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Investigations on the Cause of the Nephrotic Syndrome in Renal Amyloidosis

A Discussion of Electron Microscopic Findings

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Summary. Systematic electron microscopic investigation of glomeruli of 35 patients with renal amyloidosis (grade I-III), among them 26 with the nephrotic syndrome, reveals the following:

1. The extent of the area of basement membrane denuded of its epithelial covering is correlated significantly with the reduction of plasma protein concentration at the time of renal biopsy.
2. In amyloid free regions of the glomerular capillary loops, the foot processes of the epithelial cells remain intact despite the presence of the nephrotic syndrome. From these findings we conclude that the high glomerular protein losses in amyloidosis occur in areas of the basement membrane which are penetrated by amyloid and denuded of their epithelial covering. With increasing number of these lesions per unit area, the permeability of the capillary network for protein increases to a degree which is significantly correlated with the reduced plasma protein concentration at the time of biopsy.

Key words: Renal amyloidosis – Basement membrane denudation – Cause of nephrotic syndrome.

According to Watanabe and Saniter (1975) renal amyloidosis is accompanied by the nephrotic syndrome in 57–93% of cases. The nephrotic syndrome (NS) is most frequently found in grade II and III amyloidosis (classification of Mackensen et al., 1977). The cause of the NS in renal amyloidosis remains unknown. It is well established that the NS occurring in systemic disease (secondary amyloidosis) may disappear or improve after treatment of the underlying disease and it has been shown that renal excretory function improves without disappearance of the amyloid deposits from the glomerulus (Lowenstein and Gallo, 1970; Triger and Joekes, 1973; Dikman et al., 1977). From these facts and from

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findings in a patient with renal amyloid followed during complete clinical recovery (v. Gise et al., 1978), it seemed possible to shed more light on the cause of the NS with the aid of the electron microscope.

Material and Method

Electron microscopic investigations were carried out on 35 renal biopsies from patients with grade I-III renal amyloidosis (according to the classification of Mackensen et al., 1977).

The tissue was fixed in formaldehyde, postfixed in osmium tetroxide, stained in alcoholic uranylacetate, and embedded in Araldite after dehydration. The thin sections were stained with lead citrate.

Prompted by our follow-up findings of the above-mentioned patient with secondary renal amyloidosis (v. Gise et al., 1978), we turned our attention to amyloid-infiltrated areas of the basement membrane (BM) which were denuded of their epithelial covering. We assessed these features quantitatively and tried to correlate them with the plasma protein concentration at the time of the biopsy. To us this procedure seemed most appropriate, as we had noted in the past that the foot processes of the epithelial cells in amyloid-free areas remained intact when the NS was due to infiltration by glomerular amyloid; this finding has not been reported in cases where the NS was a result of other glomerular lesions (minimal-change nephrotic syndrome, focal sclerosing glomerulonephritis; Bohle et al., 1974; Rumpelt et al., 1974).

The enormous loss of protein could therefore hardly be considered to result from a generalised damage to the basement membrane of the glomerular capillaries. It seemed reasonable to assume that the protein losses were a direct consequence of circumscribed areas of amyloid deposition in the BM causing a disruption of the epithelial cells with subsequent partial denudation of the basement membrane.

In order to confirm our assumption, we selected for electron microscopic study patients with amyloidosis on whom we possessed the following data: serum protein concentration, serum creatinine concentration and the 24 h protein excretion at the time of biopsy. The patients suffered from grade I-III amyloid disease, i.e. up to 75% of the glomerular capillaries were infiltrated with amyloid. 7 patients were classified as grade I disease, 10 as grade II and 18 as grade III. 26 of the 35 patients suffered from the NS. Proteinurea varied extremely from case to case, ranging from 1.15 g to 20 g/day. Total serum protein concentration ranged between 2.9 g% and 7.45 g%; the serum creatinine concentration ranged from 0.7 mg% to 6.6 mg%.

For a rough orientation we chose from the 35 patients one glomerulus from a patient with the lowest total serum protein concentration and one glomerulus from a patient with the highest serum protein concentration. On these 2 glomeruli 1800 consecutive sections were cut. The extent as well as the distribution of amyloid infiltrated areas of the basement membrane with denudation of the urinary aspect were evaluated in 9 different planes respectively, with a distance of about 15 µm between each section.

