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Early Glomerular Lesions in Amyloidosis

Electronmicroscopic Findings

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Summary. Our investigations on early glomerular changes in renal amyloidosis showed the following:

1. In some renal biopsies, amyloid was demonstrated in the mesangial matrix predominantly and could be seen penetrating through the basememt membrane of the mesangial region into the subepithelial space of adjacent glomerular capillaries.

2. In other biopsies, showing the same severity of amyloidosis, deposits were demonstrated in the mesangium and in the capillary walls distant from the mesangium on both sides of the basememt membrane. There was no apparent connection between the deposits in these two areas.

3. On the basis of the morphological changes found in the cytoplasm of the mesangial cells, the glomerular epithelial cells and partly also in the endothelial cells, supported by our electron microscopic findings in the immediate vicinity of these cells, we come to the conclusion that amyloid in the glomerulus is formed from amyloid precursors brought via the blood stream.

4. Amyloid fibrils may be formed in the extracellular space of the glomerulus under the influence of lysosomal enzymes released from epithelial, mesangial and perhaps endothelial cells, by action of these enzymes on extracellularly deposited amyloid precursors.

Key words: Renal amyloidosis – Early lesions – Amyloid by electron microscopy

Introduction

In the past reports of histological changes in glomerular amyloidosis were predominantly from patients with advanced disease. Only a few exist in which

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early glomerular lesions are discussed (Hinglais 1964; Shirahama and Cohen 1967; Jao and Pirani 1972; Schneider and Thoenes 1977). This is probably due to the fact that subtle changes in the glomeruli in the early stage of amyloidosis are easily overlooked on routine staining. Not infrequently therefore, the diagnosis of minimal change lesion with the nephrotic syndrom (NS) is made. As early lesions may possibly contribute to the knowledge of the pathogenesis of amyloidosis, we would like to discuss the earliest changes caused by amyloid deposits and speculate on their formation.

Material and Method

Investigations were carried out on 25 biopsies from patients with grade I renal amyloidosis (classification according to Mackensen et al. 1977). Some cases were only discovered because all biopsies from patients with the NS obtained over the past few years were investigated electron-microscopically. Watanabe and Saniter (1975) demonstrated on our biopsy material, that early stages of amyloidosis are accompanied by the NS in 57% of cases.

Amyloid was demonstrated either from paraffin sections stained with Congo red or as amyloid fibrils in ultra-thin sections under the electron-microscope. The biopsy material was fixed in formaldehyde, postfixed in osmiumtetroxide, stained in alcoholic uranyl acetate and embedded in Araldite after dehydration. Sections of approx. 0.01 μ were cut and stained with lead citrate. Using a Siemens Type 102 electronmicroscope, we examined the glomerular capillary network for amyloid deposits and searched for early changes in the region of the mesangium and in the capillary wall distant from the mesangium.

Results

In early amyloidosis we found two main patterns of distribution of amyloid. In 11 cases the amyloid fibrils were deposited mainly in the mesangium (Fig. 1) but not in all regions. When the amyloid deposits were small, they were usually found in that part of the mesangium facing the capillary lumen. With increasing amounts of amyloid fibrils, larger areas of the mesangial matrix became infiltrated and loosened. Occasionally only a diffuse loosening of the mesangial matrix was noted, without demonstration of amyloid fibrils (Fig. 2). With increasing amounts of amyloid, the mesangium became widened and the mesangial cell cytoplasm diminihed in amount. Amyloid fibrils in the mesangium showed a marked tendency to penetrate through the glomerular basement membrane (BM) into the subepithelial space (Fig. 1). The epithelial cell processes overlying the infiltrated BM were always lost. With increasing amounts of subepithelial amyloid, the cytoplasm appeared more swollen. The subepithelial amyloid fibrils were often arranged in a conical fashion and tended to invaginate the epithelial cell cytoplasm. Occasionally one could demonstrate that these amyloid cones had broken through the epithelial cell cytoplasm into the urinary space (Fig. 2, Fig. 7d). In the presence of the NS, sections of BM infiltrated with amyloid were frequently seen to be completely denuded of their epithelial covering. On the urinary side of the BM in these areas, the residues of the conical amyloid structures were often demonstrated. In cases with amyloid deposits predominantly located in the mesangium, the walls of the capillaries distant from the mesangium appeared unchanged and showed normal epithelial foot processes, even in the presence of the NS (Fig. 1).

