The Development and Resolution of Glomerular Basement Membrane Changes Associated With Subepithelial Immune Deposits

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The morphogenesis of glomerular basement membrane changes associated with subepithelial immune deposits was studied in kidney biopsies from patients with gold-induced membranous glomerulonephritis. Serial biopsies showed focal accumulations of additional basement membrane material around the deposits, suggesting that the deposited material stimulated the epithelium to increased synthesis. Moreover, the deposits were gradually displaced towards the inner (endothelial) side of the basement membrane during the course of the disease, suggesting that this layer undergoes a slow continuous turnover, with removal at its endothelial aspect. The two processes—increased epithelial synthesis and turnover—are suggested to constitute the basis of a natural healing process resulting in elimination of the deposits and structural restoration of the basement membrane. The epithelial slit membranes were dislocated externally by the deposits or the excessive basement membrane material, indicating that their barrier function is preserved even in this pathologic condition. (Am J Pathol 79:219–236, 1975)

MEMBRANOUS GLOMERULONEPHRITIS (MGN) is a histopathologic entity.[1,2] The classic ultrastructural picture shows a combination of two types of changes[3–7]: a) extraneous deposits (which stain for both IgG and C3 on immunofluorescence[7]) located on the external surface of or within the glomerular capillary basement membrane; and b) irregularities, including projections and irregular thickening of the basement membrane proper. Only when they are well developed are the changes recognizable, and even separable from each other, in specially stained sections studied by light microscopy.[8,9] The changes are not static, but evolve in a regular manner with time.[5–7,10]

Despite extensive studies of MGN, two questions have remained unsolved: What is the origin of the changes of the basement membrane proper and when do they develop in relation to the deposits? and Which mechanisms form the basis of the evolutionary process?

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Preliminary results of a recent study [11] based on observations of isolated biopsies from patients with gold-induced MGN (gold nephropathy, GNP) of varying duration indicated that the changes of the basement membrane proper—projections and irregular thickening—develop secondarily to and as a direct consequence of deposition of immune globulins (immune complexes?) on the external surface of the basement membrane proper. In the light of these observations and the results obtained from experimental studies of others,[12–14] increased synthesis of basement membrane material by the visceral epithelial cells (stimulated by the deposited material?) was suggested to be the mechanism responsible for the accumulation of excessive basement membrane material around the deposits.

The present study is an extension of our previous study [11] and is mainly based on observations of serial biopsies from patients with GNP. Its purpose is twofold: first, to furnish conclusive evidence for the precedence of the deposits in relation to the basement membrane changes and to discuss the origin of the changes; and second, to describe the evolutionary sequence of the combined changes (deposits and basement membrane irregularities) in relation to the duration of the disease and to discuss the mechanisms involved.

Materials and Methods

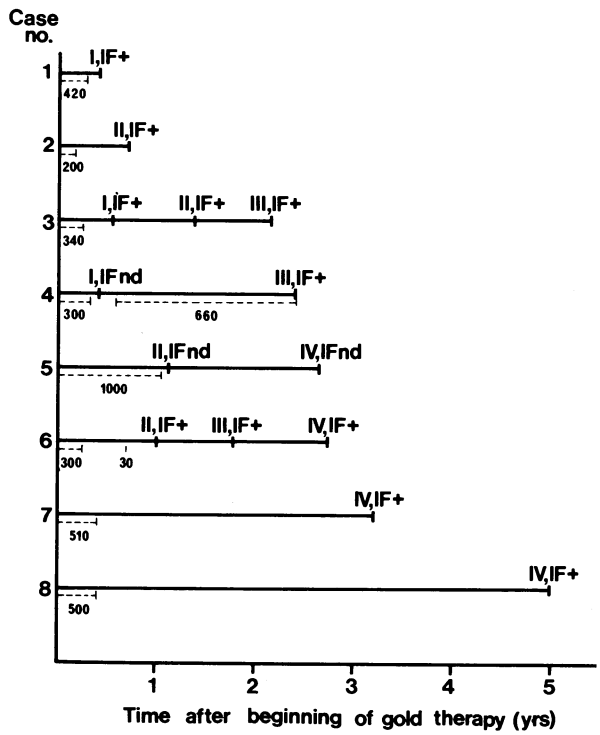
Patients and Biopsies

In all, 14 percutaneous renal biopsies with ultrastructurally demonstrable MGN (see below) were obtained from 8 patients with classic erosive rheumatoid arthritis who had developed proteinuria or nephrotic syndrome during treatment with sodium aurothiomalate (Myocrisine®, May & Baker). Four patients were biopsied once, 2 patients twice, and 2 patients three times during the course of the study (Text-figure 1). The MGN was attributed to the gold therapy principally because of a) the close time relations between the gold treatment and the appearance of proteinuria, and b) careful exclusion of other possible causes (this question was discussed at greater length in a previous communication [11]). Tissue for immunofluorescent study was available from 11 biopsies.

Electron Microscopy

The methods used for electron microscopy have been described previously.[11] Briefly, the biopsy was fixed in 1.5% cacodylate-buffered glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in ethanol, stained *en bloc* with uranyl acetate, and embedded in Epon 812. Sections with dark gray interference color were mounted on bare 200-mesh copper grids, stained with lead citrate and uranyl acetate and viewed in a Siemens Elmiskop I or a JEM 100B electron microscope.

At least three glomeruli from each biopsy were studied in numerous sections. The diagnosis of MGN was assessed on the basis of the following ultrastructural criteria: a) few or many electron-dense deposits or electron-lucent areas, either on the epithelial surface of (deposits) or within (deposits and lucent areas) the



TEXT-FIG 1—Correlative ultrastructural, immunofluorescent and clinical data. (Ultrastructural stages of GNP, *I, II, III, IV;* granular deposits of IgG and *C*3 in the glomerular capillary walls, *If+;* immunofluorescence not done, *IFnd;* duration of gold therapy and total dose in mg of Myocrisine, *dotted lines.*)

basement membrane proper, associated or not associated with b) irregularities such as projections and irregular thickenings of the basement membrane proper. As must be emphasized, these criteria imply that the diagnosis of MGN was assessed on a qualitative rather than a quantitative basis. In fact, the extent of the changes varied considerably from patient to patient and glomerulus to glomerulus, as could be demonstrated most clearly with the immunofluorescent technic (see below and Figure 6). The changes were classified according to a staging system introduced in a previous communication.[11]

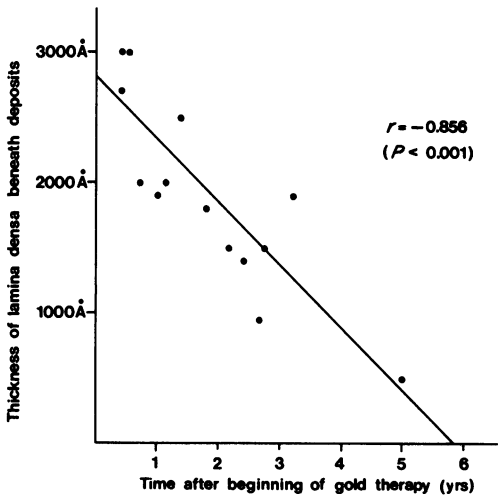
Measurements of the Thickness of the Lamina Densa

The degree of displacement of the deposits within the lamina densa was roughly estimated by measuring the distance between the most basal part of the deposits and the inner border of the lamina densa. The measurements were made on paper copies photographically enlarged three times. The primary magnifications were 5,000 to 30,000. The number of copies (different images) from each biopsy varied between 50 and 150. Measurements were made in all places where the peripheral capillary wall appeared cross-sectioned, and where the base of the deposits and the inner border of the lamina densa were clearly distinguishable. The calculated mean values for each biopsy were plotted on a graph (Text-figure 2).

