Gold Nephropathy Prototype of Membranous Glomerulonephritis

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In 7 of 10 kidney biopsies from patients with seronegative rheumatoid arthritis who had developed proteinuria during treatment with gold, electron microscopy showed changes typical of membranous glomerulonephritis. When the disease was of short duration, the only lesions seen were subepithelial deposits. The deposits were often located between intact epithelial foot processes and were demarcated externally by the slit membranes. In disease of longer duration, basement membrane changes occurred; these included projections and a layer of basement membrane over the deposits. The findings indicate that subepithelial deposits are primarily formed between intact foot processes, which would explain their unique discrete character (the basis of the typical granular immunofluorescent staining pattern of immune complex glomerulonephritis). The secondary basement membrane changes seem to evolve according to a constant pattern. The evolutionary process, probably signifying a healing process, is believed to be governed primarily by a synthesis of basement membrane performed by the epithelial cells (Am J Pathol 75:573–590, 1974).

TREATMENT OF RHEUMATOID ARTHRITIS (RA) with gold salts may result in renal complications, manifested clinically as proteinuria or nephrotic syndrome [1,2] and pathologically as membranous glomerulonephritis (MG) [2,3] or tubular necrosis.[4] Ultrastructurally, the glomerular lesions [2,3] are identical with other forms of MG in man [5] and in experimental animals.[6] In this study, gold nephropathy (GNP) refers only to those cases with gold-induced renal complications which ultrastructurally are characterized by MG. The term MG is used in a strictly ultrastructural sense to distinguish all glomerular disorders with abundant subepithelial or intramembranous deposits in the glomerular capillaries, with or without concomitant changes in the basement membrane proper.

It is commonly believed that MG is caused by circulating immune complexes,[5] although the exact pathogenetic mechanisms have been defined only in a minority of cases. Recently, the glomeruli of patients

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with GNP have been reported to contain granular deposits of immune globulins and complement along the capillary walls.[7] These findings give further support to the hypothesis that immune complexes are involved also in the pathogenesis of GNP.

Because the initiating factor is known, the maximum duration of GNP until the time of kidney biopsy can be assessed with great accuracy. Therefore, GNP offers a model for studying the changes in human MG in relation to time. A knowledge of the early changes is essential both for an understanding of the initial pathogenetic mechanisms and for a correct interpretation of the more complex capillary wall changes developing during later phases of MG. The purpose of this paper is to describe the ultrastructural changes in GNP and discuss their possible genesis with special emphasis on the very early changes, to make an attempt to classify the changes, and to discuss their causal relation to gold and RA.

Material and Methods

Patients

Ten patients, 9 women and 1 man, with RA of a few months' to several years' duration, were investigated because of signs of gold-induced nephropathy. The criteria for including patients in the group to be studied were: a) classic erosive RA, b) proteinuria beginning at various intervals after institution of gold therapy, c) no evidence of previous kidney disease, and d) no other plausible immediate explanation of the proteinuria (*eg*, streptococcal infection, amyloidosis, etc). The patients were carefully examined for any signs of other systemic diseases, notably systemic lupus erythematosus (SLE).

Kidney Biopsy

Percutaneous kidney biopsy with a Menghini needle was performed on each patient; the intervals after onset of proteinuria varied from 2 weeks to 6 years. (Table 1). A small part of the biopsy was set aside for immunofluorescent study (5 cases). The main part was rinsed rapidly in 1.5% cacodylate-buffered glutaraldehyde (0.1 M, pH 7.2) and then immersed in the same fixative for 1 or more days at 4 C. The biopsy cylinder was examined under a dissecting microscope for the recognition of glomeruli, and a small part, 1 to 2 cu mm, was separated for electron microscopic study. The tissue fragment was rinsed in 0.2 M cacodylate buffer containing 7% sucrose, minced into several pieces about ½ cu mm, postfixed in 2% *s*-collidine-buffered osmium tetroxide for 1.5 hour at room temperature, dehydrated in ethanol and embedded in Epon 812 according to a modification (Ladd Research Industries, Burlington, Vt) of the technic of Luft.[8] Uranyl acetate, 1%, was added to the last dehydrating bath for *en bloc* staining of the tissue. Ultrathin sections, cut on a LKB III ultramicrotome fitted with glass or diamond knives, were picked up on formvar-coated single hole grids (hole diameter, 1 mm) or uncoated 200-mesh grids, stained with lead citrate and uranyl acetate and examined in a Siemens Elmiskop I electron microscope at 80 kV.

From each patient, two to five representative glomeruli were studied. Where

Table 1—Summary of Patient Data

Case No.	Age/ Sex	Duration of RA prior to kidney biopsy	Gold therapy: Duration before onset of proteinuria	Gold therapy: Total dose (mg)*	Kidney biopsy: Time after beginning of gold therapy	Kidney biopsy: Glomeruli: EM stage	Kidney biopsy: Glomeruli: IF	Kidney biopsy: Glomeruli: LM	Rheumatoid factor†
1	52F	2 yrs	1 wk	10	2 wks	0	—	normal	pos
2	38F	1 yr	2 wks	90	5 wks	0	—	normal	neg
3	31F	12 yrs	1 mon	250	2 mons	0	negative	normal	neg
4	27F	1 yr	3 mons	430	5 mons	I	—	normal	neg
5	46F	1 yr	1½ mons	340	6½ mons	I	granular	normal	neg
6	40F	5 yrs	7 mons	310	9 mons	II	granular	normal	neg
7	43F	1½ yr	8 mons	300	11 mons	II	—‡	normal	neg
8	24F	1½ yr	1 yr	1000	14 mons	II	—	normal	neg
9	56M	17 yrs	2 mons	330	6 yrs§	III	granular	MG	neg
10	37F	5 yrs	9 mons	500	5 yrs§	IV	granular	normal	neg
11	36M	8 yrs	—	—	1 yr¶	0	—	normal	neg
12	24M	4 mons	—	—	4 mons¶	0	negative	normal	neg