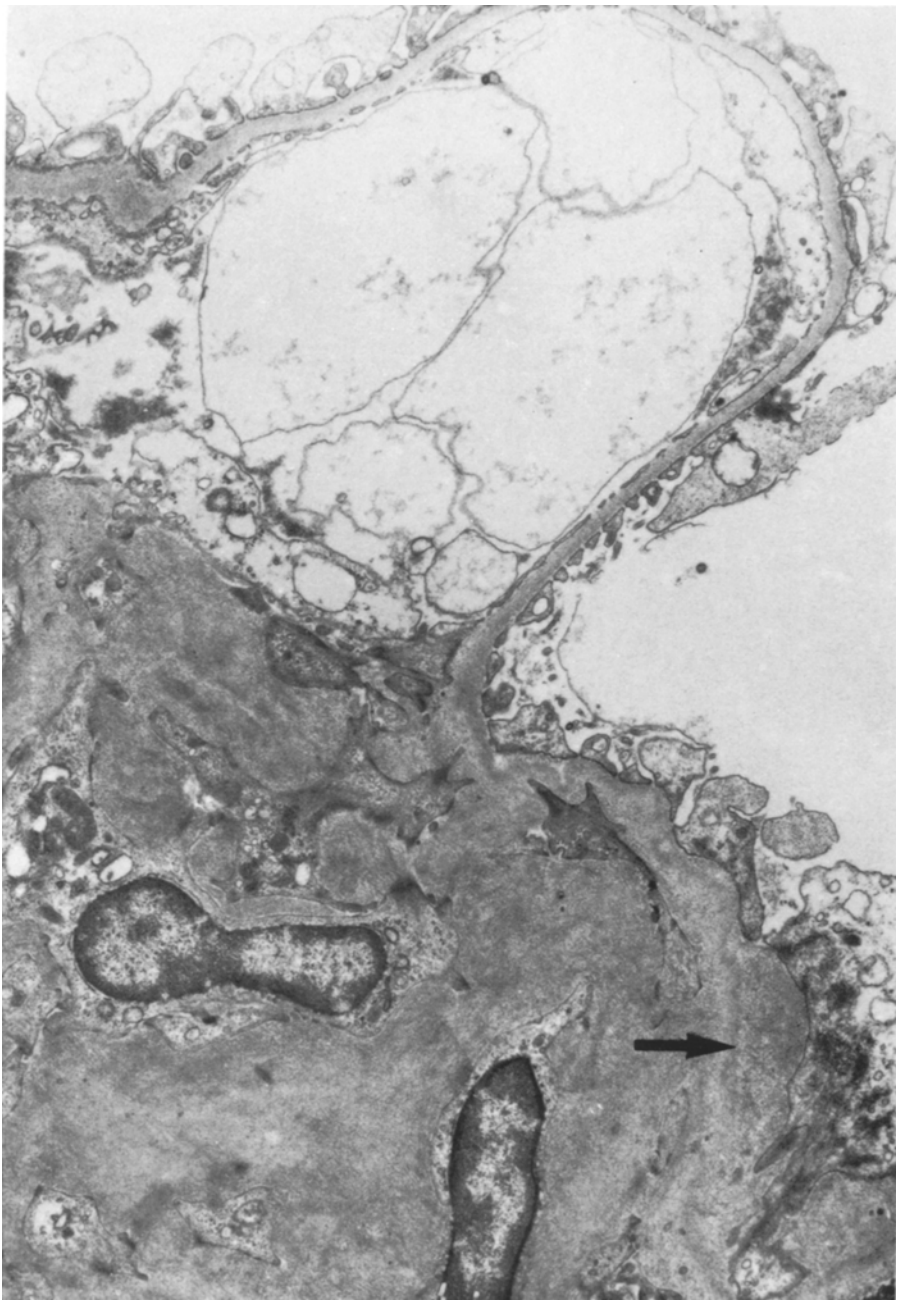

Fig. 1. Grade I renal amyloidosis with amyloid deposition predominantly in the mesangium. Capillary loop free of amyloid, basement membrane and podocyte pedicels normal. Reduced cytoplasm in relation to the nucleus of the mesangial cell. Loss of podocyte pedicels where amyloid is demonstrable in the subepithelial space (↗). 7,500:1