Results

Subepithelial Deposits Without Changes in the Basement Membrane Proper (Stage I)

This pattern was seen in the initial biopsies from 3 patients, taken



TEXT-FIG 2—Correlation between the degree of displacement of deposits within the lamina densa and the duration of GNP.

5 to 6½ months after institution of gold therapy (Figures 1 and 4). The deposits were located within the space delimited internally by the basement membrane and externally by the portions of the epithelial cytoplasmic membrane united by the slit membranes. Deposits were never seen extending beyond the slit membranes (see below). The deposits were moderately electron dense, slightly darker than the lamina densa, and homogeneously finely granular.

Secondary Basement Membrane Changes: Accumulation of Basement-Membrane-Like Material Around the Deposits (Stages II–IV)

Changes described under this heading were found in biopsies obtained after a minimum of 9 months after institution of gold therapy, that is in all remaining biopsies (including follow-up biopsies from 2 of the 3 patients described above). The changes covered a wide spectrum. In some biopsies, the only changes seen were very short projections of basement-membrane-like material protruding from the basement membrane proper on one or both sides of the deposits (Stage II). In other biopsies, the projections were taller and partly embraced the deposits laterally (Stage III, Figures 2, 5 and 13). In still other biopsies, a continuous layer of basement-membrane-like material totally surrounded ("encapsulated") the deposits (Stage IV, Figures 3, 8, 9 and 14). Often (in Stages III–IV), the material also extended into adjacent, irregular-shaped spaces between the epithelial foot processes (Figures 8 and 10–12). The material, whether forming projections or extending into adjacent intercytoplasmic (interpedicellar) spaces, always seemed to be in direct continuity with the lamina densa of the

basement membrane proper. Furthermore, the material, like the deposits, was always located internally to those portions of the epithelial plasma membrane that were connected by the slit membranes (Figures 8 and 10–12). It must be stressed that no projections or any of the other changes described above were ever seen in capillary segments devoid of deposits; the deposits and the excessive basement-membrane-like material appeared to be intimately associated.

The basement-membrane-like material seemed to accumulate around the deposits in a regular time-dependent manner. In early biopsies, 9 to 26 months after the institution of gold therapy, the projections, although varying in height, rarely made contact external to the deposits. Total encapsulation, on the other hand, was the dominant finding in biopsies obtained later (Text-figure 1). The reverse sequence was not seen in the serial biopsies, nor was a reverse pattern observed in isolated biopsies (*ie*, a layer of basement-membrane-like material external to the deposits without concomitant projections).

Displacement of Deposits Within the Basement Membrane

In biopsies obtained 5 to 14 months after the beginning of gold therapy, there was no appreciable thinning of the lamina densa beneath the deposits (the "normal" thickness ranged from 2000 to 3000 Å) (Text-figure 2). In biopsies obtained later, the distance between the inner side of the deposits and the lamina rara interna was diminished. It was approximately halved in biopsies obtained 2 to 3 years after the beginning of gold therapy, and after 5 years it was diminished to about 500 Å. In these late biopsies it was often impossible to distinguish clearly between the inner aspects of the deposits and the lamina rara interna, first because these deep deposits stained faintly and resembled the lamina rare in electron density, and second because swelling and increased granularity of the latter obscured its exact borders. In many places the deposits actually seemed to coalesce with the lamina rara interna.

Foot Processes and the Epithelial Slit Membranes

As a rule, there was some degree of focal loss ("fusion") of the foot (terminal) processes (Figures 1 and 4), which characteristically was confined to capillary segments containing deposits, the processes being intact elsewhere. Wherever slit membranes (foot processes) over deposits were preserved, they were dislocated externally either by the deposits themselves or by the excessive amounts of basement-membrane-like material, sometimes by as much as 4000 Å from the normal level

(Figures 8 and 10–12). The deposits and the basement-membrane-like material extended into the near proximity of the slit membranes (being separated from them only by a thin layer resembling the lamina rara externa; Figures 10 and 12), but were never seen to penetrate them. Thus, the slit membranes seemed to constitute effective barriers to both the deposits and the basement-membrane-like material.

Immunofluorescence Findings

Tissue from 11 biopsies was available for immunofluorescent study (Text-figure 1). In all biopsies the glomerular capillary walls contained small granules of IgG and C3 (Figures 6 and 7). In general, the immunofluorescent distribution of the deposits (diffuse or segmental) mimicked the ultrastructural distribution, but immunofluorescence usually revealed a more heavy deposition than did electron microscopy, probably because of the greater thickness of the cryostat sections (50 to 100 times thicker than the ultrathin sections). In biopsies taken late in the course of the disease the glomeruli appeared to stain more weakly than in the early biopsies, but it must be remembered that quantitative comparisons of different immunofluorescent preparations are not very reliable for technical reasons (differences in section thickness, illumination, etc).

Discussion

Glomerular subepithelial deposits—the hallmark of immune complex glomerulonephritis [15]—have long been known to be associated with projections and irregular thickening of the capillary basement membrane.[5,13,16] The fact that such changes have never been reported to occur without concomitant deposits has been taken to suggest a direct causal connection between the deposits and the basement membrane changes.[5,7,10,11,13] The evidence for such a connection is greatly strengthened by the results of our present study of GNP, which showed the characteristically segmental distribution of the changes: irregularities of the basement membrane were never seen in capillary segments devoid of deposits.

Hitherto, for lack of biopsies taken sufficiently early after the onset of the disease, it has remained uncertain which of the two components, deposits or basement membrane changes, develop first. Our present findings confirm that the sequence is as expected by previous workers [5,7,10,11,13]: The 2 cases with deposits without basement membrane changes in the initial biopsies but with both changes in the later follow-up biopsies, leave no doubt that the deposits preceded the basement membrane changes.

Origin of the Excessive Basement Membrane Material

The structural identity of the excessive basement membrane material (projections), on the one hand, and the basement membrane proper on the other, as well as the direct continuity between these two, suggest that they are one and the same material. Therefore, the excessive basement membrane material may logically be considered to have originated as part of the basement membrane. Embryologic,[17] ultrastructural [18–20] and immunohistochemical [14] studies have indicated that the basement membrane is synthesized by the visceral epithelial cells, either alone or in association with other cells of the glomerulus. Our findings are in keeping with this hypothesis: The excessive material was located on the epithelial side of the basement membrane proper, which indicates that it was formed on that side as a result of locally increased epithelial synthesis.

