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Correlations Between the Morphological and Clinical Findings in a Patient Recovering from Secondary Generalised Amyloidosis with Renal Involvement

Light- and Electron Microscopic Investigations on Serial Biopsies

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Summary. We report light- and electron microscopic findings in glomerular amyloidosis (secondary amyloidosis), which occured after recurrent empyema of the pleura. After healing of the empyema, the clinical symptoms disappeared, over a period of eight years.

During the acute stage of the disease (grade II-III amyloidosis) when the nephrotic syndrome was present, amyloid deposits were seen in the mesangium and on both sides of the basement membrane of the glomerular capillaries. Furthermore, denuded basement membrane areas showing the passage of amyloid into the urinary space, and invaginations of the podocyte by straightened amyloid fibrils were found. After clinical recovery (except for a trace of proteinuria), the renal amyloidosis had electronmicroscopically transformed from an active into an inactive or resting form, while the amount of amyloid present was almost the same. In the areas of amyloid deposits, reparative changes were observed, especially in the area of the mesangial cells and of the podocytes. The podocytes were separated from the persisting amyloid deposits by newly formed basement membrane material.

Key words: Secondary amyloidosis – Healing of nephrotic syndrome – Electron microscopic investigations.

There are few reports of histologically documented improvement of secondary generalized amyloidosis with renal involvement (Waldenström, 1928; Lowenstein and Gallo, 1970; Triger and Joekes, 1973).

We report here a patient whose renal biopsies were investigated by light- and electron microscopy:

The patient is a 32 year old male who developed pneumonia with an accompanying effusion. In March 1966 an empyema of the pleura was discovered and decortication was performed. Recurrences of the empyema later led to hospitalisation. A complicating bronchial fistula was irrigated

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Table 1. L.H., born 25,12,1933, male. Biopsies: 699139, 752405 and 7612160. December 1965 pneumonia with accompanying effusion. March 1966 to May 1969 recurrent empyema of the pleura. Complete healing after several surgical interventions and antibiotic therapy

Month/year	5–9/1969	1970	9/1971	4/1972	2/1975	12/1976
Renal biopsy	+				+	+
Rectal biopsy	+		+			
Blood pressure (mm Hg)	120/80	130/90	120/80	130/80	140/85	140/95
Serum creatinine (mg/100 ml)	2.6	—	normal	1.2	1.2–1.6	1.3
Creatinine clearance (ml)	—	—	—	—	47	100
Proteinuria (Esbach) (‰)	3.9–12–32	12	2.3	2.0	trace	0.8–1.1
Total serum protein (g/100 ml)	4.3	—	4.3	6.0	9.3	—
Nephrotic syndrome +	+	+	+	(+)	—	—
Triglycerides (mg/100 ml)	1595	—	820	440	148	—
Cholesterol (mg/100 ml)	325	612	280	292	278	—
B.S.R. (Westergreen) (mm)	77/99–99/120	78/88	9/14	22/44	7/19	5/21
Therapy:						
Resochin	=	=	=	=	=(9/75)	
Aldactone	=	=	=	=	=	
Hydromedin	=	=	=	=	=	

several times, and in February 1967 the lower lobe of the right lung was resected. In April 1968 a further acute empyema with fever and an elevated erythrocyte sedimentation rate was found. For the first time proteinuria and microhematuria were observed. In May 1969 the empyema recurred and was tapped. Subsequent antibiotic therapy led to complete recovery. At the beginning of this hospitalisation edema and proteinuria (up to 3.9‰) were observed (Table). The Congo-red test revealed 89 % retention of dye. In September 1969, at the time of the first renal biopsy, a nephrotic syndrome was found (proteinuria up 32‰). The serum creatinine concentration was elevated at 2.6 mg per 100 ml. The rectal biopsy revealed severe amyloidosis. The prognosis was regarded as unfavourable, the patient was declared unfit for work.