In all 35 cases the BM denudation per centrally cut plane in up to five glomeruli were counted; an average value per section and per case was then assessed. The quantification of amyloid infiltrated areas of the BM characterized by denudation of the urinary aspect was performed with a Siemens Type 102 electron microscope (magnification 8000:1). Each cut plane of the glomerulus was examined systematically in a ZIG-ZAG fashion from one end of Bowman's capsule to the other and

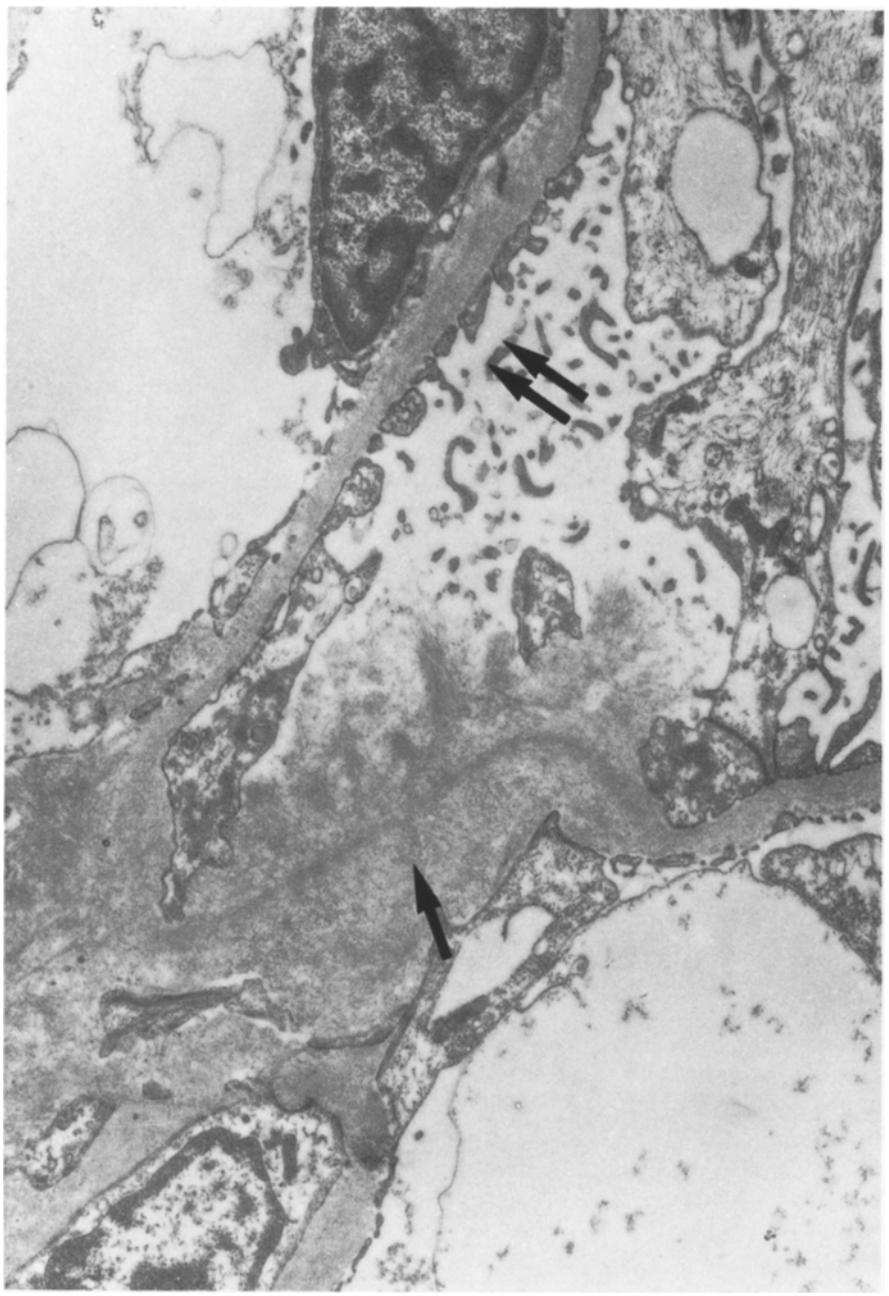


Fig. 1. Biopsy 77/10/304, 37 Year old female, grade I amyloidosis with nephrotic syndrome. Total serum protein 5.9 g/100 ml, albumin 46% rel., proteinuria 14‰, serum creatinine 1.17 mg/100 ml. Amyloid infiltrated BM area which is denuded of their epithelial covering (↗). Normal podocyte pedicels (↗ ↗). 12,000:1

the lesions that appeared on the central screen were counted. Extended areas of amyloid interspersed basement membranes showing extensive denudation of the urinary aspect were counted repeatedly when they reappeared in a neighbouring section. The average number of areas with basement membrane denudation per cut plane and per case we considered a measure of the extent of capillary wall damage. This was correlated with the total serum protein of the respective patient at the time of biopsy. The correlation coefficient r and the probable error of the t-test were calculated and linear as well as parabolic and hyperbolic regression curves were drawn up.

Results

In the early stage of amyloidosis (grade I disease), one finds limited areas of BM infiltrated with amyloid spread across the whole glomerulus showing denudation on the urinary aspect (Fig. 1) These are barely detectable on light microscopy or in semi-thin sections. Electronmicroscopically the "aggressiveness" of these often minute amounts of amyloid toward the glomerular BM and especially toward the epithelial cells becomes apparent. The originally, probably subendothelial deposits of amyloid fibrils, infiltrate the BM and then perforate into