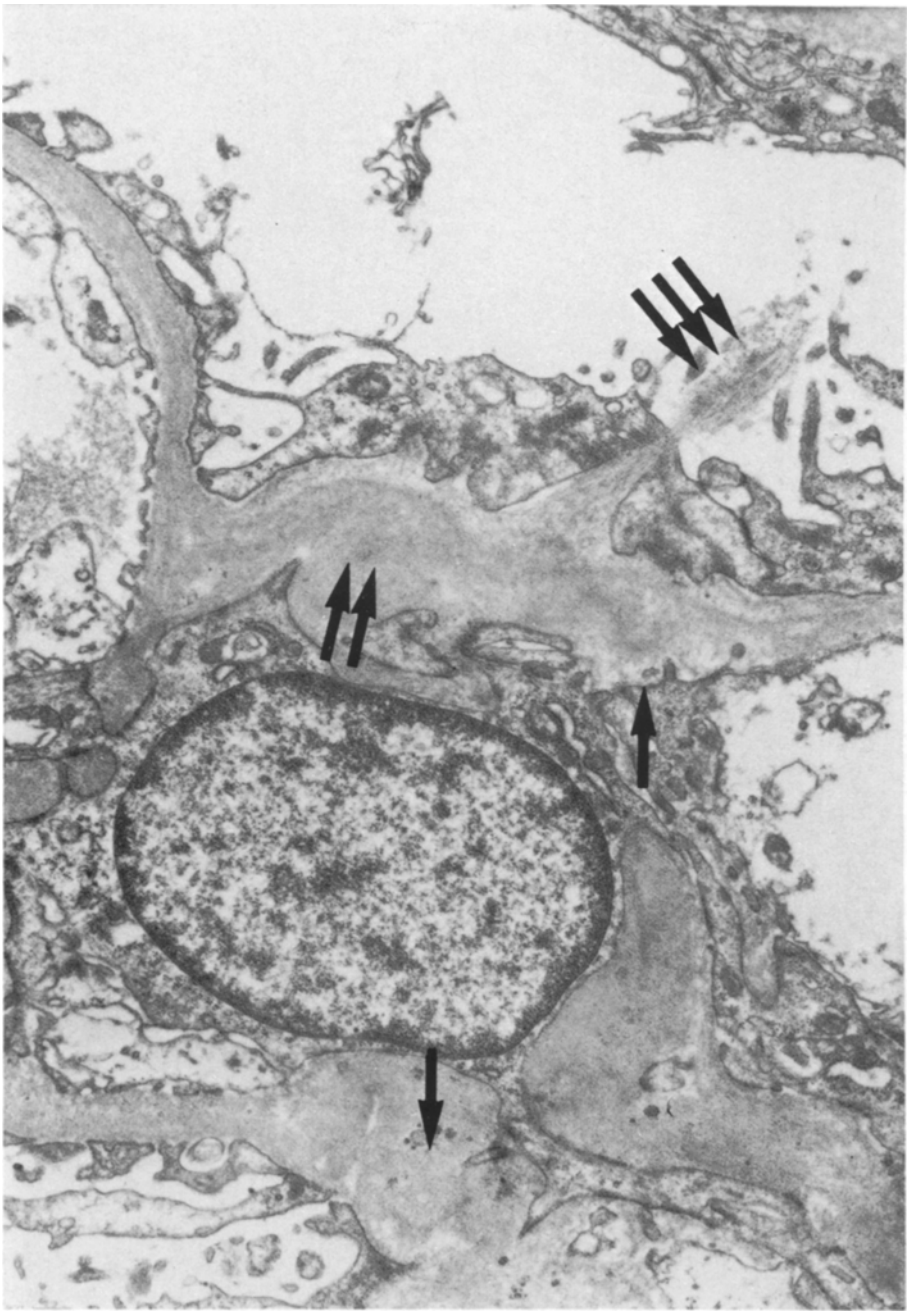

Fig. 2. Mesangial area with loosening of the structure of the mesangial matrix (↗), partly interspersed with amyloid fibrils (↗ ↗). Cone-shaped invagination of the epithelial cytoplasm by straightened amyloid fibrils which have broken through into the urinary space (↗ ↗ ↗). 15,000:1

In contrast to those patients showing amyloid deposits mainly in the mesangium, the 14 remaining cases had in addition disseminated amyloid deposits in the capillary walls distant from the mesangium (Fig. 3). In these patients the BM of these vessels revealed a segmental loosening of the subendothelial BM area, characteristic of very early involvement (Fig. 4). Later changes consisted of minute subendothelial and subepithelial accumulation of amyloid fibrils. With increasing number of fibrils in the subendothelial areas, one could follow their penetration through the lamina densa into the subepithelial space. The adjacent epithelial cells showed a loss of their foot processes dependant on the extent of the BM damage; a change which is also seen in the mesangial region (Fig. 4, Fig. 5). Between the cytoplasm and the amyloid-infiltrated BM, electronlucid and loosened subepithelial zones which contained varying amounts of amyloid fibrils were seen (Fig. 5). Small areas contained fewer fibrils than large ones. In these loosened zones, the ratio of amyloid fibrils to matrix grew with increasing size of the involved area. In these areas of the capillaries with disseminated amyloid deposits, the lamina densa showed loosening when infiltrated with amyloid fibrils.

Areas in which the BM of the glomerular capillaries was most severely affected by amyloid deposits and denuded by its epithelial covering often showed a markedly thinned or loosened lamina densa only. The amyloid masses, which had broken into the urinary space, often still showed densely packed fibrils at their base, pointing to their previous epithelial cell invagination.

The epithelial cells in the region of the subepithelial amyloid deposits showed an electron dense cytoplasm in addition to the loss of their foot processes (Fig. 4, Fig. 5). Apart from an increased number of intracellular vesicular structures and free ribosomal elements, no morphological signs of an elevated metabolic turnover of the epithelial cell cytoplasm in the region of amyloid deposits was seen. When one epithelial cell was found to cover an area of both normal glomerular BM and one that was infiltrated with amyloid, then the foot processes overlying the normal area were preserved but were lost in the other area (Fig. 6). The cytoplasm of the epithelial cell was electron-dense and rich in intracellular vesicles and ribosomal elements only where it was in close contact with amyloid fibrils or adjacent to zones of the loosened BM. In amyloid interspersed areas of the BM where there was denudation of the epithelial covering, fragments of epithelial cell processes could be seen occasionally. In the regions of loosened or amyloid infiltrated mesangial matrix, the mesangial cells demonstrated an increased number of intracellular vesicles and a dense network of microfilaments near the cell membrane (Fig. 7a). Cytoplasmic membrane invagination of mesangial cells was a rare finding. In mesangial areas with severe amyloid infiltration, we found a change in the ratio of cell nucleus to cytoplasm caused by redution of the cytoplasm (Fig. 1). In some places the cytoplasmic membrane of the mesangial cells had disappeared completely and cell organelles could be seen in between the surrounding amyloid (Fig. 7c).

In contrast to the changes described in the epithelial and mesangial cells, no changes indicative of an increased metabolic turnover could be detected in the endothelial cells adjacent to areas of loosened BM or subendothelial amyloid deposits. The endothelial cell cytoplasm appeared neither swollen nor