* Myocrisine, May & Baker
† neg = Waaler-Rose <128
‡ Granular in second biopsy 10 months later
§ Proteinuria ceased gradually after gold therapy was discontinued
|| Time after onset of proteinuria
MG = membranous glomerulonephritis

glomerular changes were few or unevenly distributed, at least three glomeruli were studied.

Controls

Two patients with RA of 4 months' and 8 years' duration, respectively, and proteinuria of unknown cause who had received no gold therapy served as controls (Table 1). Their kidney biopsies were processed and examined as described above.

Results

Electron Microscopic Findings

The overall architecture of the glomeruli was normal. No proliferation, sclerosis or infiltration of the glomerular tufts by leukocytes was seen.

Membranous Glomerulonephritis

In 7 of the 10 patients treated with gold, capillary wall changes typical of MG were seen. The cases were classified into four different stages according to the following criteria.

Stage I (Cases 4 and 5, Figure 1). The external surface of the base-

ment membrane was smooth. On it were seen few or many hemispherical or wedge-shaped electron-dense deposits. There were no projections from the basement membrane on either side of the deposits. Mostly, the deposits were separated from the lamina densa of the basement membrane by a clearly visible lamina rara externa. The terminal (foot) processes were in many places focally lost ("fused") over the deposits.

Stage II (*Cases 6, 7 and 8, Figure 2*). The deposits were of the same size, shape and electron density as in stage I. However, the external surface of the basement membrane had lost its normal smoothness because of small projections extending towards the epithelial cells on either or both sides of the deposits. The terminal processes and the lamina rara externa were principally as in stage I.

Stage III (*Case 9, Figure 3*). The deposits were deeply embedded between tall projections ("spikes") from the basement membrane. As a result, the external contour of the basement membrane was toothed or wavy and the basement membrane was clearly thickened. The terminal processes were lost over the deposits. A few deposits were in fact intramembranous, as they were covered externally and separated from the epithelial cells by a thin (newly formed?) layer of basement membrane. These intramembranous deposits were mostly electron dense and finely granular, like the subepithelial deposits. A few of them, however, were coarsely granular and rather electron lucent (see stage IV). A lamina rara externa was not discernible between the deposits and the lamina densa. When the deposits were covered with a continuous layer of basement membrane, the lamina rara externa was seen on the epithelial side of that layer.

Stage IV (*Case 10, Figure 4*). The "deposits" were exclusively intramembranous, electron lucent and coarsely granular, suggesting that the deposited material had been partially resorbed. The basement membrane was irregularly thickened or greatly attenuated in places with deposits. A lamina rara externa of normal width ran along the periphery of the basement membrane. Whereas Figure 4 shows a segment with total loss of terminal processes, it must be emphasized, however, that the terminal processes were mostly intact and delicate over the segments with deposits.

Frequency of Deposits

In all four stages the number of deposits varied. They usually occurred in clusters, many capillary loops or the greater part of the glomerulus being totally free from deposits. In some cases it was necessary

to make a thorough examination of two or three glomeruli before deposits were found. We did not make any attempt to quantify the findings.

Relationship Between Deposits and Epithelial Cells

In many places the terminal processes of the epithelial cells were intact over the subepithelial deposits (Figures 8–13 and 15). In these places the slit membranes between the terminal processes, when discernible, demarcated the deposits externally (Figures 8–12). Thus the slit membranes were pushed out from their normal location in the vicinity of the basement membrane. Occasionally, a deposit was in direct contact with the urinary space through a wider gap between the terminal processes, suggesting a disruption of the slit membrane. In a few places the terminal processes covering the deposits were joined by tight junctions or desmosomes (Figure 15). In stages I and II the lamina rara externa sometimes contained small granules, measuring 400 to 1000 Å in diameter (Figure 14). They were of the same texture and electron density as the larger subepithelial deposits. The granules probably represented early small deposits.

Clinicopathologic Correlations

Early Lesions (Stages I and II). In this group, the time interval between the first gold injection and the day of kidney biopsy averaged 9.5 months. Stages I and II taken separately, the corresponding intervals were 5.7 and 11.3 months. We considered it more significant to correlate the different morphologic stages with the above exact parameters, rather than with the onset of proteinuria, in the first place because urinalyses were not done frequently enough, and in the second place because it is uncertain whether early glomerular abnormalities always manifest themselves by proteinuria.[6]

Late Lesions (Stages III and IV). In this group the described glomerular lesions occurred at an average of 5.5 years after the beginning of gold therapy and at an average of 5 years after the first clinical signs (proteinuria) of glomerular disease.

Stage 0

The changes described below were seen in preparations from all 10 gold-treated patients as well as in the 2 controls. The cases assigned to this stage, however, did not show any changes characteristic of MG.

The changes consisted of irregular focal thickening of the internal—subendothelial—part of the basement membrane (Figures 5 and 6).