the subepithelial space. During the course of this process, the foot processes of the epithelial cells disappear and are replaced by a continuous sheet of cytoplasm. The cytoplasm of the podocyte becomes invaginated by radially arranged fibrils of amyloid (Figs. 2 and 3). One can demonstrate on serial sections by electron microscopy that these conical bundles of amyloid fibrils "burrow" through the entire epithelial cell cytoplasm and penetrate into the urinary space, being surrounded by a thin cytoplasmic membrane. Despite the presence of severe renal insufficiency in some cases of grade III disease the protein excretion was highest in this group. The average daily excretion was 11,79 g with a range of 4 to 20 g/day – this was the highest in all grades. Noticeable in this group, with severe proteinuria, was an extensive destruction and infiltration of the glomerular BM with a dense network of amyloid fibrils. The basement membrane in such areas was often extensively denuded of its epithelial covering. In the two extreme cases, the BM denudation was spread diffusely across the entire glomerulus. Despite the same degree of amyloid infiltration in both cases (grade II disease), the quantitative results differed.

In the patient with the lowest serum protein concentration (2.9 g/100 ml – the patient had a severe form of the nephrotic syndrome – we found a total of 152 basement membrane denudations in 9 planes; in the other patient suffering from a similarly severe degree of renal amyloidosis, who did not have the nephrotic syndrome (serum protein concentration 7.45 g/100 ml) we found in 9 planes only 24 of such lesions.

The correlation between the average number of amyloid infiltrated basement membrane areas per case which are denuded of their epithelial covering and the level of the serum protein concentration at the time of biopsy is shown in the Table 1. It can be seen from this table that a significantly negative correlation exists between the lesions and the extent of the reduction of the serum protein concentration.