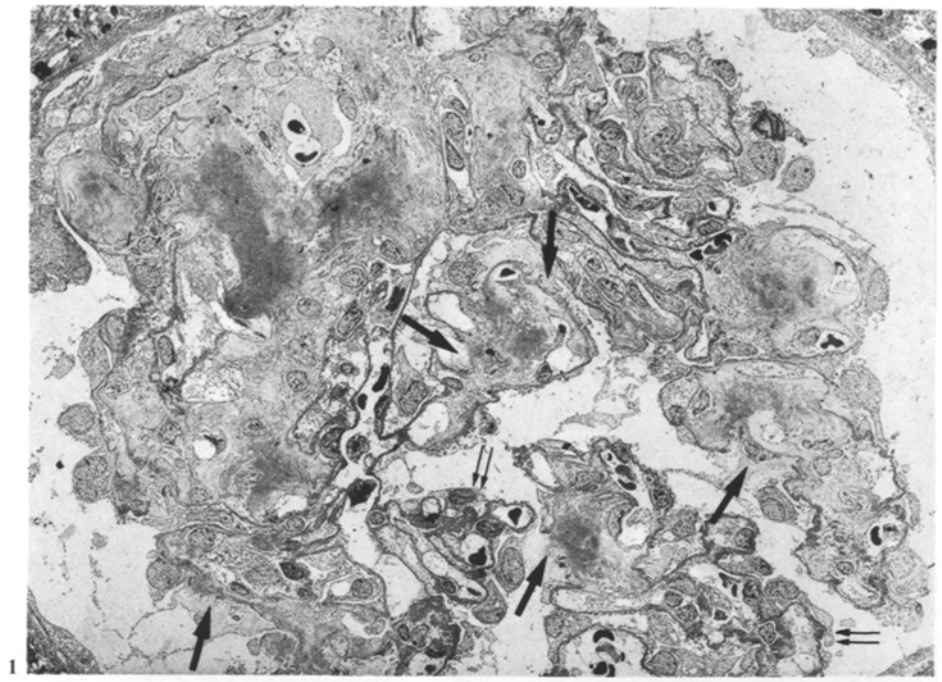
In the following two years the patient's condition improved (Table 1). The second rectal biopsy, in September 1971, showed only minimal amyloid deposits.

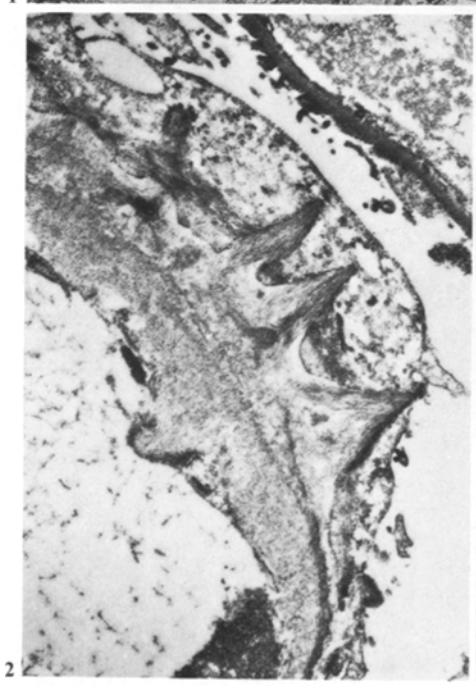
In April 1972 the patient was free of symptoms. In February 1975, at the time of the second renal biopsy, the laboratory findings showed improvement, except for the creatinine clearance which was reduced at 47 ml/min and the serum creatinine concentration which was elevated (1.6 mg per 100 ml). Most notable were the improvement of the proteinuria and the normal level of the serum triglycerides (see table).

When the patient was biopsied in February 1975 and in December 1976 for the second and the third time, all clinical findings were found to be normal except for a slightly elevated serum creatinine concentration and a trace of proteinuria (see table).

Light- and Electron Microscopic Findings

The first biopsy-cylinder (September 1969, 69/9/139) which contained 10 glomeruli, showed a grade II-III glomerular amyloidosis with focal amyloid deposits, predominantly mesangial (Fig. 1). The most prominent deposits were found near the vascular pole. Amyloid was also seen in the vasa afferentia and efferentia. Toward the glomerular periphery a continual decrease in the prominence of amyloid deposits was observed. Lightmicroscopically some areas of the glomeruli showed no





