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Human Glomerular Amyloidosis – with Special Regard to Proteinuria and Amyloidogenesis*

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Glomeruläre Amyloidose des Menschen – unter besonderer Berücksichtigung der Proteinurie und der Amyloidogenese

Zusammenfassung. An Nierenbiopsien bei Nierenamyloidose wurden licht- und elektronenmikroskopische Untersuchungen ausgeführt, insbesondere im Hinblick auf die Zusammenhänge zwischen Amyloidose des Glomerulus und Proteinurie.

Auf der Grundlage einer Graduierung der amyloidotischen Glomeruli (Grad G 1: rein mesangialer Befall; G 2: mesangialer Befall mit Beteiligung der Schlingen weniger als 50%; C III mesangialer Befall mit Beteiligung der Schlingen mehr als 50%; G 4: amyloidotische Verödung) wurden die einzelnen Fälle je nach Vorherrschen des G-Grades graduiert (C I bis C IV). Es zeigte sich, daß die Mehrzahl der untersuchten Fälle den Graden C II und C III angehören. Es wurden aber auch 4 Fälle des Grades C I (ganz überwiegend rein mesangialer Befall, d.h. amyloidfreie Kapillarschlingen) mit Proteinurie bis 12 g/24 h beobachtet. Alle vier Gruppen (C I bis C IV) zeigten trotz extremer Unterschiede im Amyloidbefall vergleichbare Mittelwerte für die Proteinurie.

Bei der gemeinsamen (gepoolten) Auswertung der elektronenmikroskopischen Aufnahmen von Kapillarschlingen inclusive Mesangium aller untersuchten Fälle (C I bis C III) ergab sich folgendes: 1. 90% der getroffenen Mesangien enthielten fibrilläres Amyloid. 2. Amyloidhaltige Mesangien können in Verbindung stehen entweder mit amyloidhaltigen Schlingen (Typ a = 34%) oder mit Borderline-Schlingen (Typ b = 35%) oder mit normal strukturierten Schlingen (Typ n = 31%). Unter der vereinfachenden Voraussetzung, daß das Schnittbild für die gesamte Schlinge repräsentativ ist, bedeutet das, daß bei Nierenamyloidosen mit erheblicher Proteinurie im Mittel weit mehr als die Hälfte der glomerulären Kapillarschlingen amyloidfrei gefunden wird. In der Gruppe mit dem geringsten Amyloidbefall (C I) erweisen sich trotz Proteinurie bis 12 g/24 h im Mittel sogar 92% der Kapillarschlingen als amyloidfrei (Typ b und n).

Aus diesen und anderen Daten wird abgeleitet, daß die Permeabilitätserhöhung des glomerulären Filters unabhängig sein muß von der Anwesenheit morphologisch nachweisbaren fibrillären Anyloids an der Schlinge. Die wesentliche Bedeutung für den Filterdefekt wird vielmehr dem Vorgang der „Amyloidogenese" zugesprochen, der als solcher in die physikochemische Beschaffenheit des Gelfilters eingreift und der von der „Amyloidmanifestation" gefolgt ist. Auf dieser Grundlage lassen sich die erhobenen morphologischen Befunde und einige scheinbar widersprüchliche klinische Beobachtungen erklären.

Schlüsselwörter: Amyloidose – Glomerulus – Proteinurie – Elektronenmikroskopie – Amyloidogenese

Summary. Light and electron microscopic investigations were carried out on kidney biopsies in renal amyloidosis cases. Particular attention was paid to the relationships between glomerular amyloidosis and proteinuria.

On the basis of a grading of the amyloidotic glomeruli (grade G 1: only mesangium affected; G 2: mesangium affected with involvement of less than 50% of the loops; G 3: mesangium affected with involvement of more than 50% of the loops; G 4: amyloidotic obliteration), the individual cases were graded according to the predominance of the G grade into four grades (C I to C IV). It was shown that the majority of the cases investigated belong to grade C II and III. However, four cases of grade C I (predominant involvement of the mesangium only, i.e. the capillary loops were free of amyloid) with proteinuria up to 12 g/24 h were also observed. All four groups (C I to C IV) showed comparable mean values for proteinuria despite extreme differences in the degree of amyloidosis.

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In the pooled evaluation of the electron micrographs of capillary loops including the mesangium of all cases investigated (C I to C III), there was the following result:

1. 94% of the sectioned mesangia contained fibrillar amyloid.
2. Amyloid-containing mesangia can be associated either with amyloid-containing loops (type a = 34%), with borderline loops (type b = 35%) or with loops of normal structure (type n = 31%). Under the simplifying assumption that the section picture is representative for the entire loop, this means that in biopsies of renal amyloidosis grades C I to C III (proteinuria between 2.5 and 20 g/24 h) on average more than half of the glomerular capillary loops were found to be free of amyloid.
3. In relation to the individual case groups, the percentage of amyloid-containing loops is shown to differ. Indeed, in the group with the lowest degree of amyloidosis (C I) an average of 92% of the capillary loops proved to be free of amyloid despite proteinuria of up to 12 g/24 h (type b and n).