It may be asked whether the increased synthesis was caused by the deposits themselves or by some other local factors which gave rise to both the deposits and the basement membrane changes. Our immunofluorescent findings show that the deposits contained at least IgG and C3. The fact that these proteins had reached the subepithelial region may be taken as evidence for increased permeability of the basement membrane, which normally impedes molecules of that size.[18,21,22] Also, the fact that all the patients were proteinuric seems to point to an increase in permeability (although it must be kept in mind that the proteinuria might have depended solely on a disturbed function of the epithelial cells [23]). A local increase in permeability, in turn, could have rendered it possible for macromolecular substances (other than those constituting the deposits) capable of stimulating synthesis to come in contact with the epithelial cells. However, the fact that basement membrane material continued to accumulate around the deposits despite the absence of "new" (superficial) deposits (Stages III to IV) and cessation of proteinuria indicates that the deposited material itself possessed some stimulating properties. Our immunofluorescent findings support the assumption that the deposits contained immune complexes. Thus, it is tempting to attribute the stimulating effect of the deposits to their immune complex nature, including their ability to activate the injurious complement system.[24]

Turnover of the Basement Membrane

As the deposits were originally observed in a subepithelial location and were later found to have moved towards the endothelial side, the material surrounding them—the basement membrane proper—had evidently moved in that same direction. This, in turn, implies that the

basement membrane has a turnover, maintained by continuous removal at its endothelial aspect, because otherwise the basement membrane would have grown steadily thicker. This view is strongly supported by studies with the experimental argyric technic,[20] which have indicated a continuous turnover of the basement membrane, maintained by continuous synthesis at its epithelial aspect and continuous removal at its endothelial aspect. Thus, the whole evolutionary process of the changes seems to be based on the effect of increased synthesis of basement membrane material coupled with continuous turnover of the basement membrane proper.

From our findings, we roughly estimate that the rate of turnover of the basement membrane—in GNP—is more than 5 years. One obvious source of error is the difficulty of assessing the exact duration of the disease, *ie,* the interval between the moment the first deposits were formed and the moment of biopsy. The onset of proteinuria cannot be considered a reliable sign of initial laying down of deposits.[13,25] For that reason, the time of institution of gold therapy appears to be a more accurate starting point for assessing the duration, provided that the time of "immunization" was fairly constant. We must also consider the possibility that some biopsies contained deposits of different ages located at various levels within the basement membrane which would result in a too high value (slow rate) of the turnover, because all deposits were included in the measurements.

The Epithelial Slit Membranes

The deposits and the excessive basement membrane material were always located on the inner side of the slit membranes and clearly dislocated them externally from their normal levels. The slit membranes have been shown to exert a final selective effect upon the glomerular filtrate, stopping molecules larger than about 64 Å in greatest dimension.[21,22,26,27] Therefore, one likely explanation is that the material constituting the deposits was held back because of its molecular size. The topographic relationship between the slit membranes and the excessive basement membrane material, on the other hand, is more difficult to understand. Assuming that the epithelial cells synthesize basement membrane, it is likely that the transport of this material occurs only through those portions of the epithelial plasma membrane that are adjacent to the basement membrane, and that the slit membranes prevent the escape of basement membrane material into the urinary space. Our findings emphasize the important function of the slit membranes as barriers between the sub- and supraepithelial sides.

Immunologic Aspects

As shown by immunofluorescence, IgG and C3 remained in the glomerular capillary walls as long as there were ultrastructurally detectable deposits. Some removal of the immune deposits probably did occur, as indicated by the decreasing intensity of the immunofluorescence in serial biopsies and the changing staining characteristics of the deposits (from electron dense to electron lucent).

Because no technics were available for the detection of immune complexes in the circulation, it cannot be decided with certainty whether the long persistence of the deposits in the glomeruli (as revealed by immunofluorescence) depended on continuous new deposition from the circulation or a slow local removal alone. Nothing suggests, however, that the formation of immune complexes continued for very long after the cessation of gold therapy.

The local removal of the deposits could have been facilitated (except by the turnover of the basement membrane) either by phagocytosis (pinocytosis) by some cells, *eg*, the epithelial cells, or by increased solubility of the deposits. Once inside the basement membrane (Stage IV), the deposits are probably no longer accessible for phagocytosis. Assuming that the deposits represented immune complexes, their solubility could have been increased in the presence of extreme antigen excess,[28] which, however, did not seem to occur in our cases. Indeed, 1 patient (Case 4) was continuously treated with low doses of gold even after GNP had been demonstrated in her first biopsy, without any differences in the pattern of resolution as compared to the other cases. This suggests that the formation of nephritogenic complexes occurred during a relatively short period of time, after which ensuing antibody excess prevented further formation of small soluble complexes. Consequently, it may be considered that the immune deposits (complexes) remained relatively insoluble and so became fixed to the basement membrane for a long time, and that their removal was accomplished mainly as a result of the turnover of the basement membrane. However, even if the immune globulins would be resolved ("washed out") completely, the possibility remains that they could leave scars (electron-lucent areas?) in the basement membrane, which would not disappear until they reach the endothelial side.

In the light of the above considerations, it is extremely interesting to note that a morphogenesis apparently identical with that in GNP has been illustrated in a case of acute poststreptococcal glomerulonephritis,[16] another glomerular disorder thought to result from deposition of immune complexes.[29] In the patient in question—a 5-year-old boy—

four consecutive biopsies revealed gradual resolution of the deposits and basement membrane changes over a period of 560 days, although electron-lucent areas were still detectable in the inner parts of the lamina densa 560 days after clinical onset. This figure does not differ very much from our calculated rate of turnover. We have similar experiences in some cases of acute glomerulonephritis which, 2 to 4 years after clinical onset (and recovery), showed basal defects in the basement membrane identical with those we have described in Stage IV of GNP.[30] Recently, Richet *et al*[31] have documented a similar natural history of subepithelial deposits in 3 patients with acute glomerulonephritis. This leads us to believe that the process of resolution is the same for all types of subepithelial immune deposits (immune complexes?), provided that they are not resolved in the early phase of the disease. It remains to be shown whether a prolonged or chronic course, characterized by progressive thickening of the basement membrane, depends on continuous deposition of immune complexes or decreased turnover (removal) alone.

Treatment and Outcome

In all patients, gold therapy was discontinued soon after the appearance of proteinuria. In 1 patient (Case 4), however, gold therapy was reinstituted at low doses after GNP was demonstrated in her biopsy, without apparent effects on the ultrastructural pattern of resolution of the deposits.

Two patients (Cases 1 and 8) recovered spontaneously from their proteinuria. The remaining 6 patients were treated with steroids and responded promptly. Two of them are still on steroids and show slight, intermittent proteinuria; the rest have recovered completely. No effect of the steroid therapy on the ultrastructural pattern of resolution could be observed, but in this respect our material is not very representative (only 1 untreated patient was biopsied late in the course of the disease).