The thickened parts were slightly lighter than the lamina densa and often contained small granular and fibrillar structures. This type of change was possibly more common in the gold-treated cases than in the controls. In patients with MG the subendothelial changes were not confined to places with deposits but seemed to occur independently.

Virus-like Particles

In case 5 (stage I) a capillary lumen contained remnants of a necrotic (lytic) cell, probably a leukocyte. Among the cellular debris were many round particles, consisting of a finely granular electron-dense core surrounded by a triple-layered membrane (Figure 7). The particles were of uniform size and measured 1400 to 1700 Å in diameter.

In case 9 (stage III) the endothelial cells contained numerous intracytoplasmic filamentous structures reminiscent of viral nucleocapsids.[9] Similar structures were seen in the endothelial cell cytoplasm of interstitial capillaries. They were not found in other cases.

Immunofluorescence Findings

In Patients with Membranous Glomerulonephritis

Tissue for immunofluorescent microscopic study was available from 4 of the 7 patients with electron microscopically demonstrable MG. In all cases the glomeruli stained intensively with antisera against IgG and C3 (Figure 15). The staining pattern was fine granular along the capillary walls. The majority of the glomeruli stained diffusely all over the capillary tuft. In many glomeruli, however, only a part or segment of the capillary tuft stained positively, thereby closely mimicking the often focal distribution of the ultrastructural changes. In a fifth case (case 7), tissue for immunofluorescent study was not available from the initial biopsy, but a second biopsy obtained 10 months later showed the same fine granular pattern (Figure 16).

In Patients with Stage 0 Changes

Tissue from 2 patients (1 gold-treated and 1 untreated) was available. In both cases the glomeruli stained negatively for IgG and C3.

Discussion

The glomerular capillary wall lesions described in stages I to IV are in all respects identical with other forms of MG.[5,6] Subepithelial deposits, morphologically identical with those in GNP, seem to constitute a characteristic ultrastructural feature of glomerulonephritis induced by immune complexes.[10] Therefore, our ultrastructural findings,

together with the immunofluorescent findings,[7] may be taken to indicate that complexes of immunoglobulins and complement are involved also in the pathogenesis of this type of MG.

Morphogenesis of the Membranous Lesions

When the time interval between the first gold injection and the day of kidney biopsy was short, the only lesions seen were subepithelial deposits. The basement membrane was normal. When the time interval was longer, concomitant basement membrane changes occurred which were more pronounced the longer the time interval. The basement membrane changes were always distributed locally along the basement membrane (*ie*, no diffuse thickening) and were always located in the vicinity of deposits. From the above it seems evident that a) the basement membrane changes were formed secondarily to and as a result of the deposits, and b) the material forming the deposits (immune complexes?) primarily was deposited focally (not linearly). The first hypothesis is supported by previous studies of human and experimental glomerulonephritis,[5,6,11–13] which have indicated that subepithelial deposits in the course of time become incorporated in the basement membrane. The second concept gains support from studies of the filtration properties of the glomerular capillary wall. It has been shown that the slit membranes between the terminal processes of the epithelial cells constitute a final barrier for the glomerular filtrate.[14,15] Consequently, it may be assumed that immune complexes primarily are deposited in the spaces delimited by the terminal processes, the lamina rara externa and the slit membranes, a view which gains support from our findings (Figures 8–15) and from experimental studies with ferritin immune complexes as well.[16] The ferritin immune complexes were, it is true, located within the lamina rara externa, whereas our findings suggest that the material forming the deposits often had traversed this space (Figures 1, 2, 10, 12 and 13).

Our frequent observations of deposits covered by intact terminal processes show that leakage of protein through the basement membrane is not invariably associated with loss ("fusion") of terminal processes. Furthermore, when loss of terminal processes occurred, the phenomenon was usually only focal and confined to places with deposits, suggesting a focal (direct or indirect) effect of the deposited material on the cytoplasm of the epithelial cells. Recently, Arakawa and Tokunaga [17] have presented conclusive evidence that the so-called fusion in fact is a process of swelling and retraction of the terminal processes. The causes of the loss (retraction) of terminal processes re-

main obscure. If it is assumed that the epithelial cells normally are occupied both in checking excessive loss of protein through the capillary wall [17] and in synthesizing basement membrane,[18,19] these two functions might compete with each other metabolically. Retraction of terminal processes might signify an attempt of the epithelial cells to increase their over all metabolic capacity. In GNP (and other forms of MG), an enhanced synthesis of basement membrane material (see below) might overload the total metabolic capacity of epithelial cells and result in proteinuria and/or retraction of terminal processes. The formation of true desmosomes (Figure 15) between terminal processes has not been described in MG previously. The significance of this phenomenon is not clear, although it seems to be intimately related to the disarrangement of the terminal processes caused by the deposited material.

The genesis of the basement membrane changes (stages II to IV) could be explained in two ways, both based on the assumption that epithelial cells synthesize basement membrane.[18,19] The first would presume a locally increased synthesis of basement membrane material in association to the deposits based on some kind of stimulating effect exerted upon the epithelial cells by the deposited material. Epithelial cells have been shown to synthesize basement membrane in response to various kinds of injury.[19] Immune complexes, on the other hand, are known to exert a local injurious effect, inter alia through activation of the complement system, chemotaxis of polymorphonuclears and release of lysosomal enzymes.[20]

Alternatively, the deposited material might have no specific stimulating effect upon the epithelial synthesis of basement membrane. Instead, one may assume that the mere interposition of deposits between the basement membrane and the epithelial cells might interfere mechanically with the incorporation, into the basement membrane proper, of the basement membrane material which is thought to be continuously synthesized by the epithelial cells all along the capillary wall. This interference would result in local defects as projections and ultimately in a new layer of basement membrane material covering the deposits. If the mechanism were the latter, then one would expect similar changes in response to every kind of inert material accumulating for sufficient time in the subepithelial space (*eg*, globin [21]).