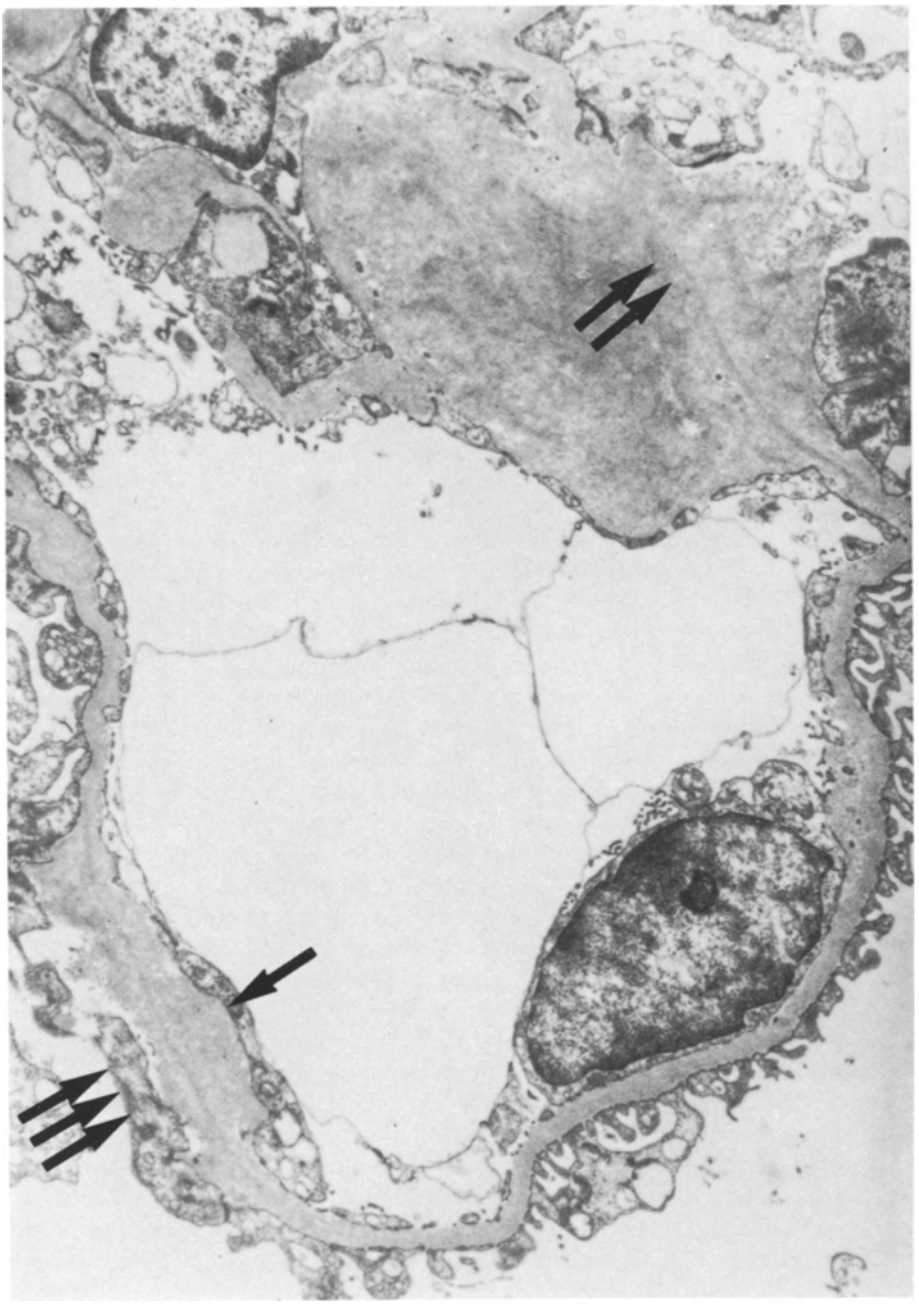

Fig. 3. Grade I renal amyloidosis with amyloid deposition in the mesangium and in the circumference (↗) of the capillary loops. Area of the BM interspersed with amyloid and denuded of epithelial covering (↗ ↗). Podocyte process already detached from the BM (↗ ↗ ↗). 7,500:1