It is inferred from these and other data that the elevation in permeability of the glomerular filter must in principle be independent of the presence of morphologically demonstrable fibrillar amyloid in the capillary loop. On the other hand, the essential importance for the filter defect is accorded to the process of "amyloidogenesis", which interferes with the physicochemical characteristics of the gel filter and which is followed by "manifestation of amyloid". The morphological findings and a few apparently contradictory clinical observations can be explained on this basis.

Key words: Amyloidosis – Glomerulus – Proteinuria – Electron microscopy – Amyloidogenesis

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In the various forms of generalized amyloidosis, the kidney is involved in a high percentage of cases. Nevertheless, renal amyloidogenesis and its functional effects are still unclear in many respects. This includes the fine localization of the amyloid in the glomerulus (mesangium/loop) and its importance for understanding the severe proteinuria which occurs clinically and which results in the nephrotic syndrome. The investigations by Bergstrand (1968), Shirahama and Cohen (1967), Hinglais (1964) and Jao (1972) which have been presented so far show various deposition forms of amyloidosis in the glomerulus. Particular importance with regard to amyloidogenesis was attached to the mesangium and the glomerular basement membrane. On the one hand, Shirahama and Cohen ascribe active involvement to the mesangium cell in the formation of amyloid and possibly also in its degradation, in which the basement membrane is thought to be secondarily altered. On the other hand, other investigators tend to believe in a passive local synthesis (Bergstrand and Bucht 1968), or in a passive accumulation of flushed in fibrillar amyloid in the mesangium and basement membrane regions (Hinglais et al., 1964). However, until today it has not been established why depositions of amyloid are associated with certain predilection sites in the tissue despite varying protein components. By comparison of various stages of glomerular amyloidosis with the aid of light microscopic, electron microscopic and immunofluorescence microscopic investigations on human renal biopsy cylinders, further insights into the dynamics of renal amyloidosis may be obtained.

Observation Materials

A total of 35 renal biopsies from 35 patients with renal amyloidosis (23 male, 12 female) aged between 12 and 68 years old were examined histologically (of these, 11 simultaneous immunofluorescence microscopically and 9 simultaneous electron microscopically. 15 patients had a typical secondary amyloidosis with different underlying diseases (rheumatoid arthritis, chronic suppurations, generalized ichthyosis and malignant tumors). In 17 patients, the underlying disease was not known ("idiopathic" amyloidosis) and four patients had familial Mediterranean fever. In almost all cases, there was a severe proteinuria with protein excretion values between 2.5 g and 39 g/24 h (in one case, 1.8 g/24 h). In 30 patients, the proteinuria was combined with a nephrotic syndrome (Table 1)[1]

The electron microscopic results which are the focus of interest in the present study were obtained from 10 patients from the observation material specified above. The biopsy tissue could be examined with the triple combination: light microscopy, electron microscopy and immunofluorescence microscopy (Thoenes et al., 1978) (Tables 1 and 3).

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Table 1. Overview of cases arranged according to degrees of severity of glomerular amyloidosis

Patient number	Biopsy number B.-No.		Age (yrs)	Sex	Case grading	Protein-uria g/24 h	Basic disease	Blood Pressure mm Hg
1	B 4254		37	♀	C-I	12	Organizing Pneumonia Pulmonary cystic disease	130/90
2	B 4756	EM	60	♀	C-I	4.2	unknown	150/100
3	B 5332	EM	52	♀	C-I	2.5	unknown	–
4	B 6863	EM	51	♂	C-I	12	unknown	110/90
						m.v. 7.7 ±5.04[a]		
5	B 244		40	♂	C-II	6.8	unknown	–
6	B 734	EM	68	♂	C-II	4.5	Rectum carcinoma	115/60
7	B 897		57	♂	C-II	8	unknown	110/75
8	B 1308		71	♂	C-II	7	Pulmonary tuberculosis	150/85
9	B 1560		61	♀	C-II	3.5	Chronic pyelonephritis	155/95
10	B 1659			♀	C-II	4.2	unknown	–
11	B 1766		54	♂	C-II	20	unknown	90/55
12	B 2011		40	♂	C-II	16	Pleural empyema	–
13	B 3042	EM	19	♀	C-II	5.4	unknown	110/75
14	B 3344	EM	27	♂	C-II	3	Chronic osteomyelitis	130/90
15	B 3949		65	♂	C-II	12	unknown	120/70
16	B 4283		65	♂	C-II	9.5	Chronic bronchitis	140/90
17	B 4504	EM	24	♀	C-II	3.5	Erythema nodosum	115/65
18	B 5890		28	♂	C-II	5	unknown	145/95
19	B 5981		12	♂	C-II	7	Familial mediterranean fever	100/60
						m.v. 7.7 ±4.9[a]		
20	B 366	EM	16	♂	C-III	1.8	Rheumatoid arthritis	115/90
21	B 615		68	♂	C-III	15	Congenital Ichthyosis	150/90
22	B 1126	EM	53	♂	C-III	7.5	Familial mediterranean fever	
23	B 1344	EM	66	♂	C-III	3	Renal cell carcinoma	110/80
24	B 1684		61	♂	C-III	9.5	unknown	–
25	B 1768		59	♀	C-III	5	unknown	–
26	B 2021		57	♀	C-III	15	unknown	140/80
27	B 3321		28	♂	C-III	5.8	unknown	170/120
28	B 4862		51	♀	C-III	4	unknown	110/70
29	B 5455		24	♀	C-III	20	Familial mediterranean fever	–
30	B 5559		45	♂	C-III	8.1	Ankylosing spondylitis (Bechterew disease)	–
31	B 5661		30	♂	C-III	6.4	Familial mediterranean fever	–
						m.v. 8.3 ±5.54[a]		
32	B 1778		34	♂	C-IV	39	Pulmonary tuberculosis	120/90
33	B 4047		66	♂	C-IV	16	Osteomyelitis	140/90
34	B 4294		13	♂	C-IV	5	Hereditary amyloidosis	130/90
35	B 5473		56	♀	C-IV	2.5	Pulmonary tuberculosis; chronic pyelonephritis	
						m.v. 15.6 ±16.65[a]		