In the light of the mechanisms discussed, the evolution of the membranous lesions would be as follows: a) Deposition of immune complexes in the subepithelial spaces between the terminal processes (variably associated with retraction of terminal processes); b) Develop-

ment of projections (spikes) as a result of either locally increased synthesis of basement membrane or locally prevented incorporation of normally synthesized basement membrane; c) External covering of the deposits by a newly formed layer of basement membrane; d) Gradual incorporation (and disappearance?) of the deposits.

We must emphasize that this evolutionary sequence is proposed for cases of MG where the precipitating factor (in our case, gold) can be eliminated at the very beginning of the disease. In the majority of patients with MG, the precipitating factors are unknown. In these patients it is conceivable that deposition of immune complexes over longer periods of time results in superimposed deposits and more complex basement membrane changes than outlined above.

Classification

Previous classifications of MG have been based on the appearance of the glomeruli in the electron microscope and, to a certain degree, in the light microscope also.[5,12,22] These classifications have indicated, quite correctly we feel, a positive correlation between the degree of basement membrane thickening and the clinical progression of the disease. Indirectly, however, the impression has been gained that a basement membrane of normal thickness (in light microscopy) is synonymous with early disease. Our findings suggest that the whole evolutionary course from the primary formation of deposits to their incorporation into the basement membrane may occur without light microscopically detectable thickening of the basement membrane. Then the features of the deposits seen in the electron microscope, *ie*, location with respect to the basement membrane, texture, etc, will provide the only clue to the evolutionary stage of the disease. We believe that these considerations are of great prognostic importance. They emphasize the need for electron microscope studies, in addition to light microscopy and immunofluorescence, in all cases of MG.

The Role of Gold

Evidence along three lines incriminates gold salts, and not the rheumatic disease, as the primary cause of the membranous lesions (stages I to IV). a) The time relationship between gold treatment and the appearance of proteinuria was too close to be purely coincidental. b) Similar deposits and basement membrane changes were not encountered in the two rheumatics who had received no gold therapy (further ultrastructural data on this interesting subject are lacking).

c) Identical membranous lesions can be induced in rats after injections of gold salts.[23]

The mechanism by which gold salts may initiate the formation of immune complexes is unknown. One possibility is that gold may alter some tissue constituent, rendering it autoantigenic. It is known, for example, that gold salts in high concentration cause injury to the epithelial cells of kidney tubules.[23,24] In this context, our demonstration by immunofluorescence of abundant deposits of immune globulins and complement in the epithelium and basement membrane of kidney tubules are of some interest (discussed more in detail in a separate paper[25]). Another possible mechanism is that gold reacting with immunoglobulins[26] may simply cause aggregation with subsequent deposition of these complexes.

The Role of Rheumatoid Arthritis

The observations by Nagi *et al*[23] indicate that gold salts alone are capable of producing nephritogenic immune complexes. That is not to say that the primary rheumatic disease might not in some way influence the development of GNP. Tests for rheumatoid factor (RF) showed normal values in all our patients with GNP. Since the completion of this study, we have investigated about ten more patients with GNP. They, too, have shown normal RF titers. RF, as detected by agglutination of immunoglobulin-coated sheep red cells, is a globulin of IgM class with antiglobulin properties directed against IgG antibodies, which presumably are complexed with antigens.[27] The following hypothesis could be constructed: If RF could interact with the nephritogenic complexes that seem to develop during gold therapy, the resulting aggregates might be large enough to be eliminated by the RE system, or they might be too large to penetrate the glomerular capillary basement membrane and instead be shunted into the mesangium.[28] The absence of RF, on the other hand, would permit small-sized complexes to penetrate the basement membrane and cause diffuse injury. Experimental work has indicated that complexes of small size are the most apt to produce diffuse capillary wall lesions, larger complexes being taken up by the mesangial cells.[28]

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[*Illustrations follow*]

Legends for Figures

Figs 1 to 4—Illustrations of the four different stages of gold-induced membranous glomerulonephritis.

Fig 1—Stage I. The external surface of the basement membrane (*bm*) is smooth without projections. The subepithelial deposits (*d*) are separated from the lamina densa by a narrow light zone (*arrow*), probably representing the lamina rara externa (Case 5, × 21,000).

Fig 2—Stage II. The deposits (*d*) are superficial. Small projections (*arrows*) protrude from the basement membrane (*bm*) on either or both sides of the deposits. A narrow light zone is partly preserved between the deposits and the lamina densa (Case 8, × 20,000).

Fig 3—Stage III. The deposits (*d*) are deeply embedded between tall projections (*arrows*) from the basement membrane (*bm*). The thickness of the basement membrane is about twice the normal (Case 9, × 21,500).

Fig 4—Stage IV. Inside the basement membrane (*bm*) are many electron-lucent areas (*d*), probably representing partially resorbed deposits. The deposits are externally covered by a continuous layer of basement membrane material (*arrows*) (Case 10, × 20,500).

Fig 5—Stage 0. The internal part of the basement membrane (*bm*) is irregularly thickened (*arrow*) and contains thin fibrillary structures (Case 8, × 21,500).