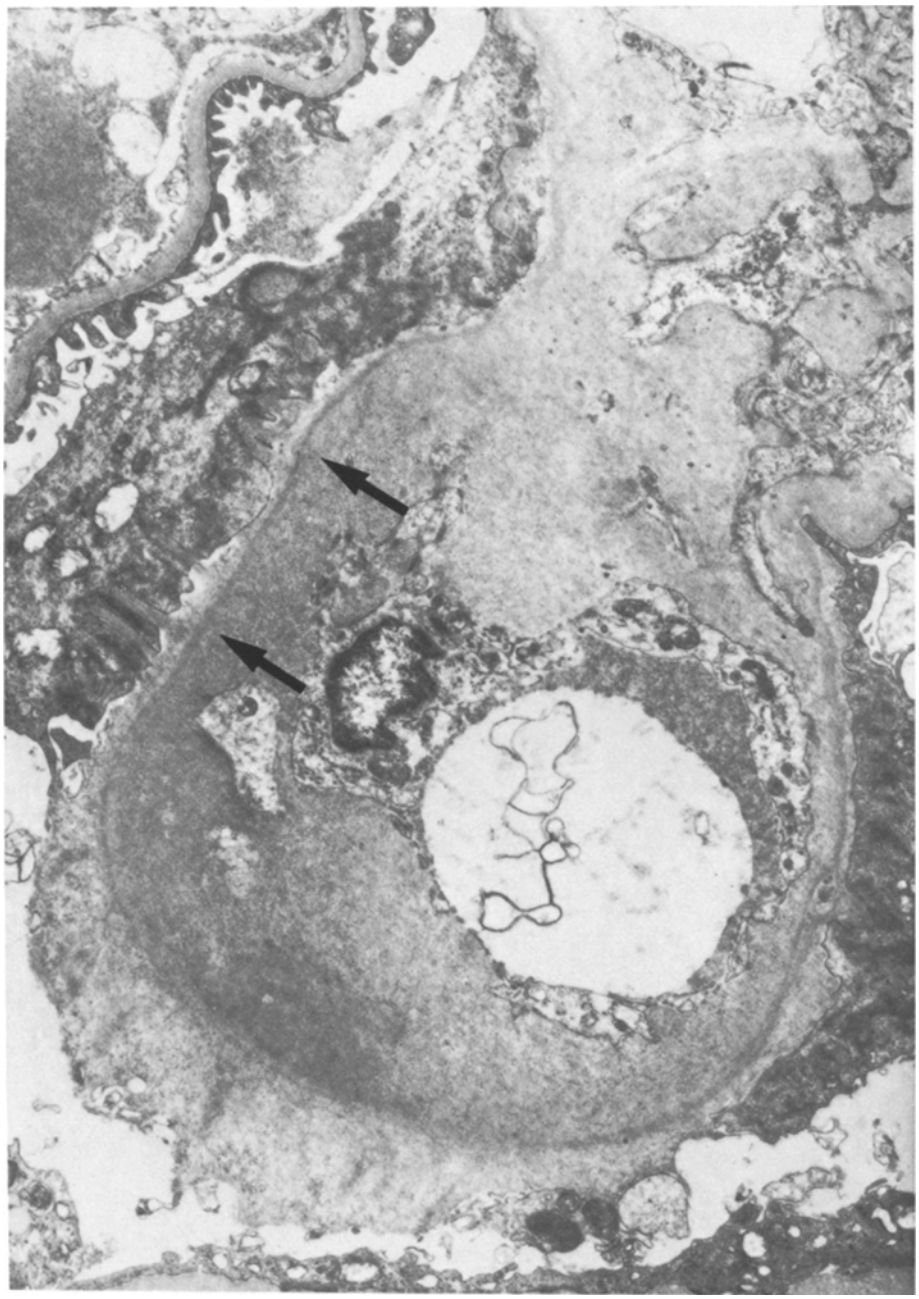


Fig. 2. Biopsy 75/7/328, 33 year old male, grade I amyloidosis with nephrotic syndrome. Total serum protein 4.4 g/100 ml, albumin 36% rel., proteinuria 2.5 g/day, serum creatinine 0.9 mg/100 ml. Capillary loop with extensive denudation of the urinary aspect of the BM. Epithelial cytoplasm invaginated by bundles of amyloid fibrils (↗). Endothelial cell detached from the BM by subendothelial amyloid masses. Upper left hand corner: normal BM with podocyte pedicels. 7 500:1