EM = Electron microscopically investigated

[a] In Proteinuria there is no significant difference between the 4 grades

Methods

Light Microscopy. Fixation with buffered 4% formaldehyde solution, embedding in Paraplast. Stains: hematoxylin-eosin, PAS-hemalum, trichrome according to Pearse (1950), in some cases Chromotrope silver methenamine (Ehrenreich and Espinoza 1971), Congo red according to Puchtler (1962). The green birefringence of the Congo red-positive tissue examined in polarized light served as a light microscopic demonstration for amyloid.

Electron Microscopy. One to three tissue cubes about 1 mm^3 in size were separated from the renal cortex of the biopsy cylinder under the stereomicroscope. Fixation in 3% buffered glutaraldehyde solution, postfixation in 1% buffered osmium tetroxide solution. Embedding in Epon. Preparation of semithin sections, staining: methylene blue and silver staining after Movat (1961). Preparation of ultrathin sections with the Reichert Ultrotome II, postcontrasting with uranyl acetate and lead citrate. Preparation of electron micrographs (original magnification 2000:1 to 40,000:1) with the Philips electron microscope 301.

Immunofluorescence Microscopy. Cryostatic sections from biopsy tissue freshly frozen in liquid nitrogen. Testing with anti-IgM, anti-IgG, anti-IgA, anti-C3, anti-C4, anti-fibrinogen from the goat (Meloy Company). The immuno-fluorescence microscopy investigations were performed by Prof. G.H. Thoenes, University Medical School, Munich-Innenstadt.

Results

Light Microscopy

In all the biopsy cylinders examined, amyloidosis of the glomeruli was revealed under the light microscope. All glomeruli displayed more or less severe amyloid deposits in the mesangium, whereas the capillary loops appear in some cases to be free of amyloid, and in some cases reveal amyloidotic changes of various extents. In order to be able to compare the individual glomeruli with each other, four grades were distinguished. These are based on the topography of the amyloidosis within the glomerulus:

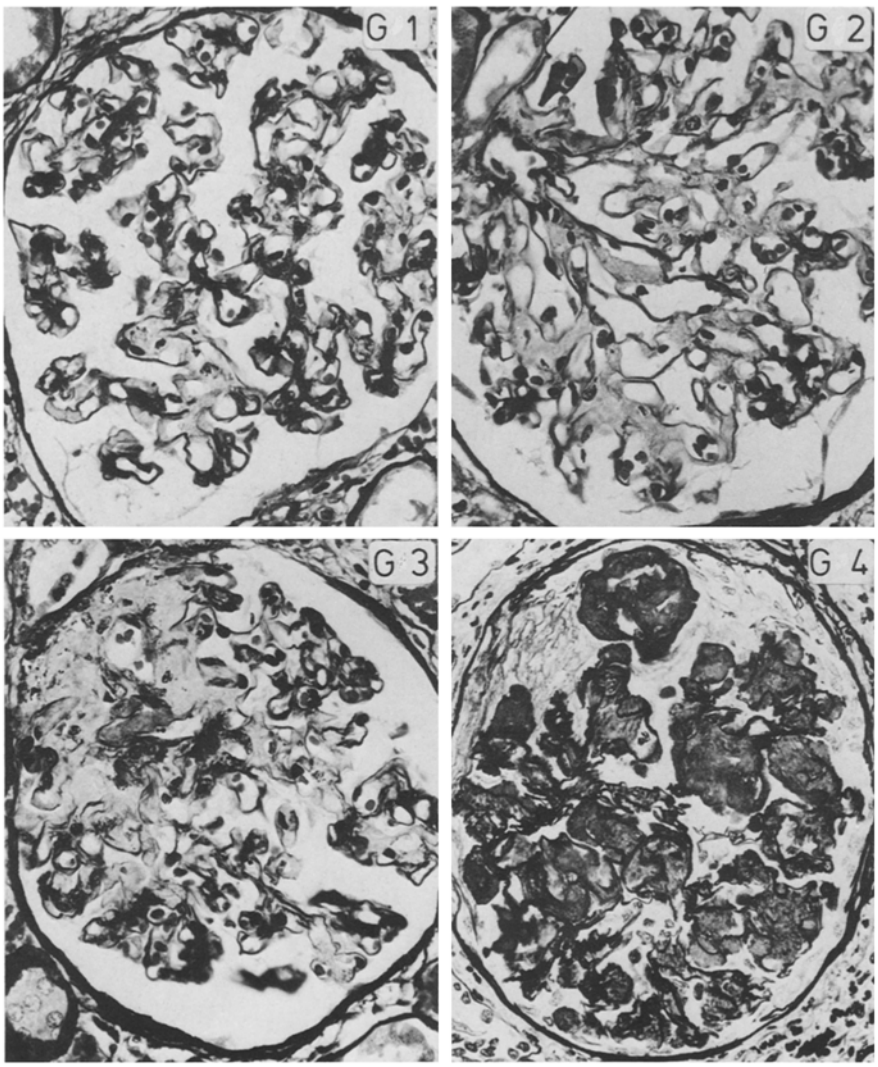

Fig. 1. Light microscopic grading of glomerular amyloidosis: *G 1* pure mesangial involvement, *G 2* mesangial amyloidosis with involvement of less than 50% of the loops, *G 3* mesangial amyloidosis with involvement of more than 50% of the loops, *G 4* total amyloidotic obliteration (Chromotrope silver methenamine, ×280

Glomerulus Grading (G 1 to G 4, Fig. 1a–d):
G 1: Only the mesangium affected under the light microscope
G 2: Mesangium affected with involvement of less than 50% of the loops
G 3: Mesangium affected with involvement of more than 50% of the loops
G 4: Total amyloidotic obliteration

This grading partially resembles that of Mackensen et al. (1977). However, their system comprised five grades and did not give special attention to the distinction of amyloidosis in the mesangium and in the loops which is significant for our study.

If the total number of glomeruli in our observation material (35 renal cylinders) is considered as a pool and subjected to the grading specified above, there is a distribution as shown in Fig. 2a. The majority of 445 glomeruli is to be assigned to the grades G 2 (120 glomeruli) and G 3 (123 glomeruli). 91 glomeruli are grade G 1 and 111 glomeruli grade G 4.

Case Grading (C I to C IV). Applying the above grading, the individual cases (biopsies) were classified with regard to the degree of severity of amyloidosis. For this, the glomeruli of one cylinder were graded and the grade which was most frequently found was assigned to the entire case.

Example. B 4504 with a total of 14 glomeruli:

Grade:	G 1	G 2	G 3	G 4
Number of glomeruli:	4	7	2	1
Case grading:	C II			

If an equal number of two grades are encountered in the evaluation, the case is assigned to the higher grade (as is usual in tumor grading).

In this way, the 35 cases which were examined light microscopically were graded as follows (Fig. 2b): the majority of the 35 cases corresponded to the grades C II (15 cases) and C III (12 cases). Only a few belong to the grades C I (4 cases) and C IV (4 cases).

Immunofluorescence Microscopy

A positive immunohistological result was obtained in a total of 9 out of the 11 cases. Intense homogenous staining was found in the mesangium. More rarely, there was delicate staining in the region of the glomerular capillary loops. All positive cases showed complement C 3 (of these, 7 cases were combined with C 4 and IgM and 4 cases were combined with IgG (see Fig. 3).

Electron Microscopy

Of the 10 cases investigated electron microscopically, 3 cases were assigned to grade C I, 4 cases to grade C II and 3 cases to grade C III in the light microscopic grading (Table 3). A total of 26 glomeruli were evaluated from these 10 cases: of these, 8 glomeruli were G 1, 9 glomeruli were G 2, 9 glomeruli were G 3. There was no glomerulus with grade G 4.

Because of the low section thickness, glomerular loops with simultaneous cutting of the mesangium and glomerular loops without cutting of the mesangium (the mesangium is in another section plane) are encountered in electron micrographs (these two situations are considered separately in Table 2). In evaluation of the electron micrograph, special attention was paid to a) the occurrence of amyloid fibrils in the mesangium and in the loops, b) alterations of the basement membrane and c) alterations of the glomerular cells, especially of the visceral epithelia (podocytes).

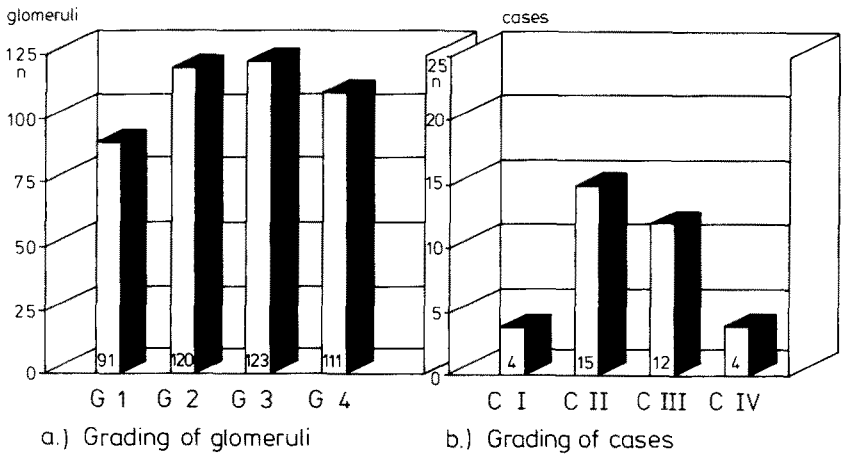

Fig. 2. Results of grading of the entire observation material a) glomeruli (pooled) b) individual cases