Fig 6—Stage 0. Larger magnification illustrating the subendothelial thickening of the basement membrane (*bm*). The swollen parts contain granular and fibrillar structures. *ld* = lamina densa (Case 2, × 42,000).

Fig 7—Portion of a destroyed cell, probably a leukocyte, filling the lumen of a glomerular capillary. Among the cytoplasmic organelles are many round virus-like particles (*arrows*) (Case 4, × 35,000).

Figs 8 to 13—Illustrations of subepithelial deposits located between intact terminal processes of epithelial cells.

Fig 8—Typical deposit of stage I occupying the triangular space delimited by two terminal processes and the basement membrane (Case 5, × 59,000).

Fig 9—The deposit has "pushed" the slit membrane (*arrow*) externally (Case 4 × 43,500).

Fig 10—Two subepithelial deposits have dislocated the terminal processes laterally and the slit membrane (*arrow*) externally. The terminal processes covering the left deposit seem to be connected by fine fibrillary structures (*arrows*). A light zone, probably the lamina rara externa, separates the deposits from the lamina densa (Case 4, × 36,000).

Fig 11—The slit membrane (*arrow*) is clearly visible on the top of the deposit. The lamina rara externa (*arrows*) appears to run external to the deposits (Case 8, × 59,000).

Fig 12—Large subepithelial deposit laying under swollen cytoplasm of epithelial cell. The slit membrane (*arrow*) is dislocated to the left (Case 8, × 36,000).

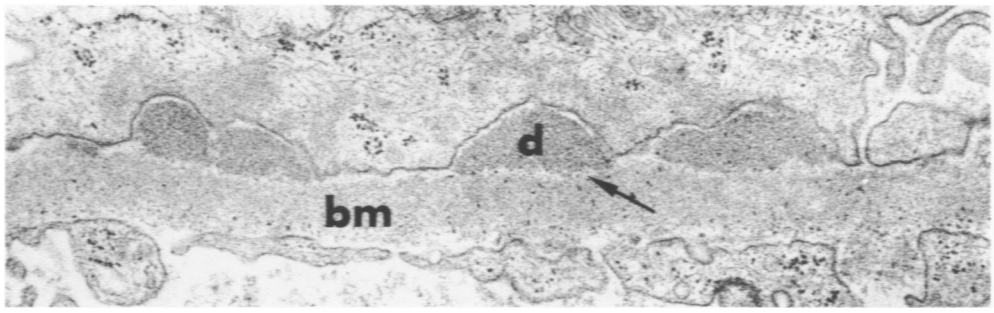
Fig 13—Subepithelial deposit extending between terminal processes of epithelial cells. The light zone under the deposit (*arrow*) probably represents the lamina rara externa (Case 4, × 49,500).

Fig 14—Many small granules (*arrows*), probably representing early deposits, are seen within the lamina rara externa of the basement membrane (*bm*). The places of the slit membranes are indicated by small dark knots in the cytoplasmic membranes of the epithelial cells (Case 5, × 30,000).

Fig 15—A Subepithelial deposit (*d*) covered by two cytoplasmic processes which are joined by a desmosome (*broad arrow*). Projections (*thin arrows*) from the basement membrane protrude on both sides of the deposit (Case 7, × 31,000).

Fig 16—Fluorescence preparation of a glomerulus stained with anti-IgG. There is diffuse fine granular deposition of IgG along the capillary walls (Case 5, × 470).

Fig 17—Part of a glomerulus, stained with anti-C3. Note the typical segmental distribution of deposits of C3 (Case 7, second biopsy, × 440).



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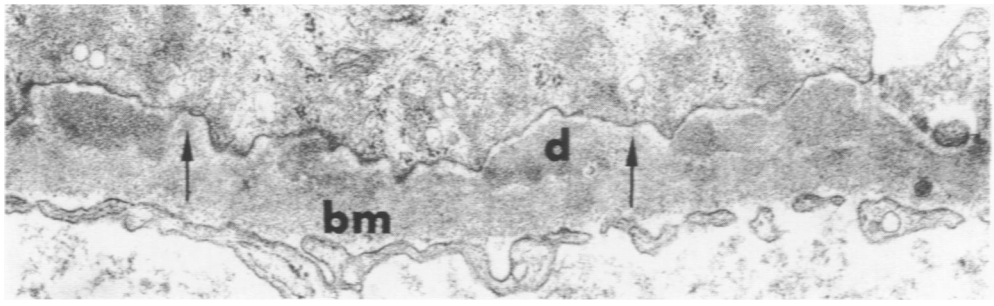

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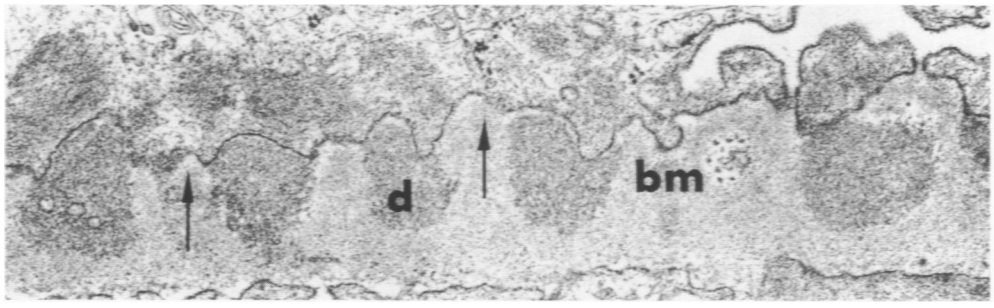