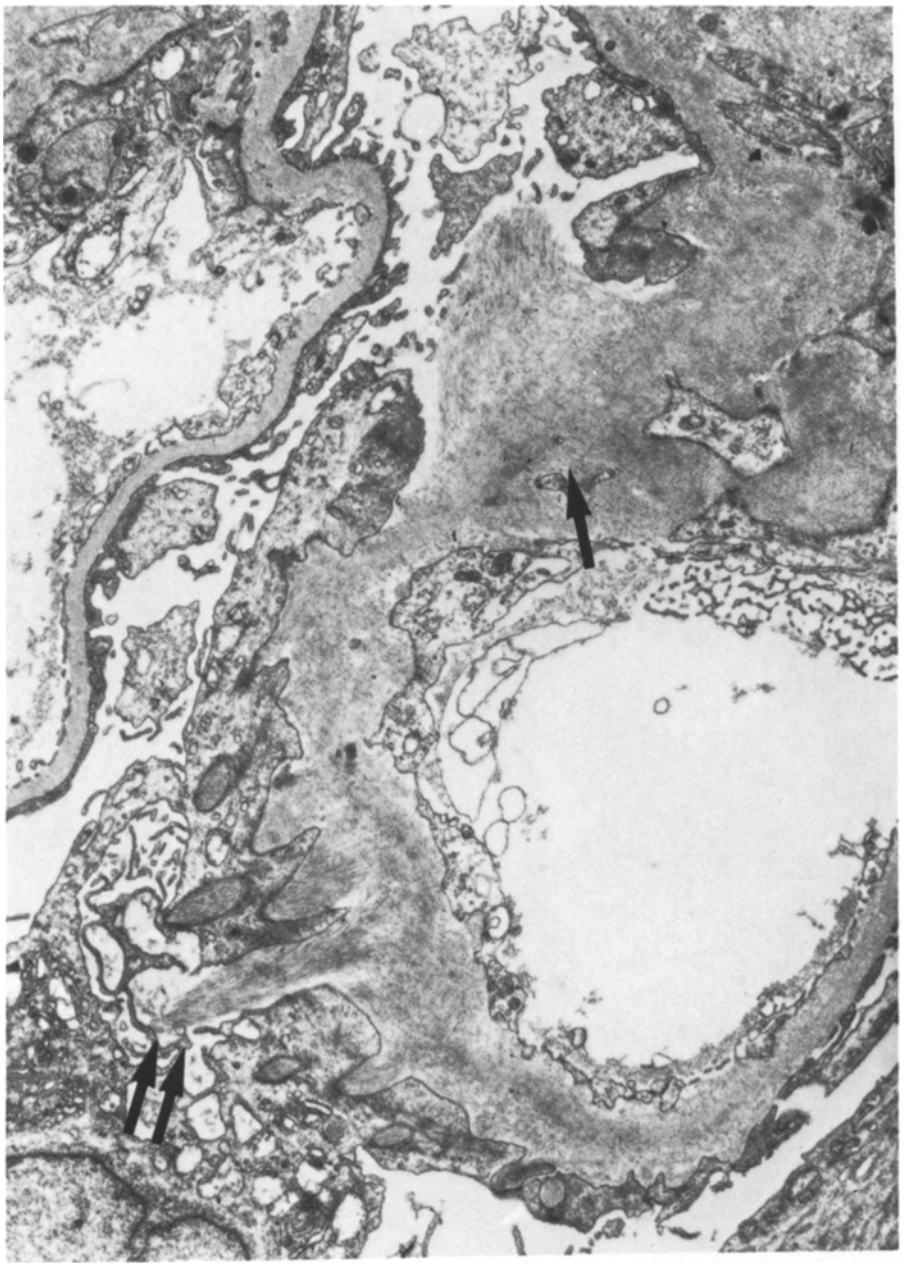
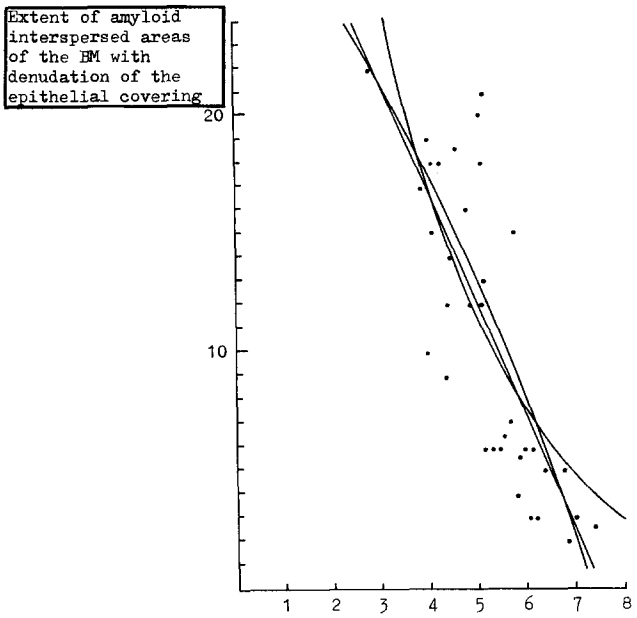