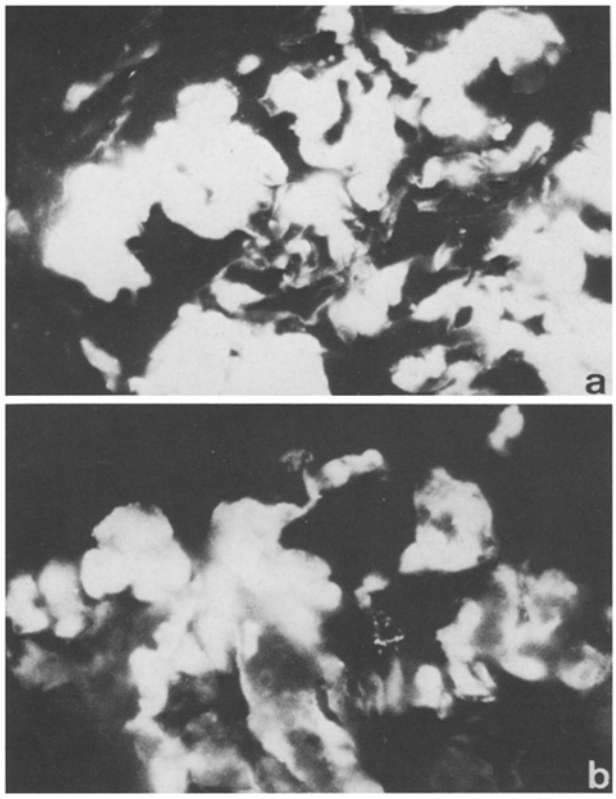

Fig. 3. Representative immunofluorescence microscopy findings (B 1344, grade C III): **a** anti-C_3 **b** anti-IgM (original × 100) (photographs: Prof. Dr. G.H. Thoenes, Munich

Mesangium. In amyloidosis of the mesangium, amyloid fibrils are found in the region of the mesangial matrix, which is apparently completely replaced by the amyloid substance (Fig. 4). The basement membrane in the region of the mesangium is in some cases still recognizable as such, and in some cases so penetrated by amyloid that it could no longer be recognized (Fig. 4b). The mesangium cells situated amid the amyloid substance are highly branched and rather rich in organelles. A detailed description will be dispensed with here.

Capillary Loops. (Fig. 5a–c, 6b): three types of capillary loops can be detected: *Loop Type a (amyloid)* (Fig. 5a): Amyloid fibrils are found in some cases in sections and in some cases on the entire circumference. With less intense amyloidosis these are only found on the side turned to the endothelium (Fig. 5a). In more intense amyloidosis, amyloid fibrils are also localized in the entire region on the epithelial side of the basement membrane; they are interwoven with the basement membrane substance and occasionally form cushions (in severe amyloidosis as found in G 3). The contours of the basement membrane may be lost. The endothelium is only altered sporadically (loss of the pores, solid cell branches instead of the pores), it is displaced in the direction of the capillary lumen above extensive amyloid deposits.

The behavior of the lining epithelium is not uniform in the amyloidotically altered loop. However, the transformation of the podocytic pedicles into broad sheets of cytoplasm (> 1.5 µm contact) predominates (Fig. 5a, Tab. 2). The epithelial lining is almost always continuous even when amyloid is deposited subepithelially (Fig. 4b). Focal breaks in the epithelial lining were found only occasionally (in our observation material only three times) in a higher grade amyloidosis (G 3) and then only in the mesangial region. We were thus unable to find regularly occurring denudation of basement membrane, which is regarded by v. Gise et al. (1978) as the cause of the proteinuria in amyloidosis

Loop Type b (borderline) (Fig. 5b, 6b): In the section plane, no amyloid can be discerned electron microscopically on the entire circumference. However, in these capillary sections one finds apart from a few lining epithelia with normal foot processes (contact breadth up to a maximum of 1.2 µm) abundant pathologically altered epithelia with broad cytoplasm sheets (contact breadth > 1.2 µm). The behavior of the basement membrane is variable. It appears morphologically to have a normal structure over long distances. However, in sectors the lamina densa can be widened, the basement membrane substance may be disintegrated and not sharply delimited on the endothelial side, so that the lamina rara interna is no longer shown up clearly. The endothelium does not show any appreciable alterations. Figure 6a shows a detail from a G 1 glomerulus and mesangial amyloid in which all cut loops correspond to the amyloid-free loop type b.

Loop Type n (normal) (Fig. 5c, 6b). Normal structure.

On the basis of this typing, the analysis of the electron micrographs obtained from the 26 glomeruli with a total of 173 capillary loops (original magnifications for this evaluation 5,000:1) leads to the results shown in Table 2.