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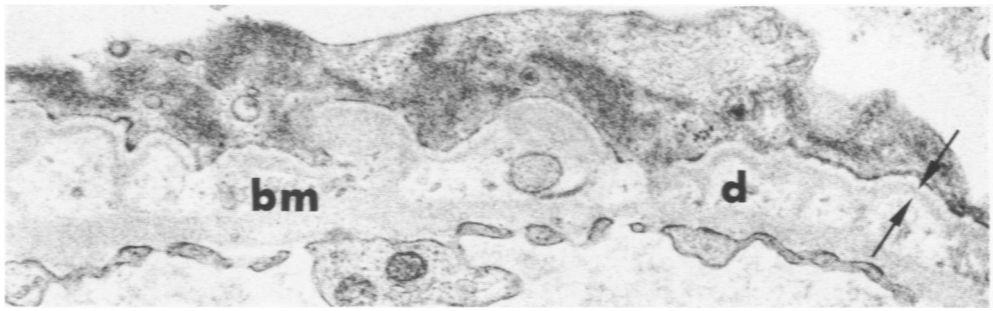

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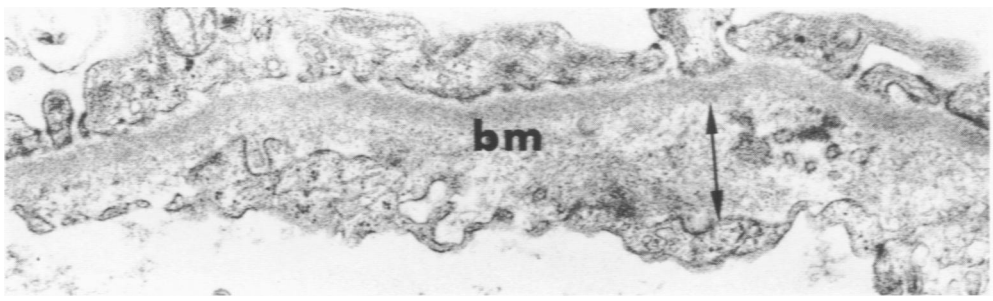

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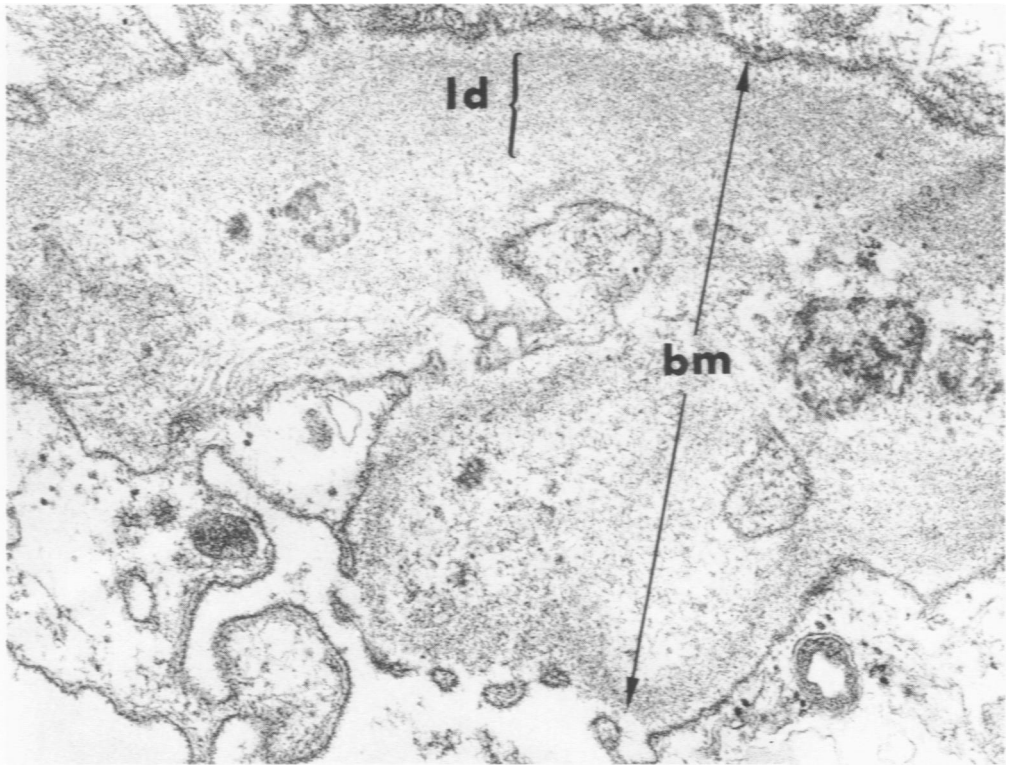