References

1. Rosen S, Pirani CL, Kark RM, Muehrcke RC, Pollak VE: "Lipoid nephrosis" and idiopathic membranous glomerulonephritis. Am J Pathol 44:14a, 1964
2. Churg J, Grishman E, Goldstein MH, Yunis SL, Porush JG: Idiopathic nephrotic syndrome in adults. N Engl J Med 272:165–174, 1965
3. Movat HZ, McGregor DD: The fine structure of the glomerulus in membranous glomerulonephritis (lipoid nephrosis) in adults. Am J Clin Pathol 32:109–127, 1959
4. Habib R, Michielsen P, deMontera E, Hinglais N, Galle P, Hamburger J: Clinical, microscopic and electronmicroscopic data in the nephrotic syndrome of unknown origin. Ciba Foundation Symposium on Renal Biopsy. Edited by

GEW Wolstenholme, MP Cameron. Boston, Little, Brown and Company, 1961, pp 70–102

5. Ehrenreich T, Churg J: Pathology of membranous nephropathy. Pathology Annual. Edited by SC Sommers. New York, Appleton-Century-Crofts, 1968, pp 145–186
6. Pollak VE, Rosen S, Pirani CL, Muehrcke RC, Kark RM: Natural history of lipoid nephrosis and of membranous glomerulonephritis. Ann Intern Med 69:1171–1196, 1968
7. Bariéty J, Druet P, Lagrue G, Samarcq P, Milliez P: Les glomérulopathies "extra-membraneuses" (G.E.M.): Étude morphologique en microscopie optique, électronique et en immunofluorescence ["Extramembranous" glomerulonephritis. Light microscopic, electronmicroscopic and immunofluorescence studies]. Pathol Biol 18:5–32, 1970
8. Churg J, Grishman E: Subacute glomerulonephritis. Am J Pathol 35:25–45, 1959
9. Jones DB: Nephrotic glomerulonephritis. Am J Pathol 33:313–329, 1957
10. Hatta I: Electron microscopic analysis of glomerular basement membrane in membranous nephropathy. Jap Circ J 36:137–152, 1972
11. Törnroth T, Skrifvars B: Gold nephropathy prototype of membranous glomerulonephritis. Am J Pathol 75:573–590, 1974
12. Oshima K, Hatano M, Maeyama Y, Sugino N, Takeuchi T: Electron microscopy of the glomerular basement membrane of the rat kidney. Proceedings of the Third International Congress of Nephrology, Vol 2. Edited by G Richet. Basel, S. Karger, 1967, pp 45–53
13. Alousi MA, Post RS, Heyman W: Experimental autoimmune nephrosis in rats: Morphogenesis of the glomerular lesion; immunohistochemical and electron microscopic studies. Am J Pathol 54:47–71, 1969
14. Pierce GB, Nakane PK: Basement membranes: Synthesis and deposition in response to cellular injury. Lab Invest 21:27–41, 1969
15. Wilson CB, Dixon FJ: Diagnosis of immunopathologic renal disease. Kidney Int 5:389–401, 1974
16. Strunk SW, Hammond WS, Benditt EP: The resolution of acute glomerulonephritis: An electron microscopic study of four sequential biopsies. Lab Invest 13:401–429, 1964
17. Vernier RL, Birch-Andersen A: Studies on the human fetal kidney. I. Development of the glomerulus. J Pediatr 60:754–768, 1962
18. Farquhar MG, Wissig SL, Palade GE: Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall. J Exp Med 113:47–66, 1961
19. Kurtz SM, Feldman JD: Experimental studies on the formation of the glomerular basement membrane. J Ultrastruct Res 6:19–27, 1962
20. Walker F: The origin, turnover and removal of glomerular basement-membrane. J Pathol 110:233–244, 1973
21. Graham RC Jr, Karnovsky MJ: Glomerular permeability: Ultrastructural cytochemical studies using peroxidases as protein tracers. J Exp Med 124: 1123–1134, 1966
22. Venkatachalam MA, Karnovsky MJ, Fahimi HD, Cotran RS: An ultrastructural study of glomerular permeability using catalase and peroxidase as tracer proteins. J Exp Med 132:1153–1167, 1970

23. Michael AF, Blau E, Vernier RL: Glomerular polyanion: Alteration in aminonucleoside nephrosis. Lab Invest 23:649–657, 1970
24. Cochrane, CG, Dixon FJ: Cell and tissue damage through antigen–antibody complexes. Calif Med 111:99–112, 1969
25. Sagel I, Treser G, Ty A, Yoshizawa N, Kleinberger H, Yuceoglu AM, Wasserman E, Lange K: Occurrence and nature of glomerular lesions after group A streptococci infections in children. Ann Intern Med 79:492–499, 1973
26. Latta H: The glomerular capillary wall. J Ultrastruct Res 32:526–544, 1970
27. Rodewald R, Karnovsky MJ: Porous substructure of the glomerular slit diaphragm in the rat and mouse. J Cell Biol 60:423–433, 1974
28. Valdes AJ, Senterfit LB, Pollack AD, Germuth FG Jr: The effect of antigen excess on chronic immune complex glomerulonephritis. Johns Hopkins Med J 124:9–17, 1969
29. Andres GA, Accini L, Hsu KC, Zabriskie JB, Seegal BC: Electron microscopic studies of human glomerulonephritis with ferritin-conjugated antibody: Localization of antigen-antibody complexes in glomerular structures of patients with acute glomerulonephritis. J Exp Med 123:399–412, 1966
30. Törnroth T: Unpublished observations
31. Richet G, Fillastre JP, Morel-Maroger L, Bariety J: Change from diffuse proliferative to membranous glomerulonephritis: Serial biopsies in four cases. Kidney Int 5:57–71, 1974

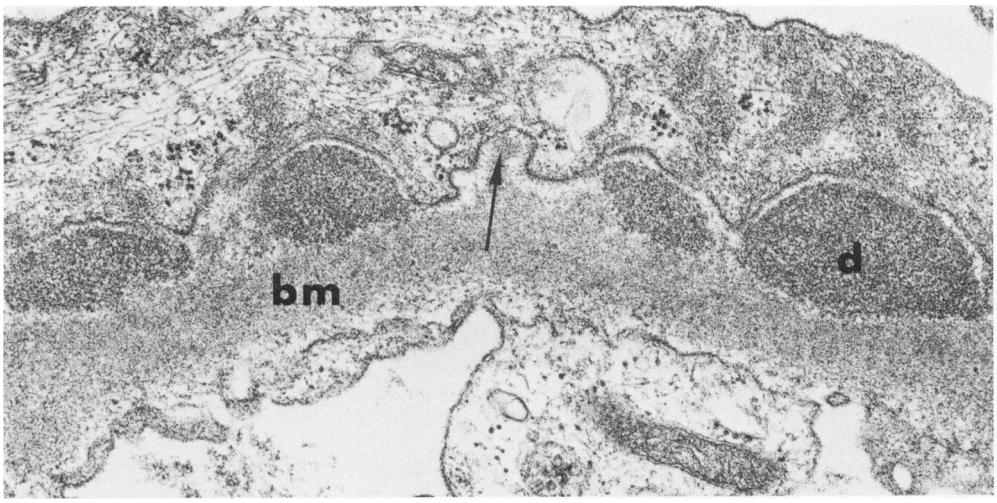
Legends for Figures

Figures 1–3 are images from three consecutive biopsies of Case 3.