It is shown that the various parameters show an appreciable variability in the mutual assignment. The following statements can be made (Table 2):

1. Amyloid is predominantly found in the mesangium (129 out of 136) (Table 2, 1.1). However, there are a small number (7 out of 136) of amyloid-free mesangial sections (Table 2, 1.2). The capillary loops belonging to these amyloid-free mesangial areas are always free of amyloid and have a normal structure (loop type n) (Table 2, 1.2).

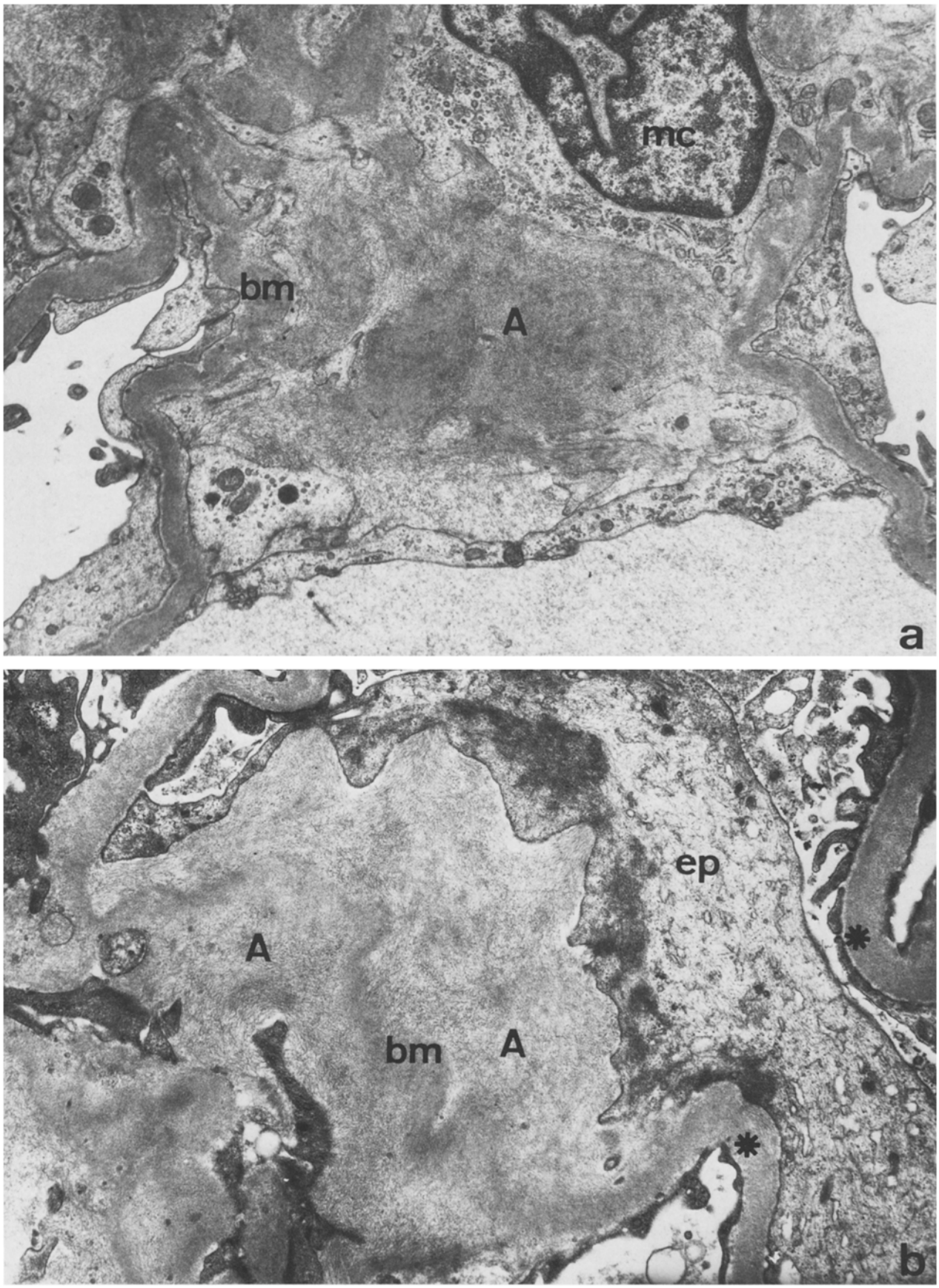

Fig. 4. **a** Amyloid (*A*) in the region of the mesangial matrix between mesangial cells (*mc*) and basement membrane (*bm*) (11,500:1) **b** mesangial amyloid (*A*) on both sides of the (in some cases) still demonstrable basement membrane (*bm*). Continuous epithelial sheat (*ep*) over the amyloid deposit. Amyloid-free portions of loops (*) (16,330:1)