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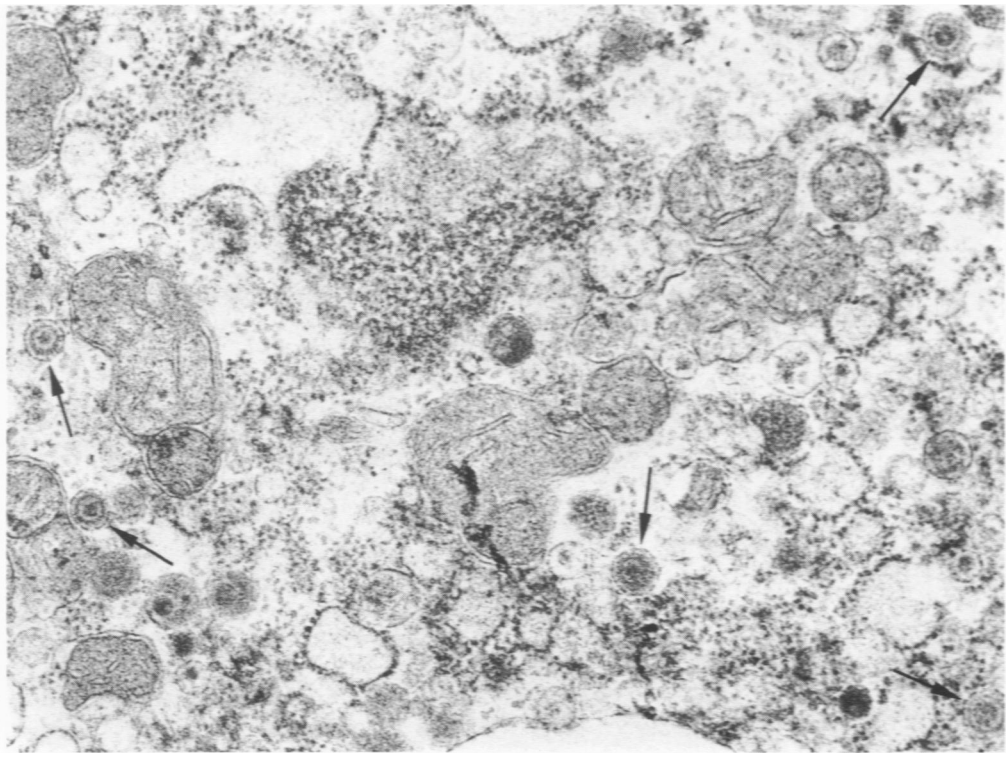

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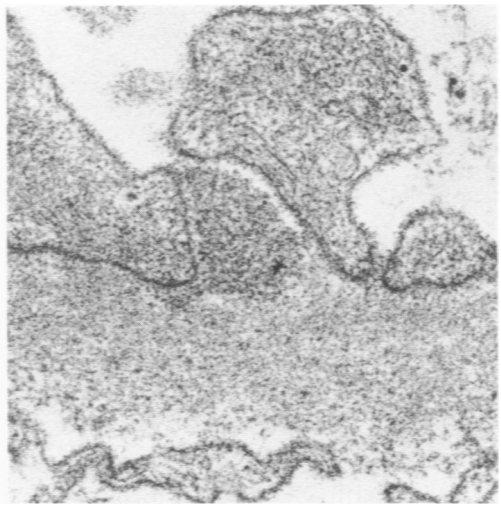

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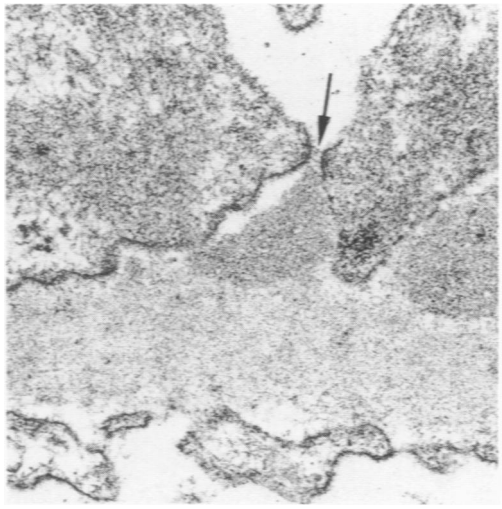

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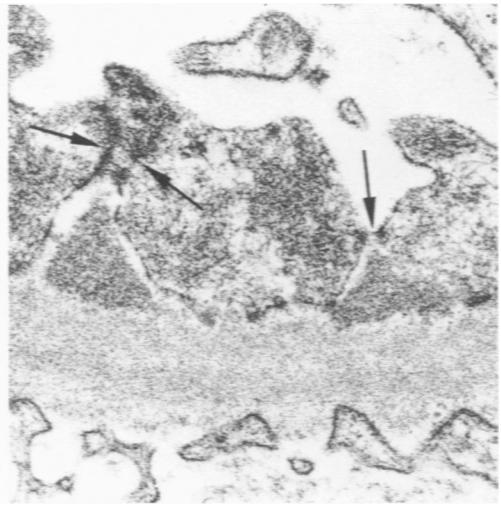

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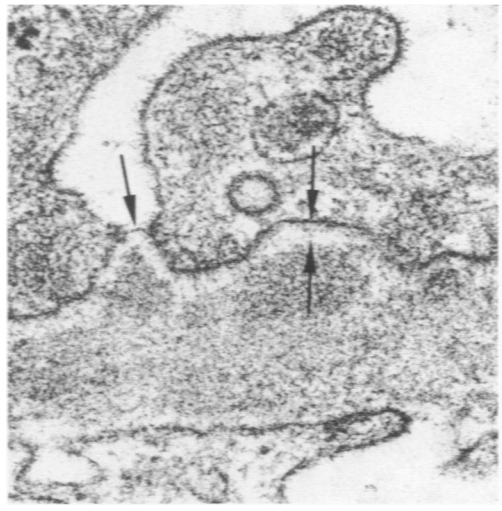