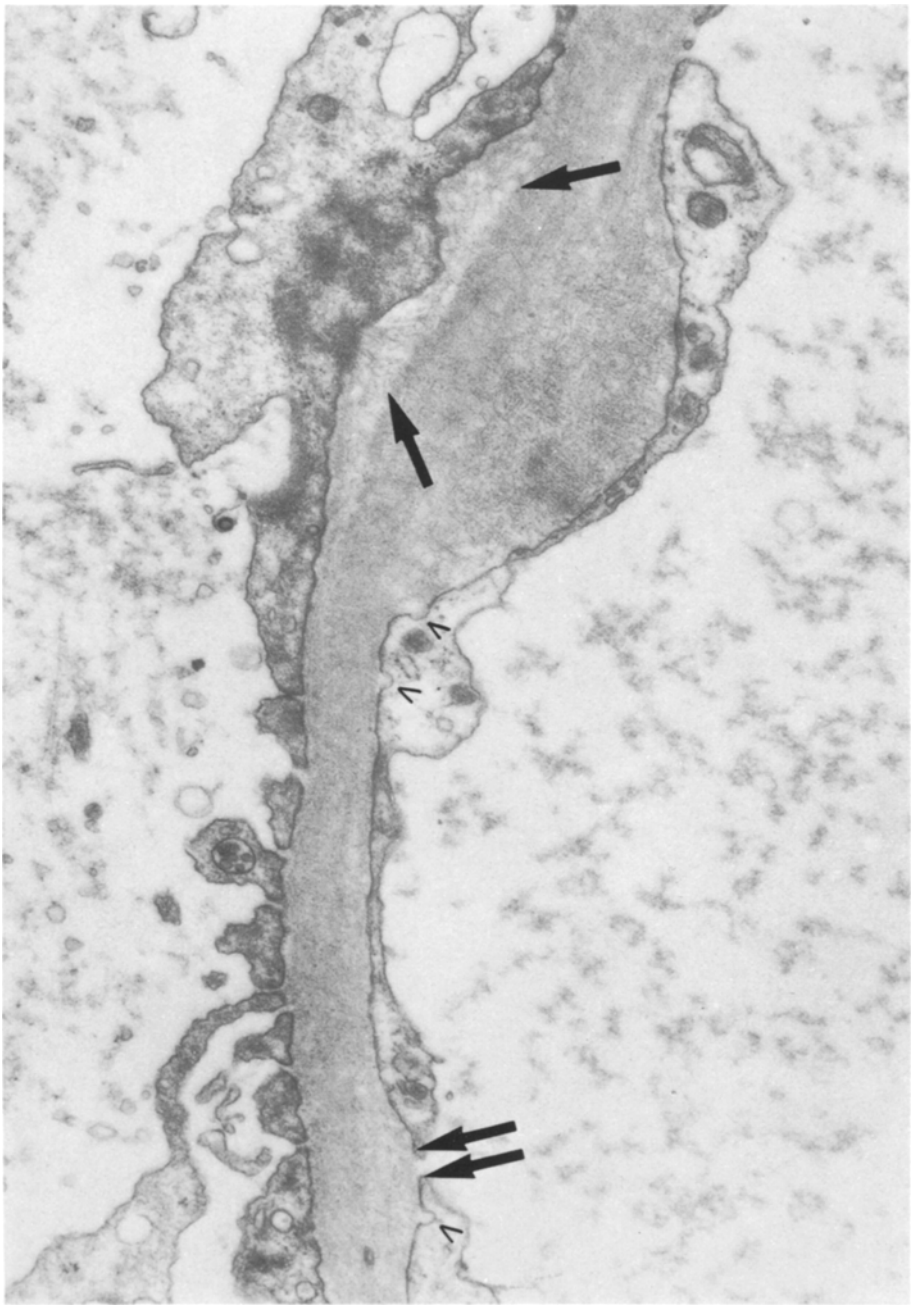

Fig. 4. Early stage of amyloidosis. BM with a small subendothelial amyloid deposit, loosened structure of the lamina densa in this area. Early formation of amyloid fibrils in the subepithelial space (↗). Small subendothelial region with loosening of the BM structure (↗ ↗). Opened vesicles which are integrated into the cell membrane (arrowheads). 20,000:1

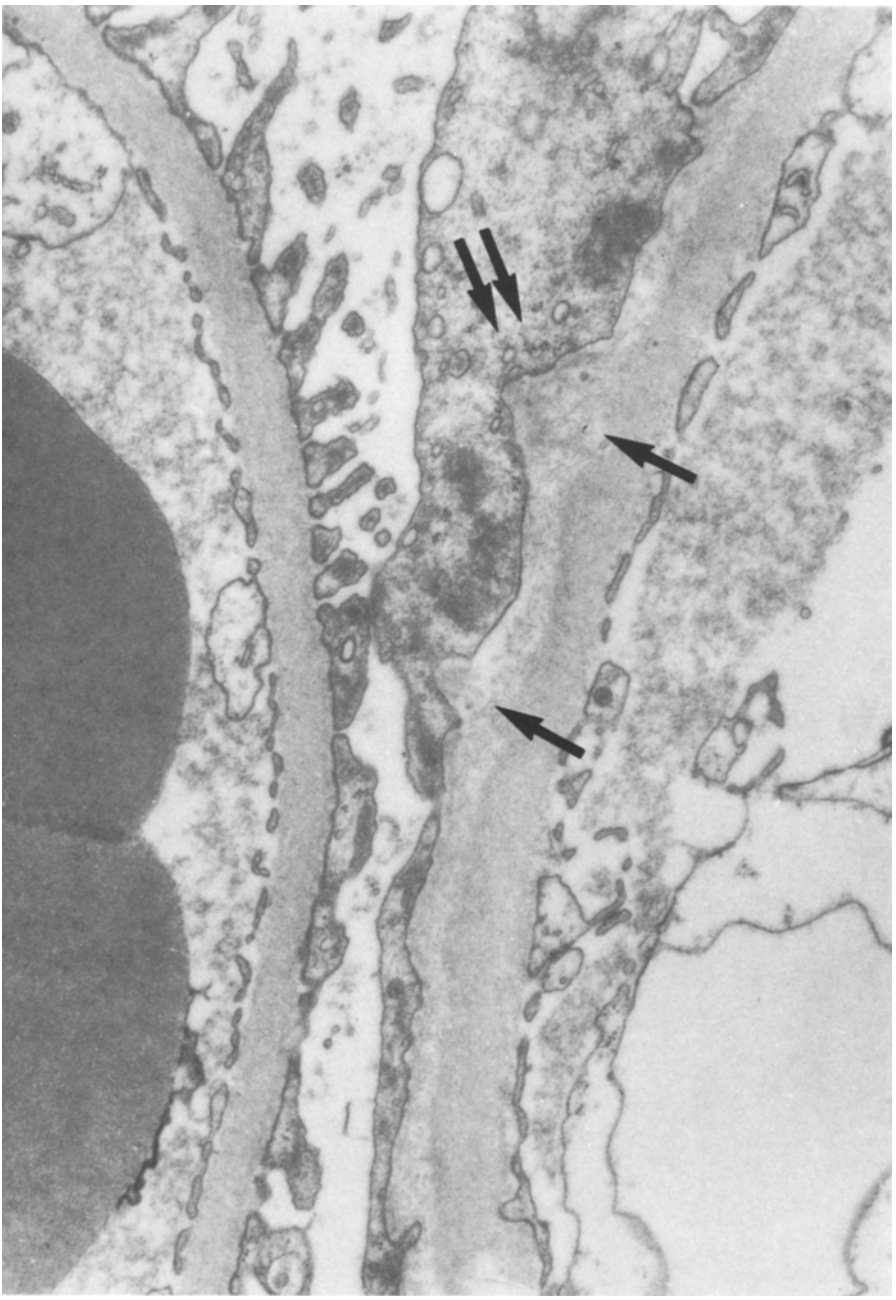

Fig. 5. Early stage of amyloidosis. Normal BM (left). Transformed and thickened BM (right) with a small loosened subendothelial zone containing no amyloid fibrils. Subepithelial space broadened and containing various amounts of amyloid fibrils. Early phase of invagination of the continuous sheet of epithelial cytoplasm (↗). Several vesicles close to the invaginated area (↗ ↗). 25,000:1