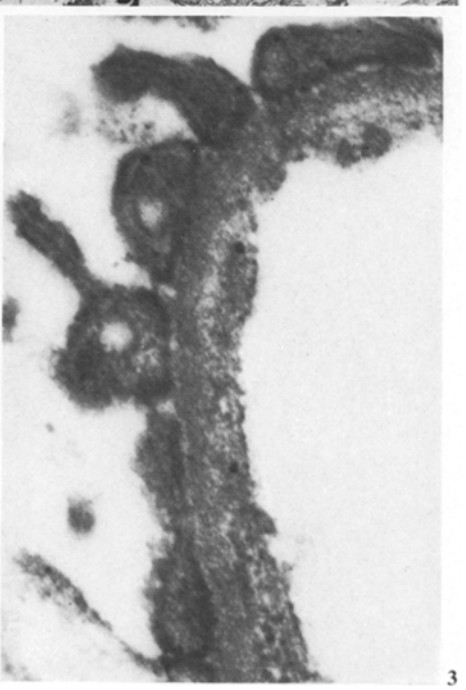
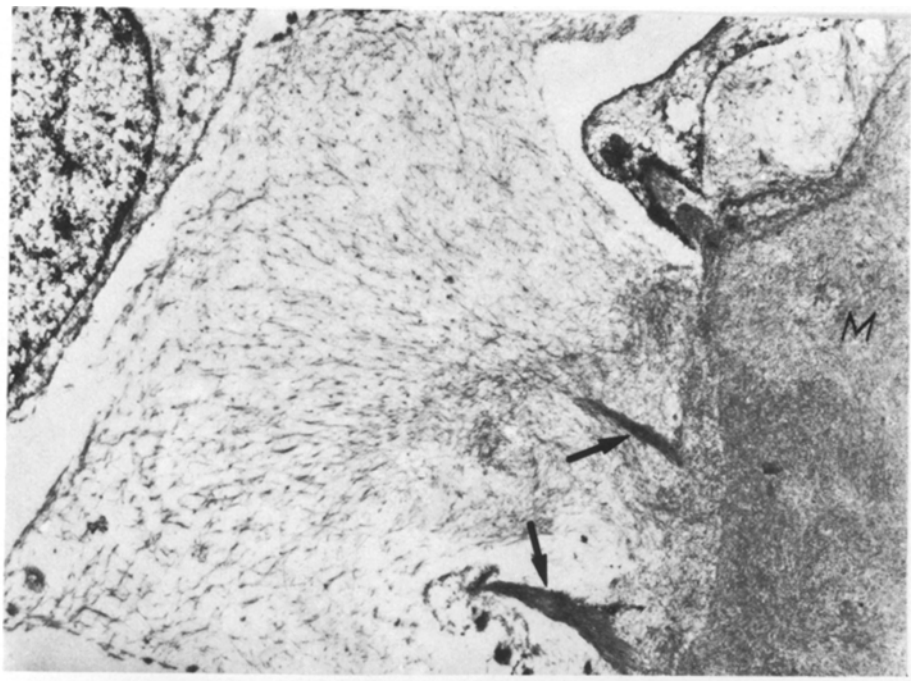


Fig. 1. First biopsy 69/9/139. Glomerulus with focal amyloid deposits (grade II–III) predominantly in the mesangium. Amyloid traversing into the urinary space in areas of denuded basement membranes (↗). Peripheral capillary loops free of amyloid (↗ ↗). 750:1

Fig. 2. First biopsy 69/9/139. Part of a capillary loop near the mesangium with amyloid deposits on both sides of the basement membrane. Cone-shaped invaginations of the podocyte caused by straightened amyloid fibrils arranged radially to the basement membrane. Lamina densa appearing narrowed and blurred. 5000:1

Fig. 3. First biopsy 69/9/139. Part of a peripheral capillary with normal pedicles. The nephrotic syndrome was present at the time of biopsy 30,000:1