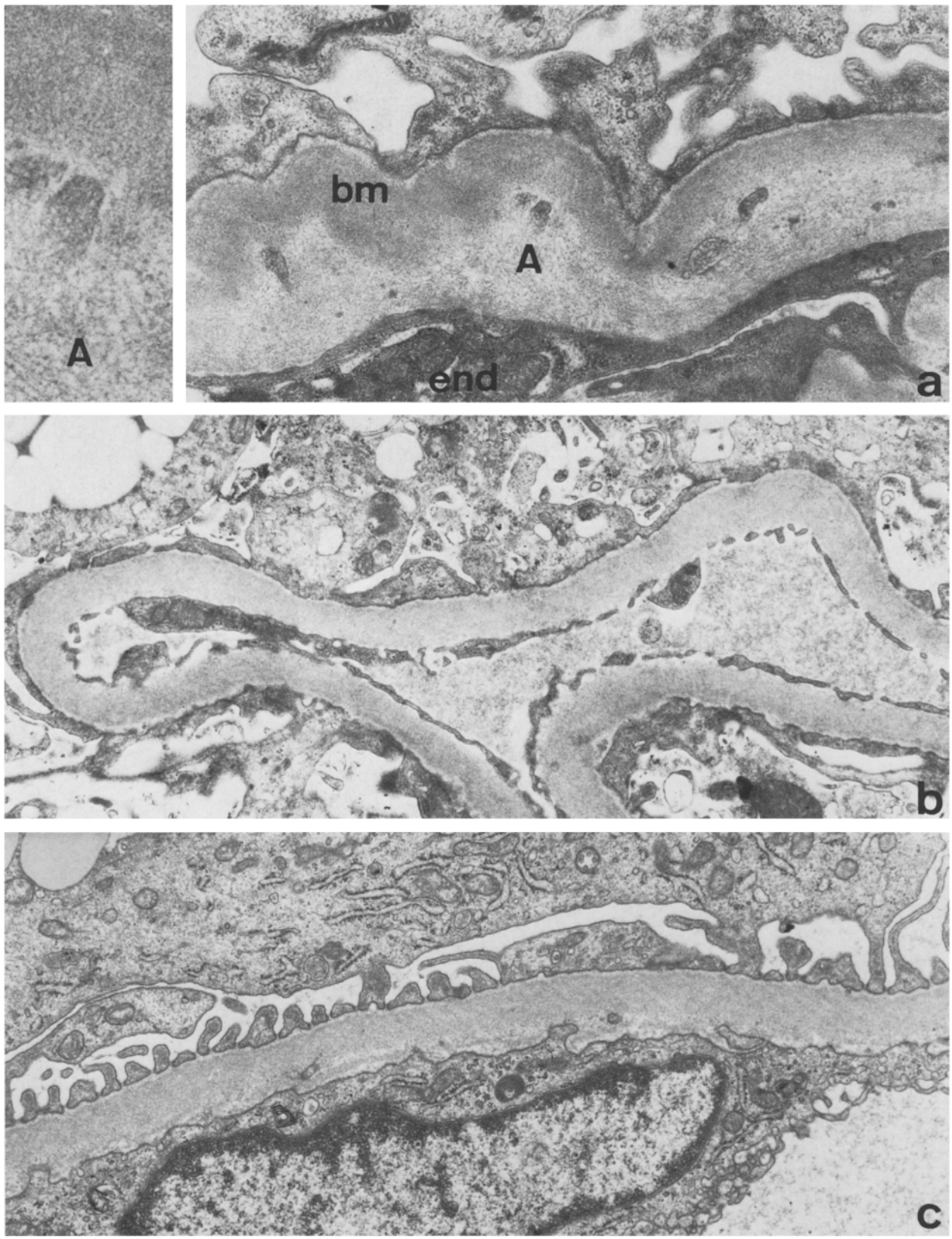

Fig. 5. Loop types from glomeruli with mesangial amyloid. **a** loop type a = amyloid (*A*) between endothelium (*end*) and basement membrane (*bm*) (25,200:1) **b** Loop type b = amyloid-free, but irregular contours of the lamina densa of the basement membrane and broadened or fused podocytes (17,000:1). **c** Loop type n = amyloid-free and normal structure (18,300:1)