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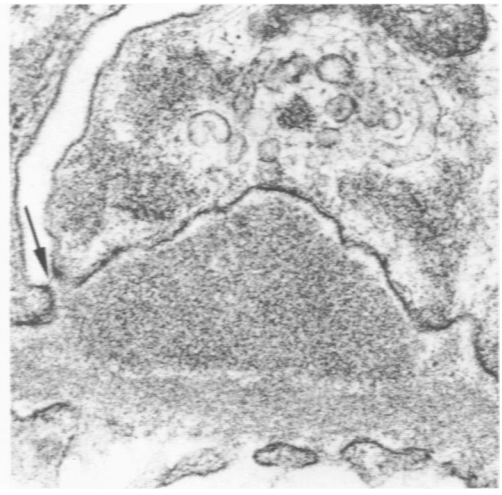

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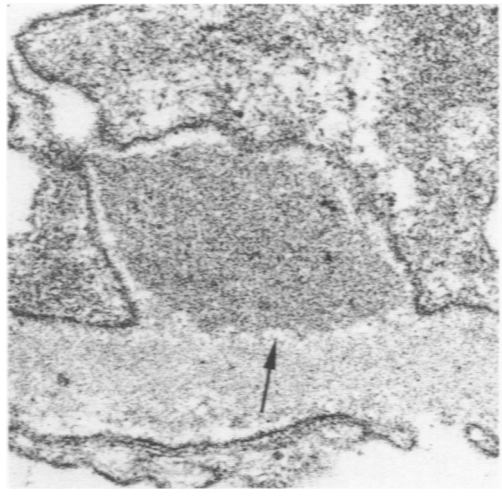

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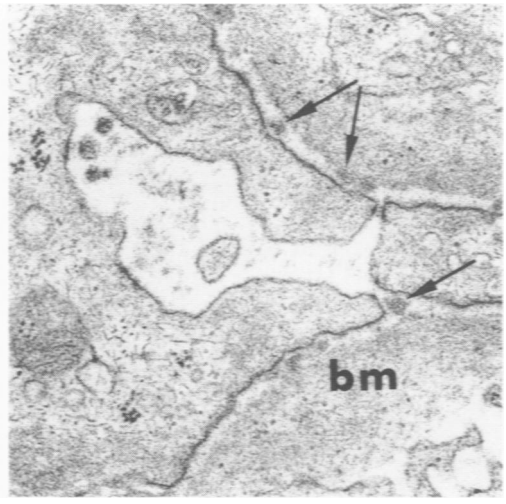

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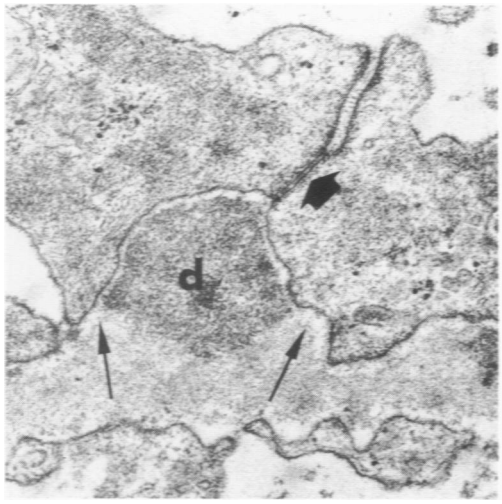

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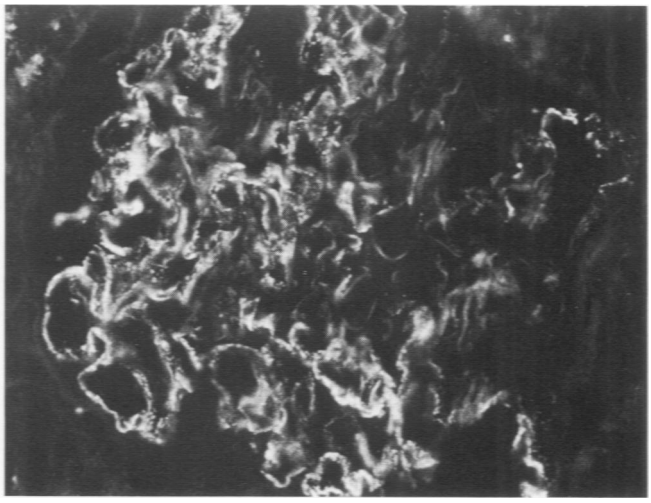

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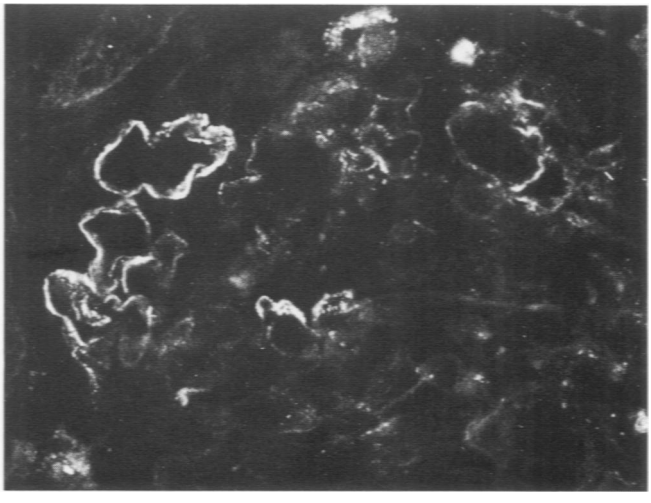

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