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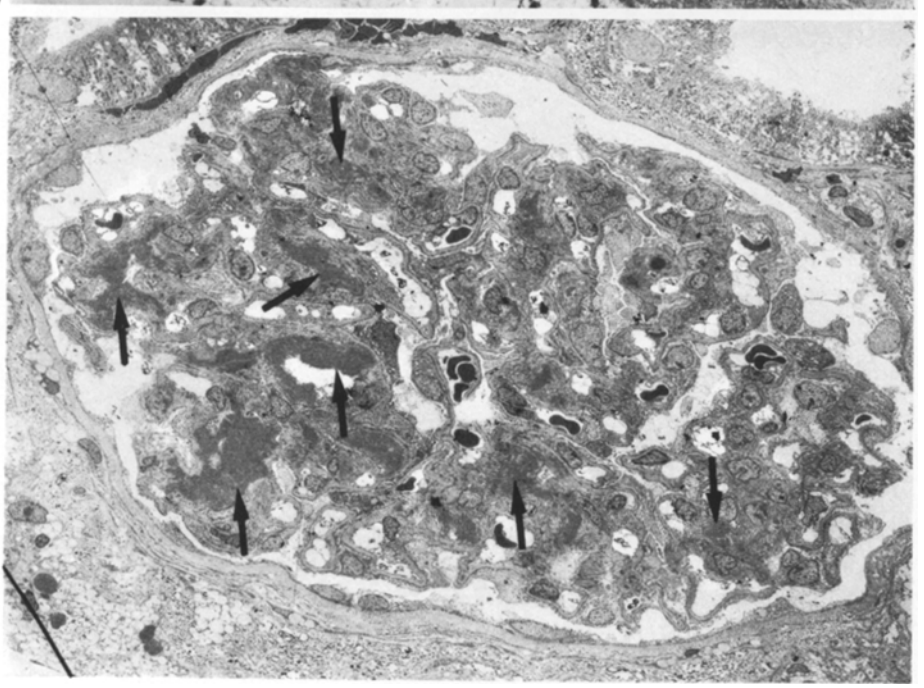

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Fig. 4. First biopsy 69/9/139. Basement membrane with denudation of the epithelial covering; amyloid breaking through into the urinary space. Rests of cone-shaped amyloid bundles (↗) in the area of the mesangium (*M*). 15,000:1

Fig. 5. Second biopsy 75/2/405. Glomerulus with dense amyloid masses (grade II) in the markedly broadened mesangium (↗) showing increased cellularity compared with 6 years before. 750:1

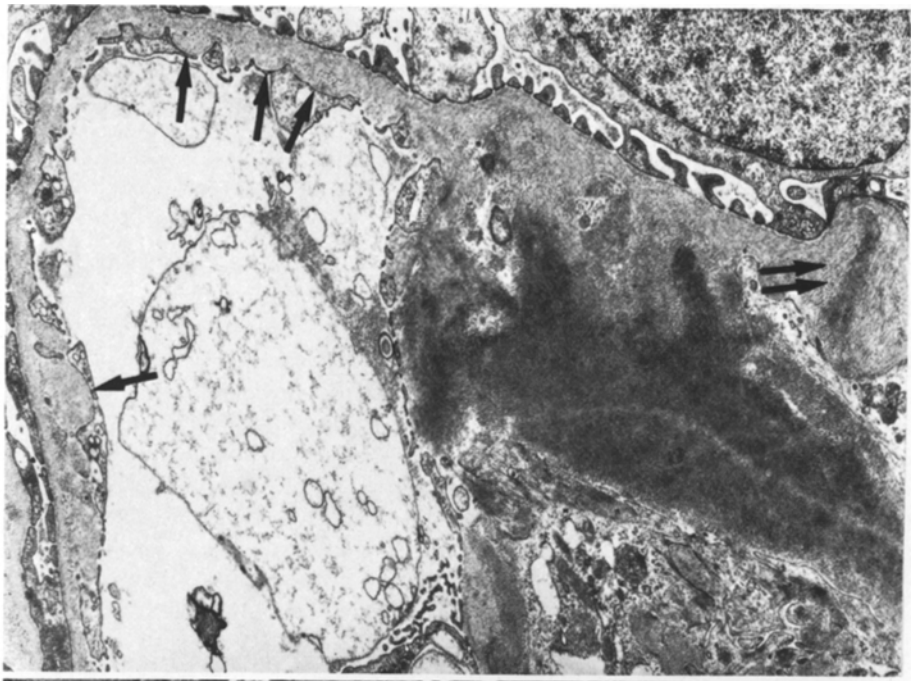
change. In the areas with distinct mesangial amyloid deposits the cytoplasm of the endothelial cells was displaced into the capillary lumina, which appeared narrowed. On semi-thin sections the basement membranes in the mesangial region and occasionally elsewhere showed a distinct decrease in silver impregnation when surrounded by amyloid. The interstitium seemed to be widened. Small seams of amyloid were observed between epithelial cells and the basement membrane of the tubules.