Fig. 3. Biopsy 77/6/1471, 17 year old male, grade II amyloidosis with severe nephrotic syndrome. Total serum protein 2.9 g/100 ml, albumin 26% rel., proteinuria 3.6 g/day, serum creatinine 0.7 mg/100 ml. Amyloid infiltrated BM area which is denuded of its epithelial covering (↗), amyloid fibrils transgressing into the urinary space. Bundle of amyloid fibrils, which has broken through into the urinary space (↗ ↗). Upper left hand corner: capillary loop with normal foot processes despite presence of nephrotic syndrome! 7 500:1

Table 1



x/y	Function	Correl. Coeff. r	t	Error probab. α
linear	$\hat{y}=35,77-4,74x$	-0,778	7,11	$< 0,0001$
parabolic	$\hat{y}=33,79-3,96x-0,07x^2$	-0,774	7,02	$< 0,0001$
hyperbolic	$\hat{y}=-10,7+\frac{109,14}{x}$	-0,739	6,30	$< 0,0001$

Discussion

The results reveal a statistically significant correlation between the extent of glomerular basement membrane damage at sites of disruption of the epithelial covering and reduction of totalum protein. These findings confirm previous assumptions (v. Gise et al., 1978) that basement membrane transformation with accompanying denudation of its urinary aspect is the most prominent histological lesion, associated with the high protein loss in glomerular amyloidosis. During the recovery stage, the membrane lesions become covered with a newly formed basement membrane, epithelial cells and foot processes (Fig. 5). Dikman et al. (1977), having studied patients that recovered from the NS due to secondary renal amyloid, have made similar observations. In the recovery stage, proteinuria disappears almost completely; nonetheless one can still demonstrate deposits of amyloid under the light, and electron microscope (Gise et al., 1978). Prerequisites for reduction of the proteinuria in amyloidosis appear to be the