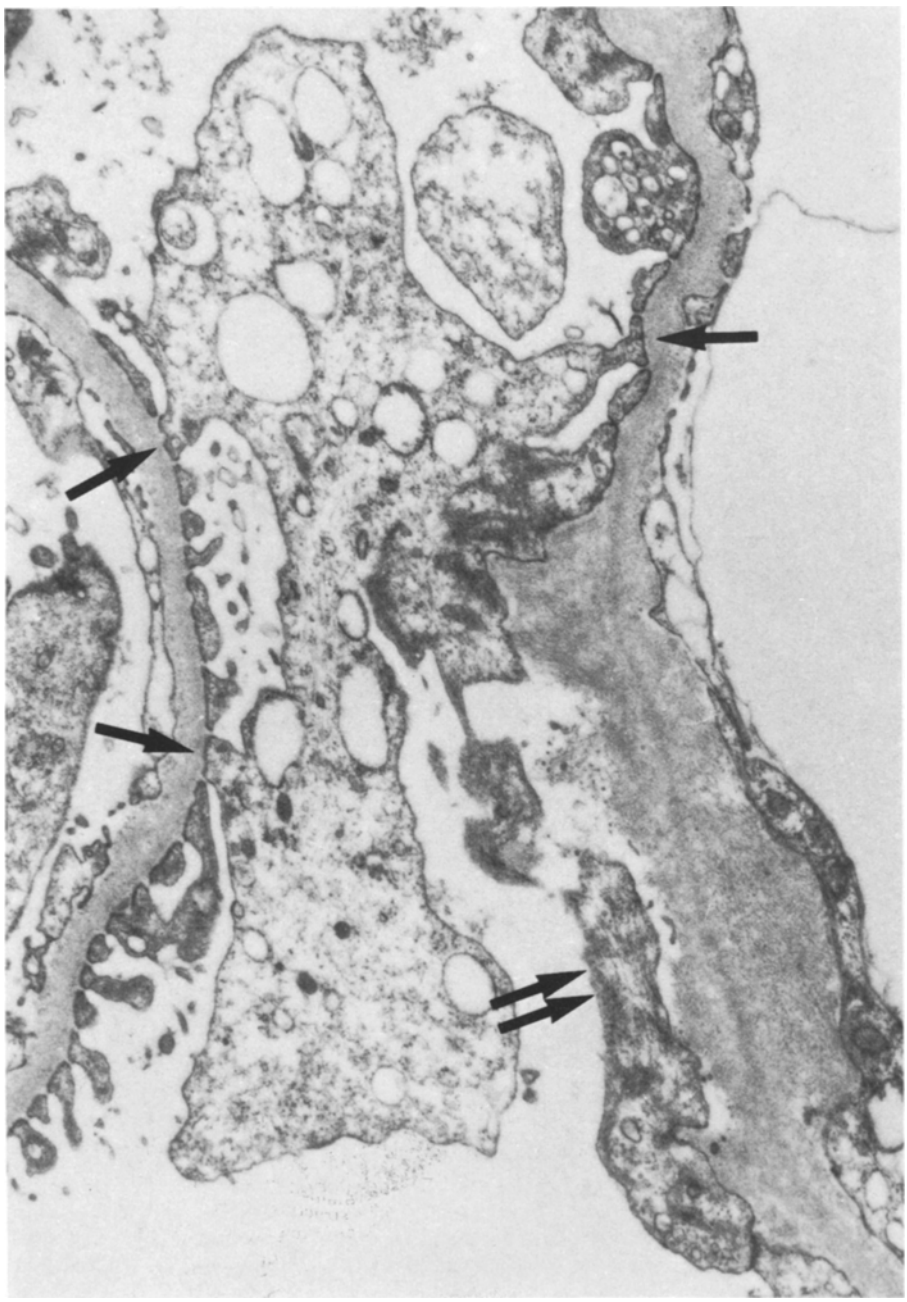

Fig. 6. Podocyte cytoplasm attached to a normal BM and to a BM which is interspersed with amyloid. Normal podocyte pedicels in BM areas free of amyloid (↗). Electron dense and swollen cytoplasm over deposited amyloid (↗ ↗), being detached from the BM by subepithelial amyloid. 12,500:1