Electron microscopic investigations (the biopsy cylinder was embedded from paraffin into plexiglass and postfixed in osmium acid) confirmed the destruction of the basement membrane in the areas of amyloid deposition (Figs. 1, 2). In the areas where the amyloid deposits were localised in or along the basement membrane, the lamina densa appeared distinctly narrowed and blurred (Fig. 2), the lamina rara externa and interna could not be identified. The focal amyloid masses in the mesangium led to an enormous enlargement of the mesangial region with a reduction of the local cell population and the matrix; the basement membranes in these areas were extensively interspersed with amyloid. The adjacent podocytes had fused foot processes, often showing characteristic invaginations (Fig. 2). These invaginations were caused by subepithelial cone-shaped amyloid deposits with straightened fibrils, radially arranged with respect to the basement membrane. Furthermore, areas where the basement membrane was infiltrated with amyloid and where there was denudation of the epithelial covering with mushroom-like prolapes of amyloid into the urinary space, could often be observed in the areas near the mesangium (Fig. 4). Such changes seldom were seen in areas away from the mesangium. Peripheral lobules appeared electron microscopically unaltered or showed only small amyloid deposits in the mesangium. If no amyloid deposits could be discovered in the basement membrane, the foot processes of the podocytes were unchanged despite the existance of the nephrotic syndrome (Fig. 3).

Biopsy (75/2/405)

In the second biopsy in February 1975 little material was obtained. The whole biopsy cylinder was therefore fixed in 4% buffered formalin (pH 7.4), then in osmium acid and was later embedded in araldite. Semi-thin sections were stained with Giemsa. The 5 glomeruli found in the biopsy cylinder showed a grade II amyloidosis. The amyloid deposits looked like plaques (Figs. 5 and 6) and were intensely stained. As in the first biopsy, the amyloid deposits were predominantly localised near the vascular pole, only few deposits were seen in the periphery of the glomeruli. It was notable—on the semi-thin sections—that the parts of glomerular basement membrane away from the mesangium contained scarcely any amyloid. The amyloid deposits were predominantly localised in the mesangium and frequently had a "chewed" appearance. In comparison with the first biopsy, however, an increased cellularity was noticeable in the markedly broadened mesangium (Fig. 5).

In many areas the glomerular basement membranes were electronmicroscopically inconspicuous, the foot processes of the podocytes being visible (Figs. 6, 8), with several showing a slight broadening of their bases. Even over large amyloid plaques in the mesangium the basement membrane appeared electron microscopically homogeneous and the foot processes of the podocytes were normal. The glomerular basement membranes distant from the mesangium showed focal "not-like" thickenings. These were covered by endothelial cyto-