Fig 1—Six and one-half months after institution of gold therapy, the deposits (*d*) are superficial and the outer contour of the basement membrane (*bm*) is smooth. In the middle of the picture is a small projection (*arrow*). Such formations, however, were extremely rare in this biopsy (× 34,500).

Fig 2—Ten months later. The deposits (*d*) are surrounded by broad projections (*arrows*), giving an irregular appearance to the external contour of the basement membrane. The slit membranes (*arrowheads*) are dislocated externally (× 33,000).

Fig 3—Nine and one-half months later. Some of the deposits (*d*) are covered externally by a layer of basement-membrane-like material (*arrow*), which dislocates the slit membranes (*arrowhead*). The illustrated pattern was seen infrequently in this biopsy, the predominant pattern being like that seen in Figure 2. Notice the layer of amorphous material (arrows) between the lamina densa and the endothelium (× 33,000).



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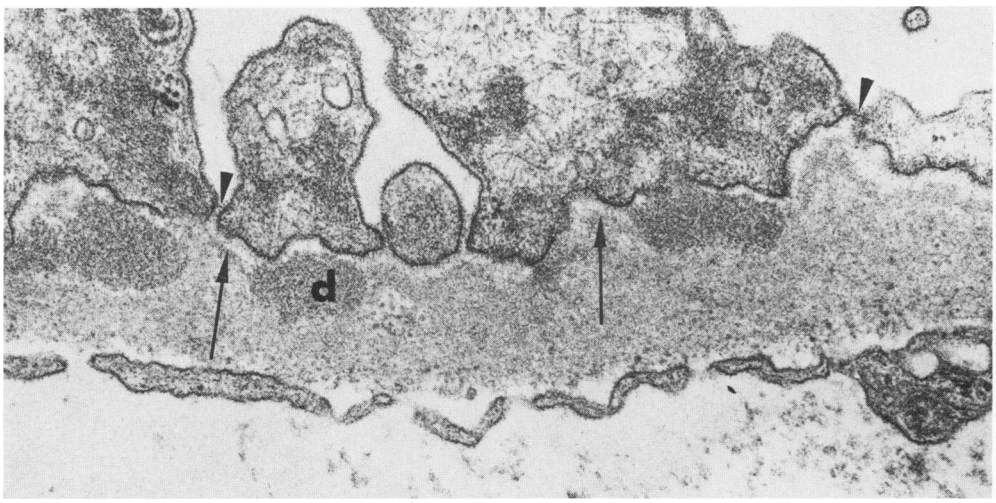

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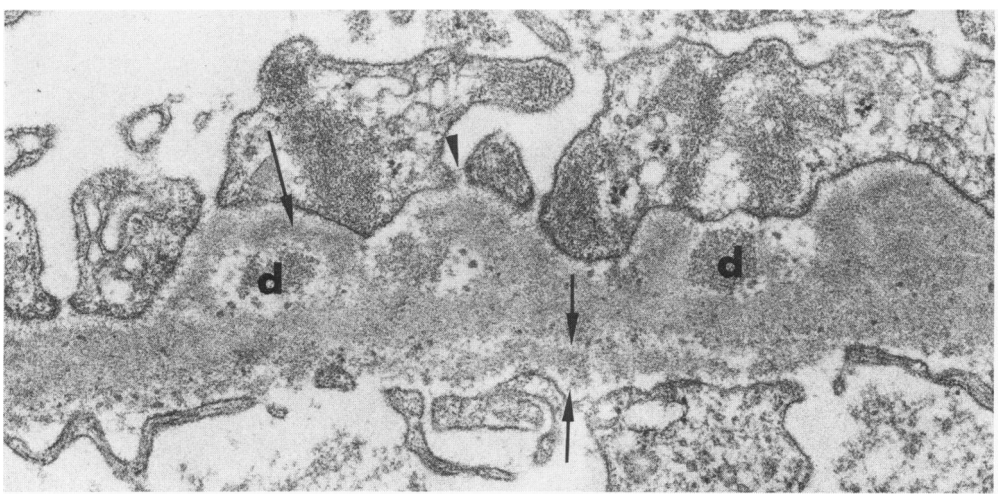

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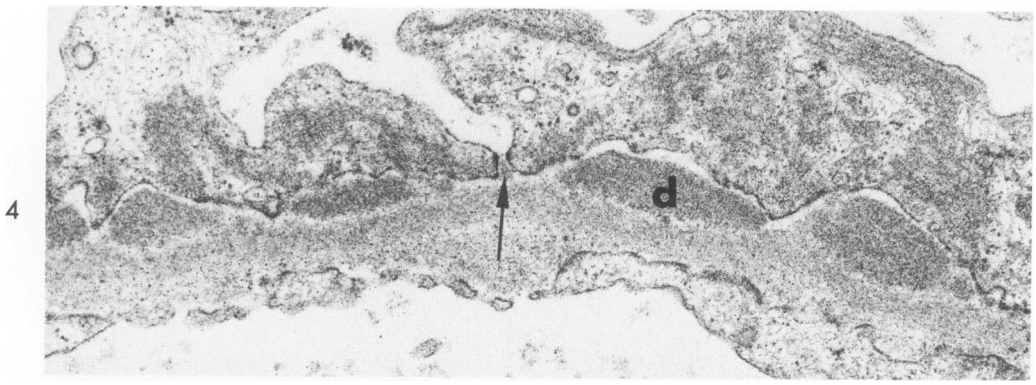

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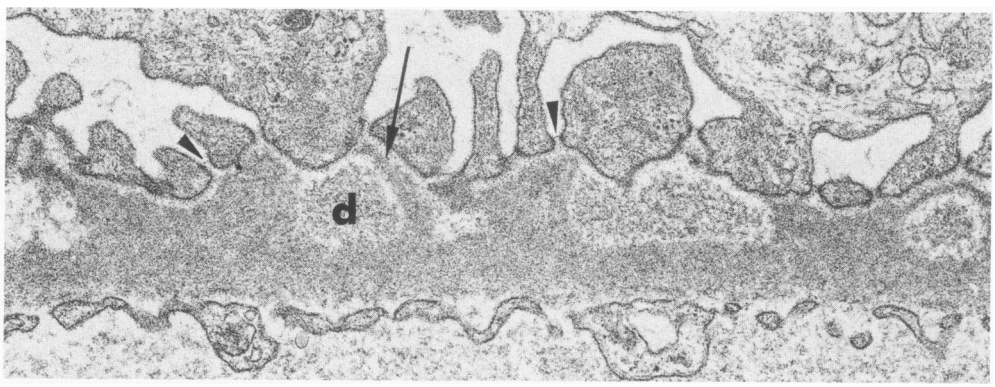

