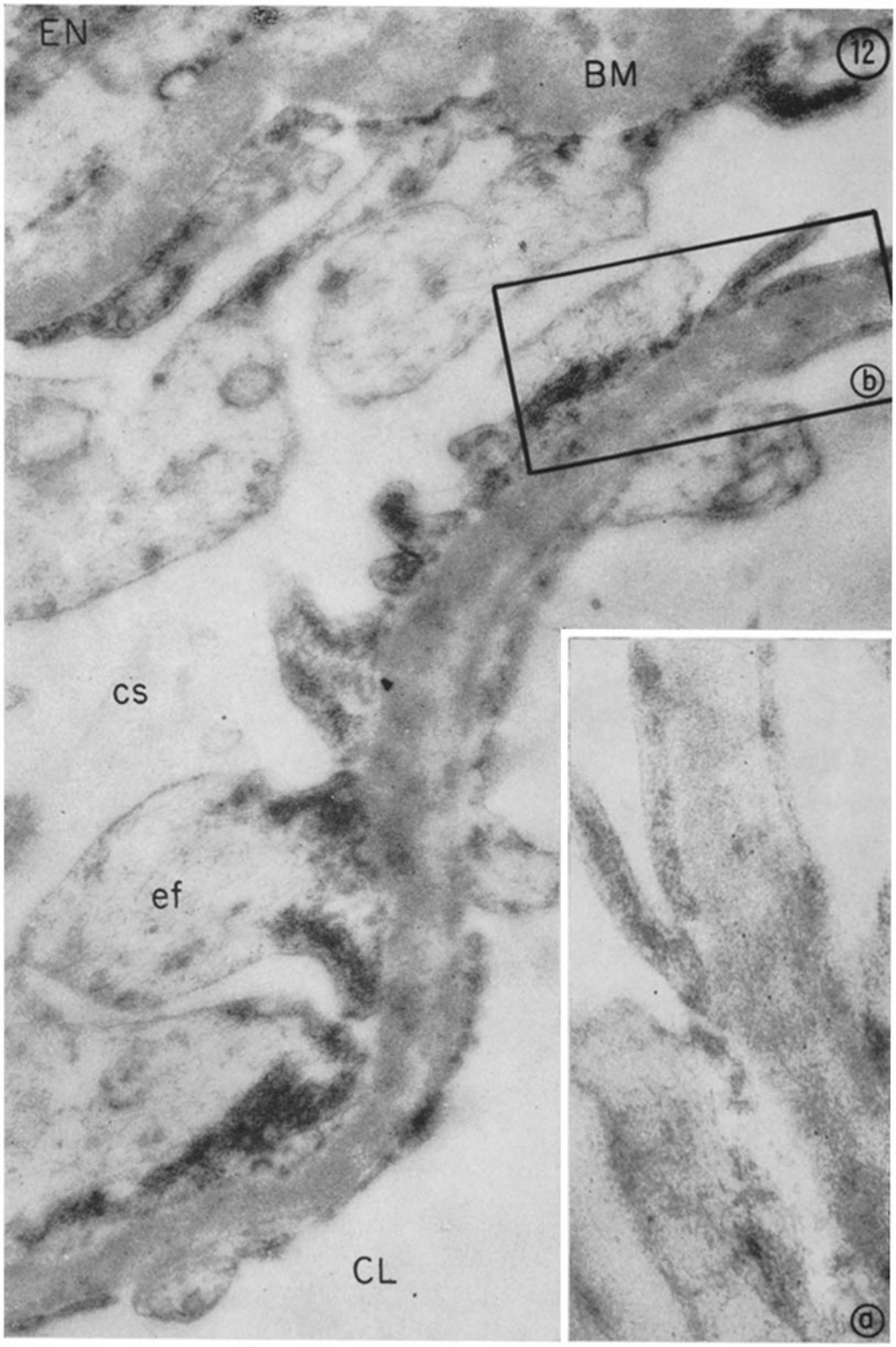


(Andres et al.: Human glomerulonephritis)

PLATE 60

FIG. 12. Normal kidney: glomerular capillary walls of normal human kidney treated with antibody to 7S γ-globulin. *EN* is endothelium; *cs* is capsular space; and *ef* is epithelial foot process. × 28,000.

Inset a illustrates an area of the basement membrane (boxed area b) at higher magnification. Only a few ferritin granules are localized in the glomerular basement membrane (*BM*). × 62,000.



(Andres et al.: Human glomerulonephritis)