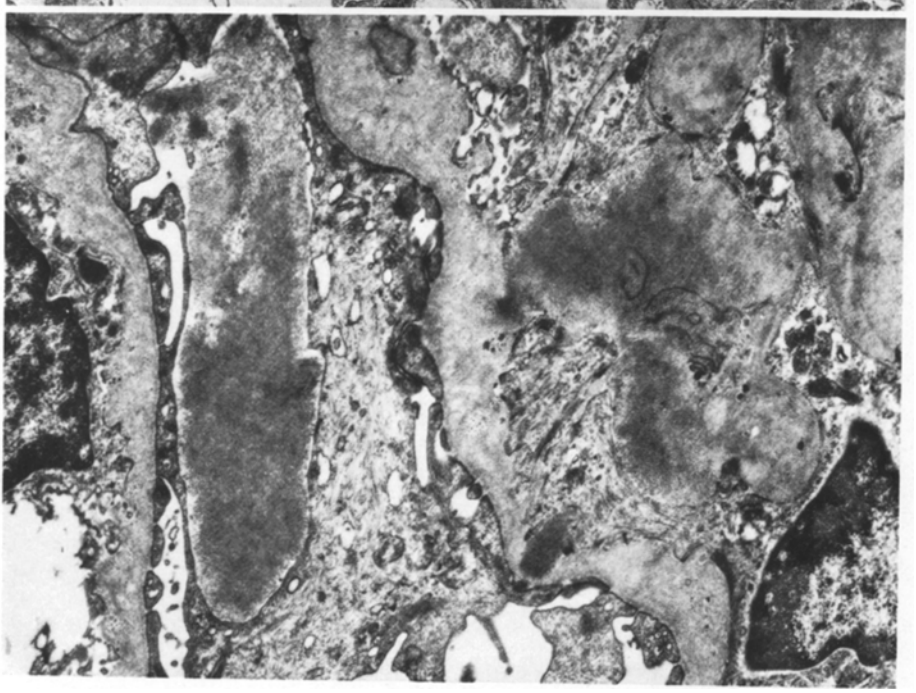
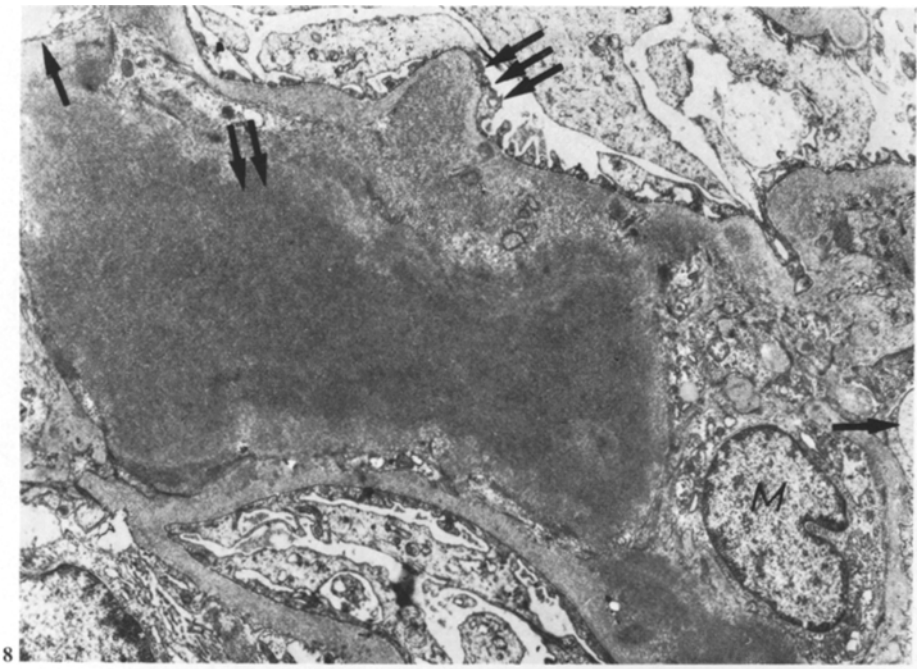


Fig. 6. Second biopsy 75/2/405. Peripheral capillary (left) with "knot-like" thickened basement membrane areas (↗), and part of the mesangium (right) with dense amyloid masses. Bundle of amyloid fibrils separated from the epithelial foot processes by basement membrane like material (↗↗). 7500:1

Fig. 7. Second biopsy 75/2/405. Amyloid masses in the urinary space surrounded by epithelial cells. Amyloid fibrils only sporadically recognizable. 10,000:1



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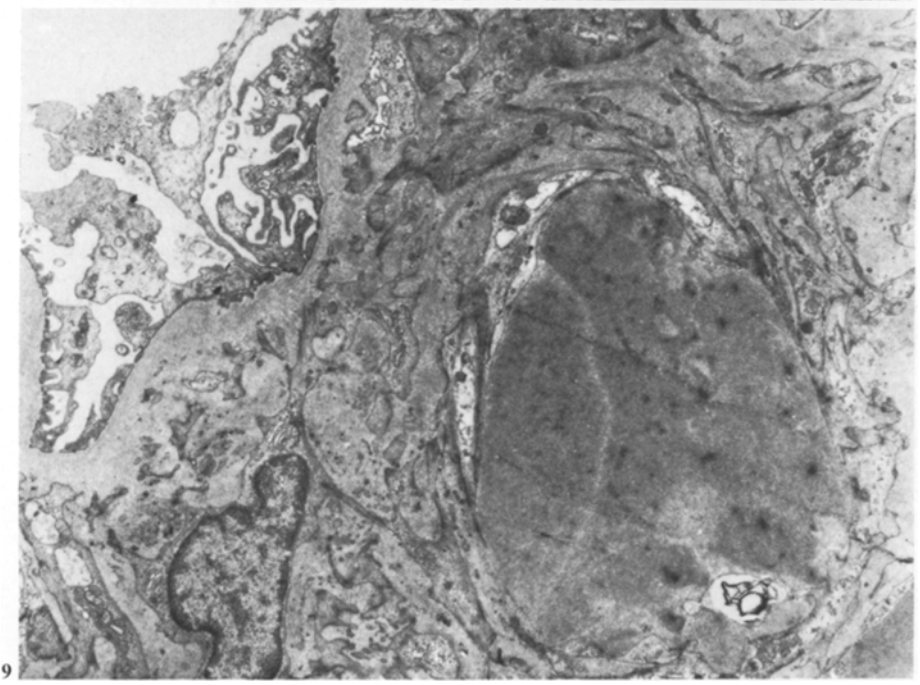