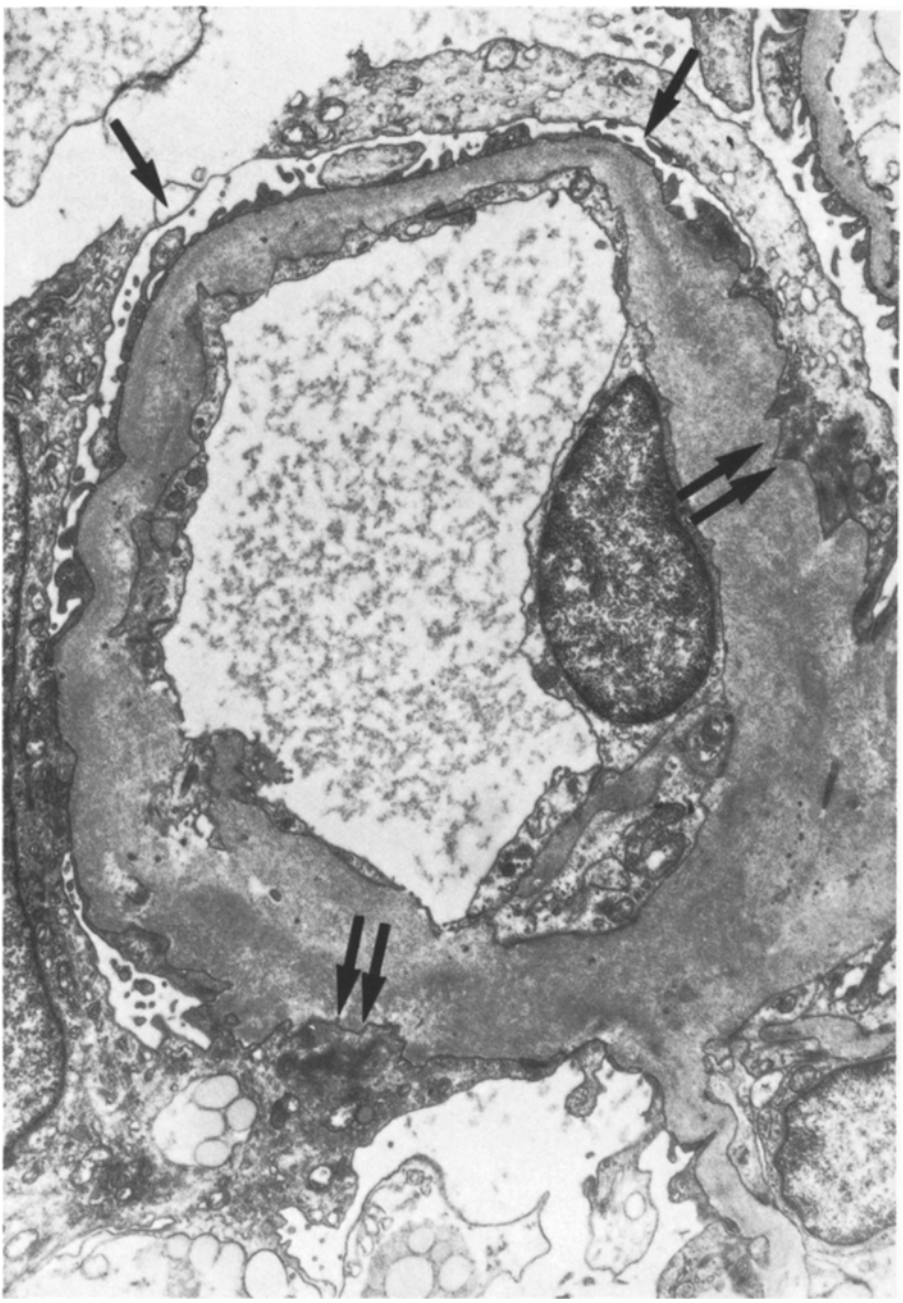


Fig. 4. Biopsy 75/7/606, 50 year old male, grade III amyloidosis without nephrotic syndrome. Total serum protein 6.2 g/100 ml, albumin 48.7% rel., proteinuria 3‰, serum creatinine 1.6 mg/100 ml. Typical capillary loop without denudation despite extensive infiltration of the BM. The podocyte pedicels are partly normal (↗), and in part replaced by a continuous sheet of cytoplasm (↗ ↗). 7 500:1