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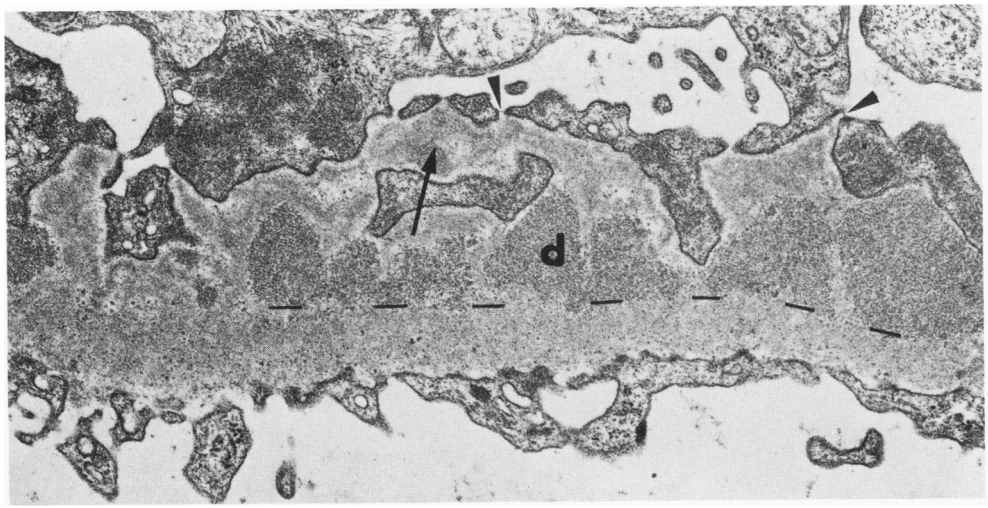
Figures 4 and 5 are representative micrographs from two sequential biopsies of Case 4. **Fig 4**—Five months after institution of gold therapy. The deposits *(d)* are superficial. A small deposit *(arrow)* is located in the interpedicellar space (× 30,000). **Fig 5**—Two years later. The deposits *(d)* are less electron dense than in the first biopsy. They are surrounded by excessive amounts of basement-membrane-like material *(arrow)*. The dislocated slit membranes *(arrowheads)* are clearly visible (× 30,000). **Fig 6**—Case 6, second biopsy. Fluorescence micrograph of a part of a glomerulus stained with anti-IgG. Notice the segmental distribution of the fine granular deposits of IgG along the capillary walls (× 470). **Fig 7**—Case 6, second biopsy. Part of another glomerulus stained with anti-C3. In this glomerulus the deposits are diffusely distributed over the whole capillary tuft. The section is rather thick, which explains the apparent thickening of the capillary walls (× 470).



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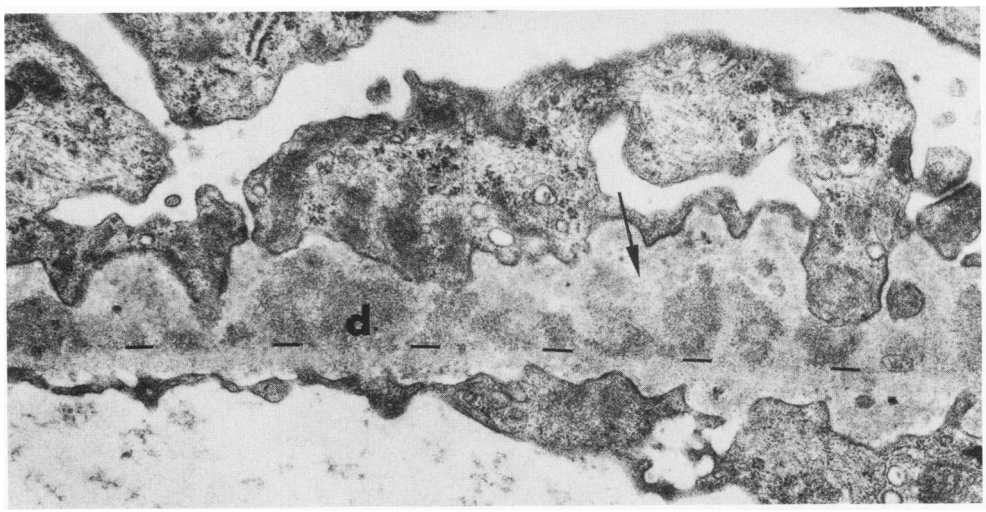

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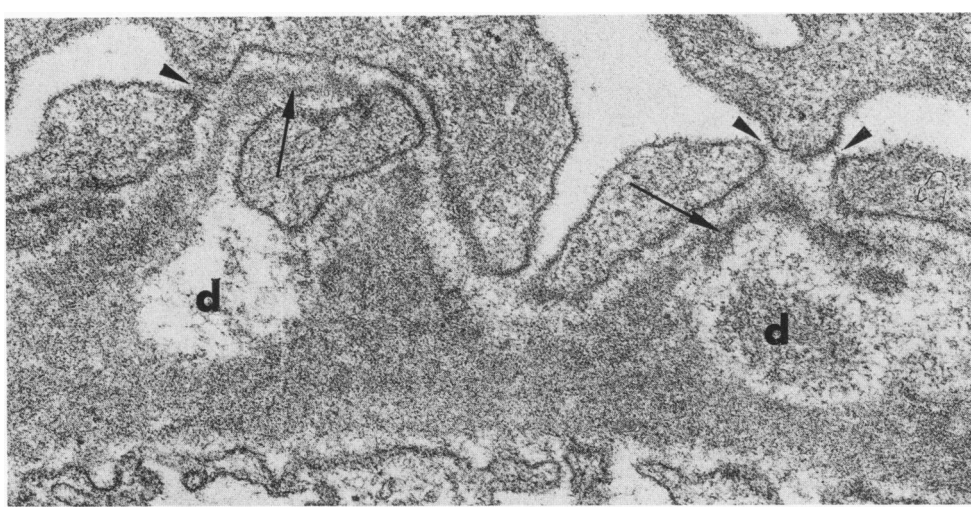