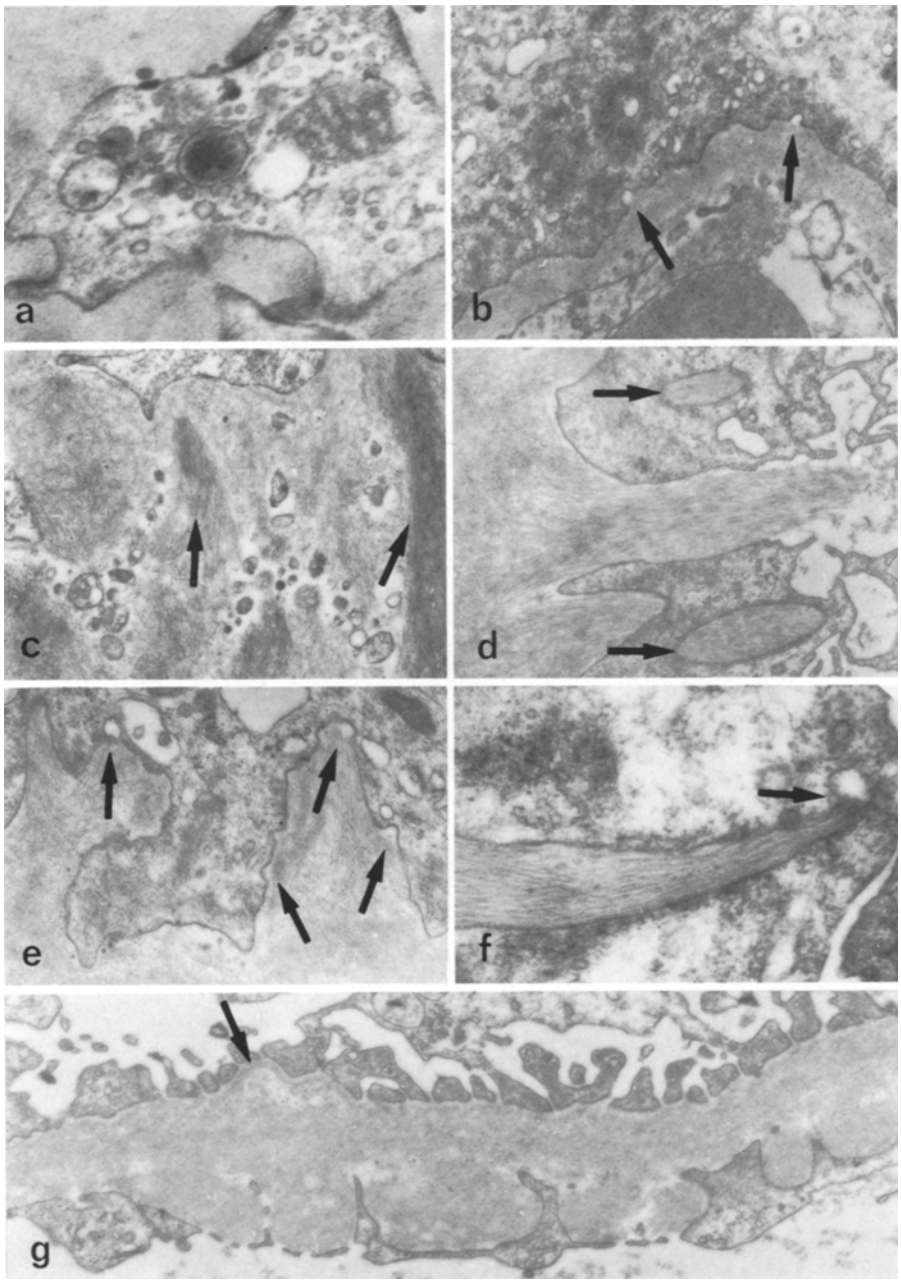

Fig. 7. a Mesangial cytoplasm with abundant vesicles and surrounded by mesangial matrix which is interspersed with amyloid. 25,000:1. **b** BM showing loosened structure, covered by a continuous sheet of cytoplasm which contains a multitude of vesicles which are integrated in the cell membrane (↗). 15,000:1. **c** Mesangial area loaded with amyloid. Mesangial cell cytoplasm seems to recede from the extending amyloid cones (↗). Free cell organelles between amyloid fibrils. 15,000:1. **d** Bundle of amyloid fibrils which has extended through the epithelial cytoplasm into the urinary space. Tangentially cut amyloid cones (↗). 15,000:1. **e** Amyloid masses extending from the BM into the epithelial cytoplasm. Several opened vesicles are integrated into the cell membrane (↗) in the area of the invaginations. 18,000:1. **f** Cone-shaped bundle of amyloid fibrils invaginating the epithelial cytoplasm in close contact with a vesicle (↗). 20,000:1. **g** Healing process of amyloidosis, seven years after recovery from a recurring pleural empyema. BM with knot-like regeneration of formerly subendothelial amyloid deposits. Small subepithelial seam of newly built basement membrane material (↗). 16,000:1

was there a loss of endothelial cell pores. There was also no invagination of the endothelial cell cytoplasm by amyloid fibrils. A new formation of BM material near amyloid deposits was not detectable on the endothelial or the epithelial side.

Discussion

Like Watanabe and Saniter (1975), we found that there are two different kinds of distribution of amyloid in the glomerular capillary network, and that these differences in distribution are detectable in grade I renal amyloidosis. Hence there are cases of early glomerular amyloidosis in which, in contrast to the observations made by Schneider and Thoenes (1977), amyloid is rarely found in the capillaries distant from the mesangium despite deposition in the mesangial region. These patients also frequently have the NS, despite lack of "fusion" of the epithelial foot processes. In these patients, the denudation of the urinary aspects of the mesangial region is due to the high protein loss resulting in the NS (Gise et al. 1978). These lesions can be observed in the mesangial region in a great number of cases with grade I amyloidosis. We could not confirm the findings of Caesar (1963), Hinglais (1964), Shirahama and Cohen (1967) that the mesangium is always the first place where amyloid is deposited. In more than half of our cases, amyloid deposits of variable size were found spread throughout the entire mesangium as well as in the walls of capillaries distant from the mesangium. There was no apparent connection between these latter deposits and those found in the mesangial region (Fig. 3). Thus it seems not to be the rule that the mesangial amyloid deposits extend laterally into the subendothelial space. The continuity of mesangial and peripheral subendothelial amyloid deposits, which may be seen in severe amyloid disease, might also be the result of a "growing together" of several peripheral amyloid masses with mesangial deposits. However, in cases showing predominantly mesangial deposits a certain degree of lateral extension became apparent. It was, however, limited in its extent and was seen mainly in the subepithelial area, where the amyloid had broken through the mesangial BM. The most remarkable finding in the early stages of amyloidosis was that the glomerular endothelial cells showed no obvious reaction towards larger subendothelial or mesangial amyloid deposits. This is a striking feature when one considers that the mesangial and epithelial cells show vigorous cytoplasmic and structural changes in the presence of only small amounts of amyloid. In areas of subendothelial or subepithelial loosening of the BM or adjacent to amyloid deposits, no evidence for BM regeneration from either the endothelial or epithelial side was found. In areas of subendothelial or subepithelial loosening of the BM or near amyloid deposits, there is a local inability of the BM to regenerate. This seems to be due to the deposition of amyloid precursors in these areas. Amyloid precursors have already been postulated by Schmidt (1904) and Randerath (1950).