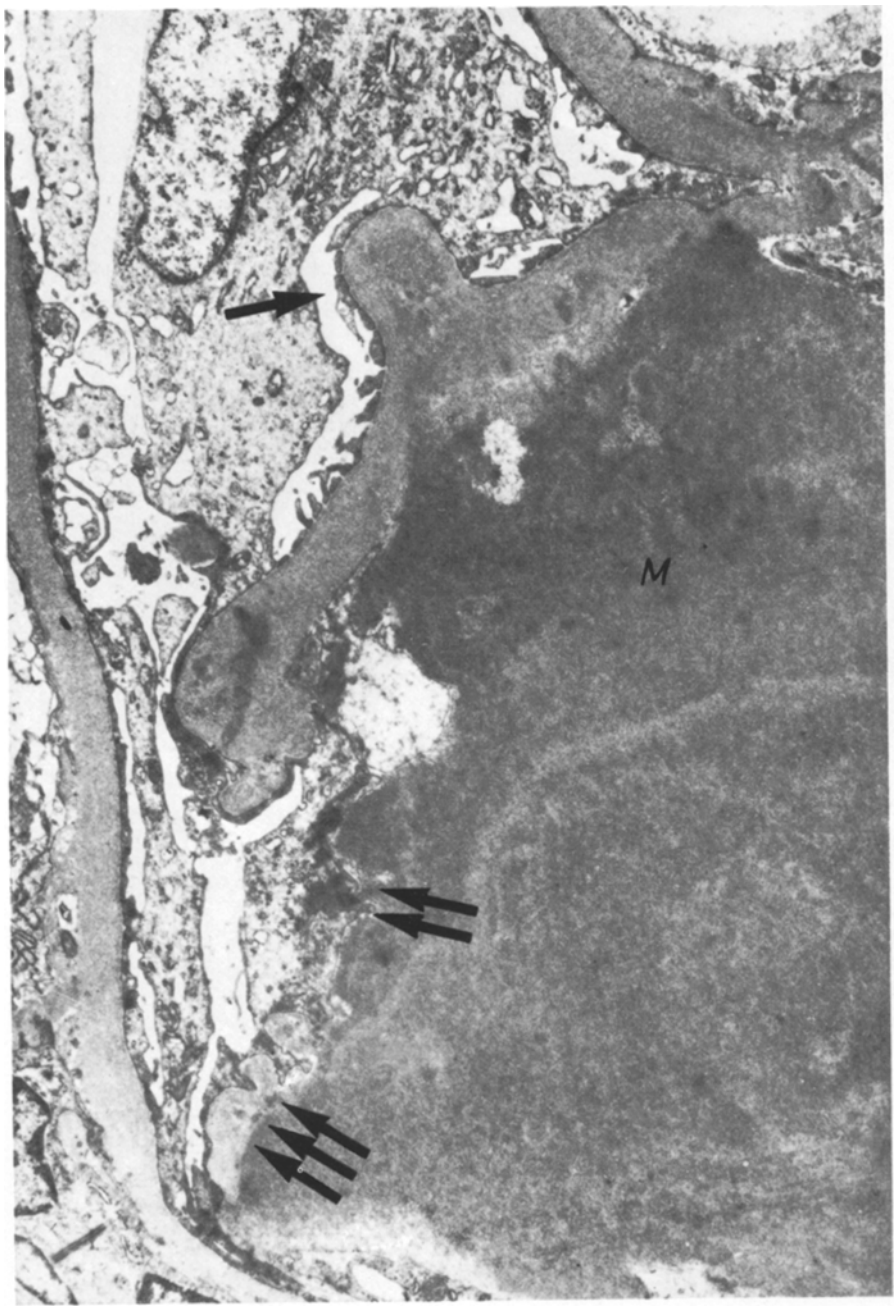


Fig. 5. Biopsy 76/12/160, 44 year old male, grade II amyloidosis during recovery, seven years after healing from a recurring pleural empyema. Total serum protein normal, proteinuria 0.8–1.1‰, serum creatinine 1.3 mg/100 ml. Extremely condensed amyloid masses in the mesangium (M). Newly generated BM surrounding an amyloid prolaps into the urinary space (↗). Epithelial cytoplasm immediately adjacent to the amyloid masses (↗↗).Site of generation of a new BM (↗↗↗). 7 500:1

covering of amyloid masses which had broken through into the urinary space by an epithelial cytoplasm, and the generation of a new basement membrane.

On our opinion the key structural changes that may explain the persistant heigh proteinuria in the stage of renal insufficiency are represented by those amyloid infiltrated areas of the BM which are completely denuded of their epithelial covering. Many of these lesions can already be observed in patients with grade I amyloidosis especially those suffering from the nephrotic syndrome (Figs. 1 and 2).

It has been demonstrated experimentally that "early amyloid" is no barrier for plasma proteins (Schultz, 1977) and that it may be perfused with plasmic substances. It is therefore not surprising that plasma proteins may escape in areas where amyloid has penetrated into the urinary space.

Our findings support the hypothesis that the epithelial cells may be the main barrier for serum albumin (Latta, 1970, 1975; Ryan and Karnovsky, 1975; Farquhar, 1975). As long as the continuity of the epithelial coverings is intact, protein loss will be small, despite advanced destruction of the BM by amyloid deposits (Fig. 4). On the other hand proteinuria will disappear when the amyloid changes from the "active" to the "inactive" form and when the amyloid deposits which had broken through into the urinary space become covered by podocytes and a new basement membrane (Fig. 5).

The reason for the enormously variable extent of basement membrane denudation in patients suffering from amyloidosis of similar severity remains unknown.

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