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Fig. 8. Third Biopsy 76/12/160. Mesangium with dense amyloid masses (↗ ↗). Here there is also a bundle of amyloid fibrils separated from the pedicles by a basement membrane like material (↗ ↗ ↗). Capillary space (↗). Nucleus of the mesangial cell (*M*). Seam of mesangial cell cytoplasm between basement membrane and amyloid masses. 7500:1

Fig. 9. Third biopsy 76/12/160. Mesangial area with an extremely dense deposit of amyloid which is surrounded in an onionlike pattern by mesangial cells and mesangial matrix. 7500:1

plasm with pores and sometimes contained residual amyloid fibrils (Fig. 6). The amyloid deposits in the mesangium and near the vascular pole were electron-dense and were distinctly demarcated from the other glomerular structures. The fibrils themselves had a crumbly appearance. The amyloid deposits in the mesangium were localised and were surrounded by mesangial cells and matrix in an "onion-skin" fashion. In 1969 however the mesangial amyloid deposits had broken through the basement membranes into the subepithelial and urinary space. Amyloid had completely disappeared from the peripheral mesangial regions except for some remaining plaques and only an enlarged mesangial matrix, containing several mesangial cells remained. A distinct cell membrane could be seen between the amyloid deposits and the surrounding mesangial cells.

In the 1975 and 1976 biopsies, one could observe phases of the healing process of the denuded basement membrane areas, first seen in the 1969 biopsy cylinder (Fig. 6). These appeared as bundles of fibrils which prolapse in a dome-like fashion into Bowman's capsule and which were separated from the podocytes on the surface by basement membrane-like structures.

Condensed amyloid was also seen in the urinary space (Fig. 7) partly surrounded by the cytoplasm of the podocytes.

Biopsy (76/12/160)

The third biopsy – performed 22 months later – provided enough material for light- and electron microscopic investigations. Light microscopically the glomeruli showed grade II amyloidosis (as in February 1975). Beside single glomerulus without any amyloid, some totally scarred glomeruli were now seen surrounded by atrophied tubules. The mesangium was broadened, but seemed to be more cellular than in 1975. The dense amyloid deposits were predominantly localised near the vascular pole and in the centre of the lobules. In the periphery of the lobules, however, no amyloid could be observed, although some single and small plaques remained in the mesangium, again being surrounded by mesangial cells and matrix in an onion-like fashion (Fig. 9). In comparison with 1975, the amyloid infiltration had slightly decreased.

Electron microscopy, however, revealed essentially no differences compared with the findings of 1975. In the areas of formerly denuded basement membranes formation of a new basement membrane was seen, whereby the prodocytes were clearly delineated from the rest of the amyloid fibrils (Fig. 8).

Discussion

In both man and animals secondary amyloidosis is said to be curable provided that the diseases which lead to the amyloidosis can be sucessfully treated (Waldenström, 1928; Rosenblatt, 1936; Richter, 1954; Williams, 1967; Lowenstein and Gallo,1970; Polliak et al., 1970; Wright et al., 1972; Triger and Joekes, 1973). In agreement with the light microscopical findings of Lowenstein and Gallo (1970) and of Triger and Joekes (1973), our investigations also show that clinical recovery from amyloidosis can occur relatively fast, while the

morphological changes persist. We were also able to define electronmicroscopically the structural changes which could be seen after the partial disappearance of amyloid deposits from the glomeruli. Moreover, the ultrastructural changes of active glomerular amyloidosis with nephrotic syndrome could be compared with an inactive or improving form of amyloidosis. The most important findings of the three serial biopsies in the course of 8 years are the following:

In the "active" form of amyloidosis with nephrotic syndrome, the foot processes are preserved in the areas of the glomerular capillaries where no amyloid is deposited. However, no foot processes can be found in the areas where amyloid is demonstrated in or surrounding the basement membrane. This observation differs from those made in minimal-change disease with the nephrotic syndrome as well as from those in focal sclerosing glomerulonephritis (Bohle et al., 1974), where the nephrotic syndrome is accompanied by a complete loss of the foot processes of the podocytes. This loss of the foot processes is regarded as a characteristic lesion of this disease entity, associated with an increased permeability of all glomerular capillaries. Other structural changes have to be present in secondary renal amyloidosis with the nephrotic syndrome. Here the basement membrane permeability is only increased in the areas where the membrane either contains amyloid fibrils or is surrounded by amyloid it is especially increased, however in areas where the amyloid affected basement membrane is denuded of its epithelial covering. This increased permeability in the areas of denuded basement membranes is evidently associated with the straightened fibrils, which project into the urinary space and which can be found in the urinary sediment (Neale et al., 1976). This supposition is supported by the experimental findings in nephrotoxic nephritis (Kühn et al., 1977). These authors showed that the glomerular basement membrane is enormously permeable to albumin and to the high molecular weight ferritin in areas showing denudation of the epithelial covering. Whether and to what degree these particular damaged areas in our case are the morphological basis for increased permeability leading to the nephrotic syndrome is a subject of further investigations.

The ultrastructural findings of the three biopsies demonstrated different reactions of the podocytes to the presence of amyloid. In the first biopsy, funnel-shaped invaginations of the podocytes in several areas were observed. Similar changes have been described by Bari et al. (1969), Shirahama and Cohen (1975), Kimura et al. (1974), Ishihara (1973), Sorensen et al. (1964) during the acute stage of experimental amyloidosis in cells of the RES, especially in Kupffer's cells. These changes were not found in the second and third biopsy. Here the alterations were separated from the podocytes by a newly formed basement membrane. Furthermore, the reduction of the amyloid is combined with an increase in the number of the mesangial cells.

We should like to speculate as to whether the podocytes are able to produce amyloid intracellulary and whether the deposition takes place in the areas of cytoplasmic invagination. We do not think that this latter hypothesis is likely, although analogous changes have however been observed in experimental amyloidosis in the cells of the RES (especially in the Kupffer's cells) and have been interpreted as being the place of amyloid production (Bari et al., 1969; Ishihara, 1973; Kimura et al., 1974; Shirahama and Cohen, 1975). We think

that it is more likely that the invaginations of the podocytes are produced by the amyloid masses, which have permeated the glomerular basement membrane and which project in the direction of Bowman's capsule. In cases of pronounced invagination, the adjacent podocytes were detached from the glomerular basement membrane which was largely destroyed by the amyloid masses. Furthermore, there are close spatial connections between invaginations of the podocytes and the denuded basement membranes. In these areas large amyloid masses, in the form of amyloid fibrils, penetrate into the urinary space.

The metabolism of the podocytes, which normally produce the basement membrane, seems to be disturbed in "active" amyloidosis. No basement membrane is produced in areas where the podocytes are in contact with amyloid fibrils. After recovery from the disease which led to the secondary amyloidosis, the metabolism of the podocytes apparently returns to normal. The remaining amyloid masses will be coated by basement membrane-like structures (s. Figs. 6 and 8), and the foot processes of the podocytes regenerate.

The origin of the masses of amyloid which are found in the mesangial region in the "active" form of amyloidosis, cannot be explained by local cellular activity alone, since increasing deposits are accompanied by a decrease in the number of mesangial cells. It is assumed, therefore, that preamyloid substances "polimerize", when they are in contact with amyloid fibrils or under the influence of still unknown factors.

Only if one proceeds from this hypothesis is it explicable that in severe amyloidosis the glomeruli are transformed into hyaline bodies consisting of amyloid without any cells, surrounded by Bowman's capsule. Based on experimental findings, as pointed out by Rosenthal and Franklin (1977), the preamyloid SAA has a tendency to aggregate with itself and with serum albumin.

In summary, it is well known that secondary amyloidosis is clinically cureable. This clinical recovery, however, does not necessarily go hand in hand with a re-establishment of normal renal morphology. The findings show that glomerular function is disturbed only in "active" but not in "inactive" glomerular amyloidosis.

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