During the recovery stage, "resting" amyloid masses in the glomerulus are covered by a BM-like structure on the endothelial and on the epithelial side (Gise et al. 1978). As this membrane-like structure (Fig. 7g) is never observed during the "active" phase, one must postulate that a disturbance in

BM regeneration exists at this time. This disturbance may be a result of a systemic immunological process affecting all vessels (Schultz and Milgrom 1973). We regard it as more likely that local changes resulting from amyloid precursors infiltrating the BM via the blood stream are the cause of the disturbed BM regeneration. This infiltration leads to the loosened areas on both sides of the glomerular BM. These lesions and the loosening of the mesangial matrix, should be regarded as the earliest morphological changes in renal amyloidosis (see also Schneider and Thoenes 1977). The actual mechanism of amyloid deposition and in particular of the production of the fibrils remains unclear. experimental results reveal that serum amyloid-A-protein (SAA) (Levin 1973) – a presumed component of amyloid fibrils – has a strong tendency to aggregate with itself and with albumin (Rosenthal and Franklin 1977). Other authors (Shirahama and Cohen 1973, 1975) mention that a link may exist between lysosomal enzymes and amyloid. According to Schultz (1977) the primary disturbance resulting in the appearance of amyloid fibrils is a disturbed vascular permeability which allows the penetration of plasma derivatives into tissue space.

We believe that amyloid precursors and substances coming from the blood stream, showing an affinity for one another and tending to aggregate, may interact with locally released lysosomal enzymes at the same site and aggregate extracellularly to form amyloid fibrils. In the mesangium this could take place in two ways: Firstly, mesangial cells near amyloid deposits show a raised metabolism with increased number of vesicular structures near the cell membranes. Our observations have shown that the vesicles often come into close contact with the cell membrane and seem to extrude their contents into the surrounding matrix. Secondly, lysosomal enzymes may be in abundant supply due to previous damage to the mesangial cells, which is seen early in the process (Fig. 7c). The fact that amyloid is concentrated in a greater amount in the mesangial area than in the peripheral subendothelial region, we regard as the consequence of the marked mesangial cell reactivity. A similar mechanism may be effective in the epithelial cells. The material infiltrating the subepithelial space appears to produce a strong local irritation on the epithelial foot processes. This stimulation results in a loss of foot processes, which are replaced by a continuous sheet of epithelial cytoplasm (Fig. 4, Fig. 5). In these areas, often showing severe cytoplasmic swelling, one finds the characteristic coneshaped bundles of amyloid fibrils which invaginate the epithelial cytoplasm (Fig. 7d, f). As we were unable to demonstrate "dense bodies" or intracellular amyloid in these epithelial cells, we regard it as unlikely that amyloid can be produced intracellularly (as suggested by Shirahama and Cohen 1973, 1975). We would like to regard the invagination of the cytoplasm as an "unsuccessful phagocytosis" in which lysosomal enzymes are released into the extracellular space (Henson et al. 1972). Jao and Pirani (1972), also regard the extracellular production of amyloid fibrils as a possibility. Observations on serial sections support our hypothesis of a reactive involvement of the epithelial cells in fibril production. Characteristic bundles of amyloid can "burrow" through the entire epithelial cell cytoplasm and penetrate into the urinary space (Gise et al. 1978). The cylindrically shaped bundle of fibrils, surrounded by a cytoplasmic membrane, with its base attached to the BM (Fig. 7d), grows further and further into the urinary space. The continual

growth of parallel arranged fibrils into the urinary space does not go in hand with the hypothesis of intracellular fibril production. Epithelial cell invagination may be regarded as a dynamic process resulting from the growing of extracellularly produced fibrils and the increasing reaction of the podocytes by liberation of lysosomal enzymes. The initial step is probably the extrusion of vesicular content as a reaction of the epithelial cell to the altered BM structure. The extruded material – probably lysosomal enzymes – could promote the aggregation of amyloid precursors and plasma components. The amyloid fibrils produced penetrate into the lumen of the vesicle because of their rigidity and prevent it from reforming. The amyloid fibrils may now produce a constant stimulus on the vesicular membrane, which has now become the new cell membrane, and induce the opening of other vesicles. In this way one could explain the apical growth and the almost parallel arrangement of the fibrils. This explanation is also in agreement with the observation that the amyloid fibril seem to "grow" into the urinary space after having perforated the urinary aspect ot the epithelial cell membrane. However, one can observe that the epithelial cell invagination stops when the corresponding epithelial cell loses contact with the BM due to subepithelial deposits of amyloid in severe cases. The epithelial cell may subsequently give way to the amyloid masses. Furthermore, one may observe that epithelial foot processes which are attached to unchanged sections of the BM appear electronmicroscopically normal whereas foot processes of the same podocyte adjacent to a changed section of the BM show alterations (Fig. 6). The amyloid precursors in the subepithelial space hence provoke a locally limited stimulus on the corresponding epithelial cell. Thus it appears that only part of the metabolism of the cell is disturbed; more changes would be expected if amyloid production were to take place intracellularly.

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