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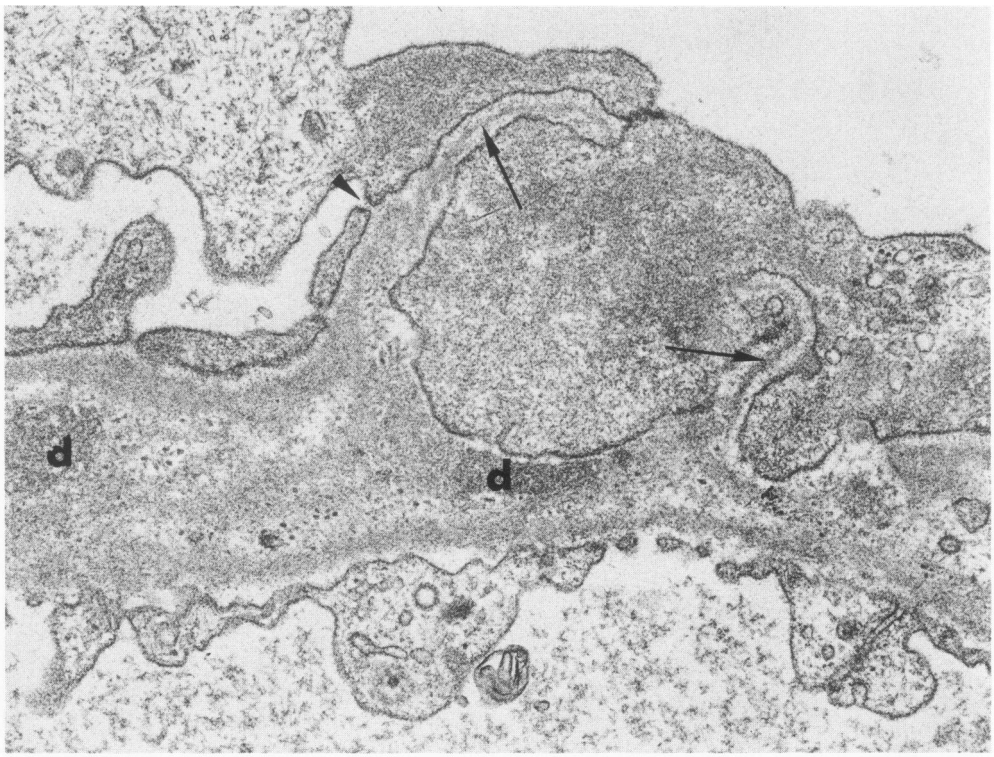
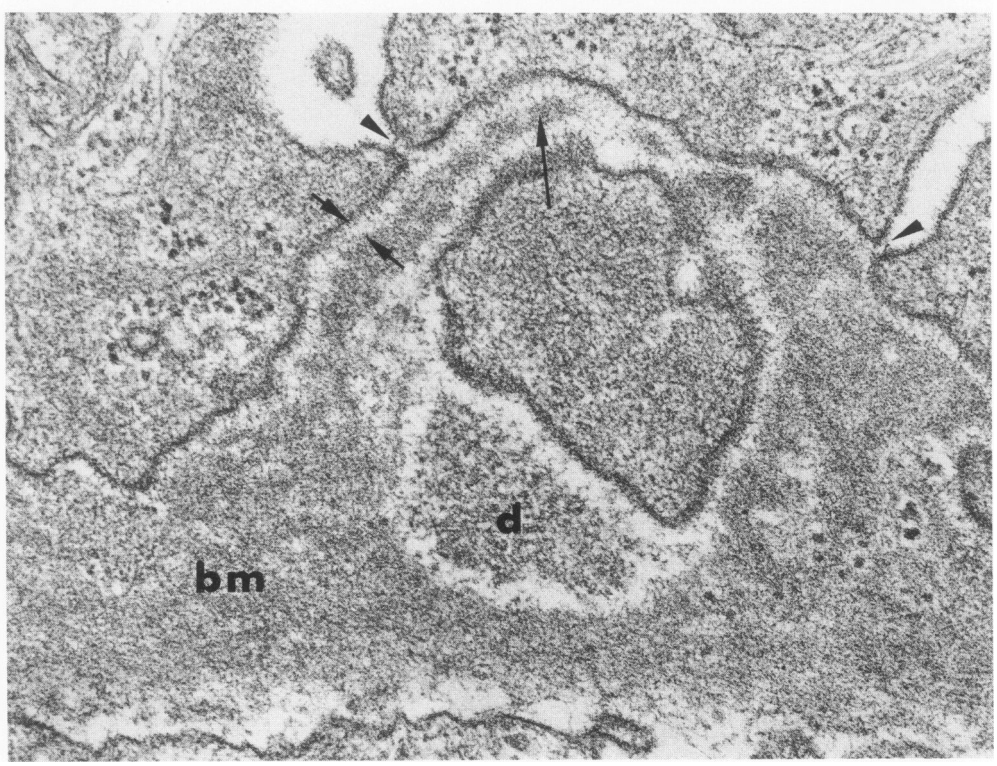
Fig 8—The deposits *(d)* are covered by basement-membrane-like material *(arrow)*, which extends into the interpedicellar spaces. The slit membranes *(arrowheads)* have been "pushed" far away from their normal levels. The deposits are located at a regular distance *(line of dashes)* from the inner surface of the basement membrane (× 16,000). **Fig 9**—This picture clearly illustrates the regular arrangement of the deposits *(d)* within the basement membrane. The intact part of the lamina densa (between the line of dashes and the lamina rara interna) is thinned. *Arrow* indicates excessive basement membrane material (× 16,500). **Fig 10**—A photomicrograph like those described in Figures 11 and 12, (× 43,500).

11



12



Figs 11 and 12—The deposits *(d)* are surrounded by basement-membrane-like material, which forms long, slender processes *(single arrows)* in the interpedicellar spaces. The slit membranes *(arrowheads)* seem to constitute barriers against the urinary space. The basement-membrane-like material is lined by a layer resembling the lamina rara externa (**12**, *arrows*), which separates the material from the slit membranes (**11**, × 30,000; **12**, × 60,000).

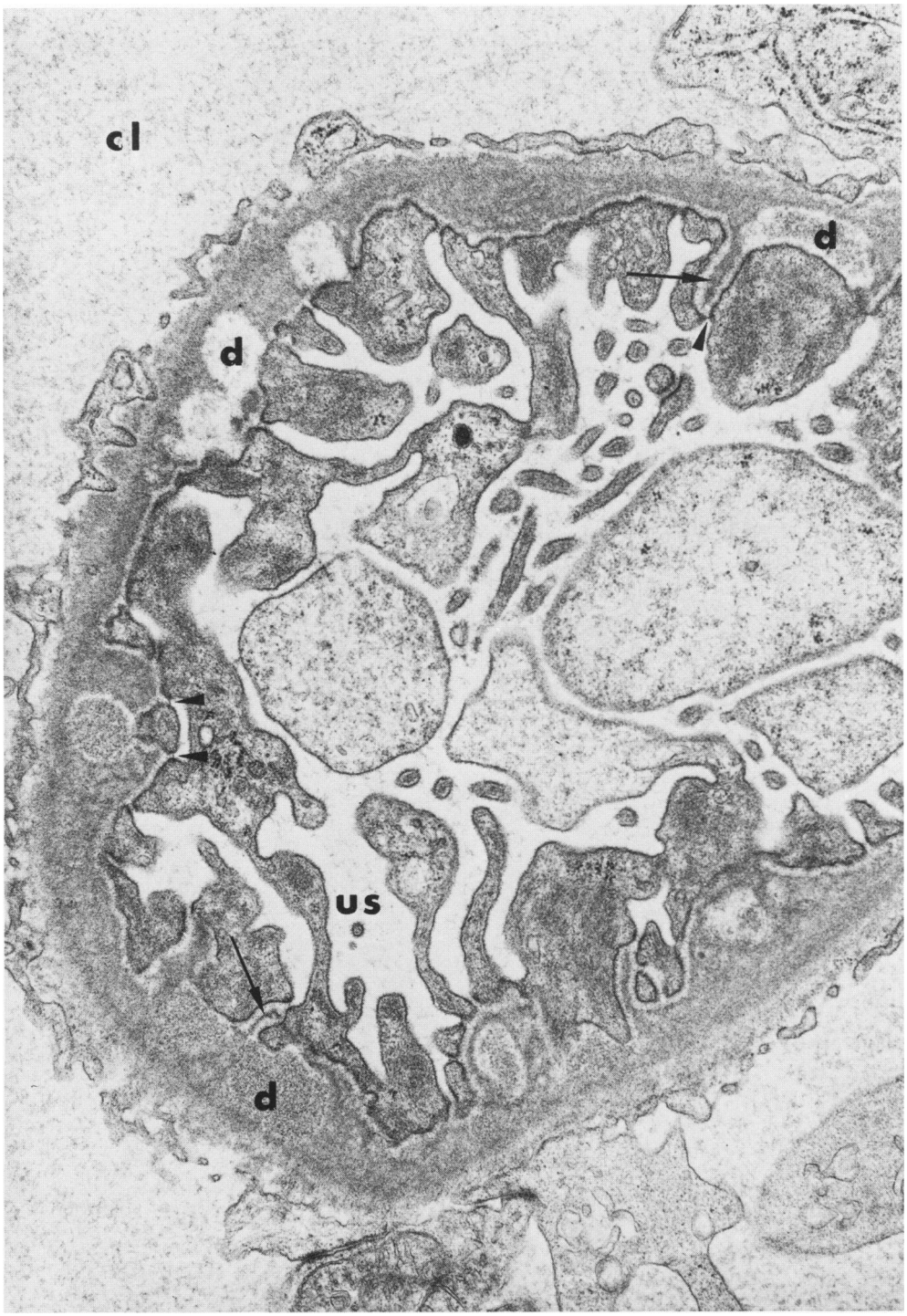


Fig 13—Case 4, 2 years and 5 months after institution of gold therapy. Survey picture showing many deposits *(d)*, some of which are electron dense and some rather electron lucent. The lamina densa is clearly thinned beneath the deposits. Projections *(arrows)* surround the deposits and dislocate the slit membranes *(arrowheads)* towards the urinary space *(us)*. *cl* = capillary lumen (× 24,000).

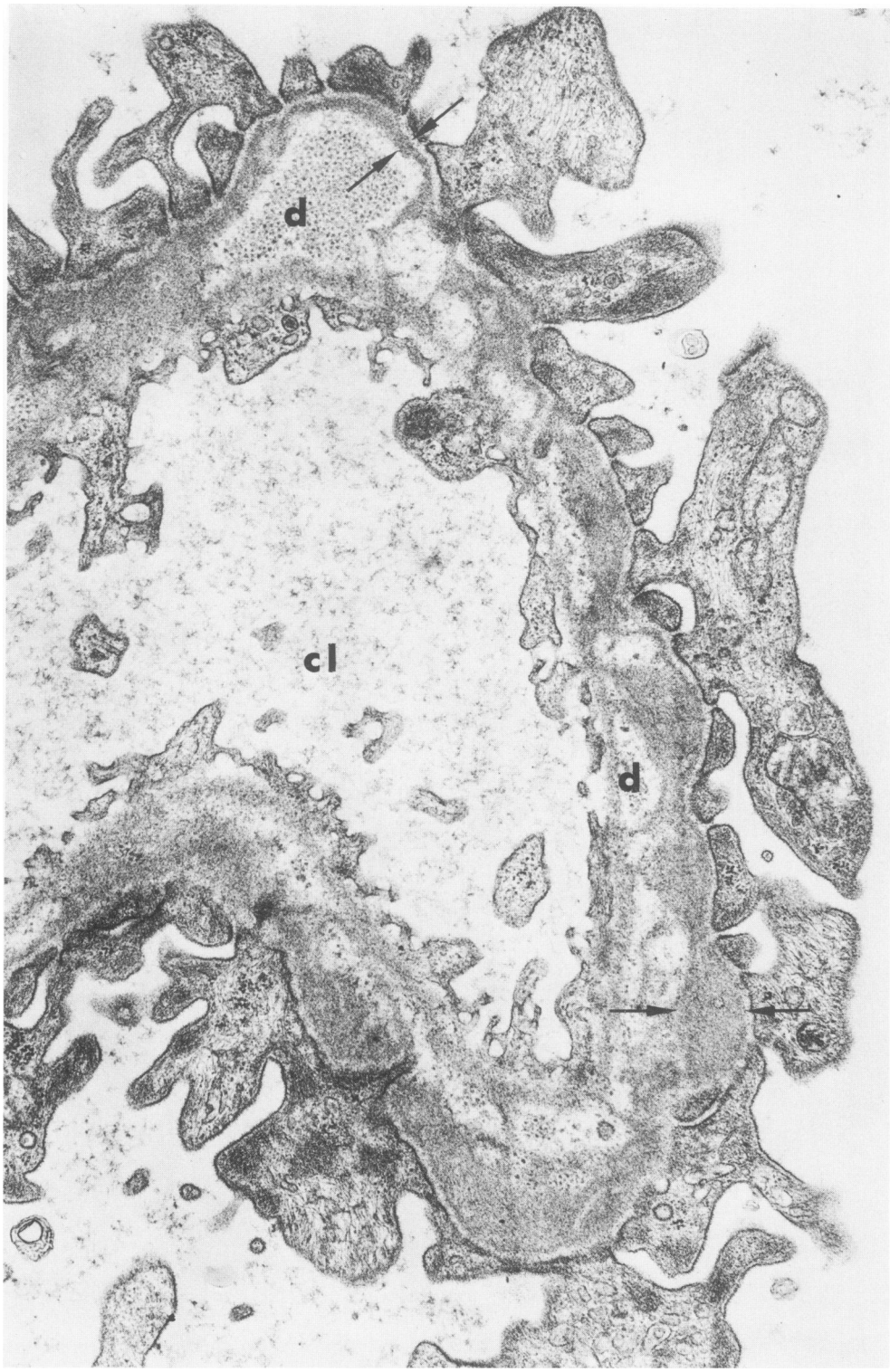


Fig 14—Case 8, 5 years after institution of gold therapy. The basement membrane is irregularly thickened. The deposits *(d)* are electron lucent and coarsely granular and are located near the inner surface of the basement membrane. They are externally covered by a continuous layer of basement membrane material of varying thickness *(arrows)*. *cl* = capillary lumen (× 22,000).