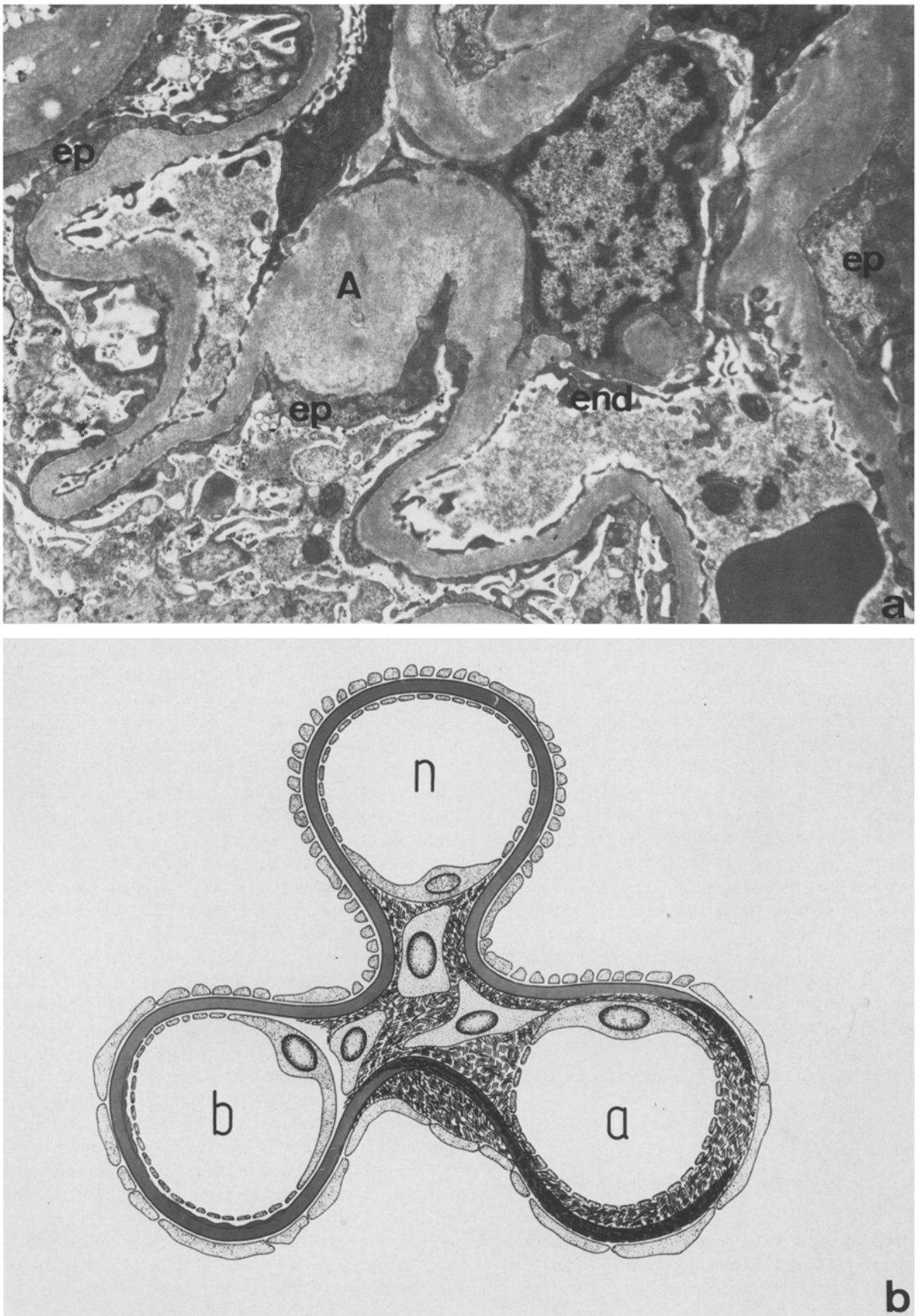

Fig. 6. **a** Detail of glomerulus with mesangial amyloid (*A*) and capillary loops of type b (*ep*=visceral epithelium, *end*=endothelium) (10,350:1) **b** Schematic representation of the possible electron microscopic findings in glomerular amyloidosis (grade G 1 to G 3) with various loop types (*n*=normal, *b*=borderline, *a*=containing amyloid)

Table 2. Results of electron microscopic evaluation on the glomerular capillary loops including mesangium

	Number of loops n	Loop type		
		Type a	Type b	Type n
1. Loops including Mesangium	136	44	45	47
1.1 Mesangium *with* Amyloid	129 (100%)	44 (34%)	45 (35%)	40 (31%)
1.2 Mesangium *without* Amyloid	7	0	0	7
2. Loops without Mesangium	77 (100%)	23 (30%)	36 (47%)	18 (23%)
3. Loops total	213	67	81	65
4. Podocytes				
4.1 normal	65	3 (4%)	0	62
4.2 altered	148	68 (96%)	80	0
Total	213	71 (100%)	80	62

2. If the sectioned area of mesangium contains amyloid, only a proportion (44 out of 129=34%) of the adjacent capillary loops likewise shows amyloid (loop type a). The others are either normal (type n: 40 out of 129=31%), or they are pathologically altered (45 out of 129=35%) corresponding to the loop type b (Table 2, 1.1).

3. Capillary loops of type a are predominant (96%), but not always associated with broadened or fused foot processes of the visceral epithelium (Table 2, 4.2). This means at the same time that amyloidotically altered basement membranes (at least events in the section plane concerned) may also have podocytes of normal structure in a small percentage of cases (3 out of 65=4%) (Table 2, 4.1). We are thereby able to confirm the results of v. Gise (1978).

Discussion

A. Light Microscopic Findings in Glomerular Amyloidosis

The results of light microscopy reveal amyloidotic alterations of the renal glomerulus of various degrees. In order to be able to characterize better the extent of this glomerular amyloidosis and in order to be able to compare the individual glomeruli with each other, we have established the four degrees of severity (G 1 to G 4) of glomerular amyloidosis defined above. In this grading, the topography of the amyloidosis within the glomerulus is at the forefront, with special emphasis given to the relation amyloid in mesangium/amyloid on the peripheral loop. This topographical relation is of special significance, since in the early stage of amyloidosis the deposition of amyloid in the mesangium precedes that at the peripheral capillary loop. This points to the mode of spreading of glomerular amyloidosis (Thoenes 1969; Beneke 1971). However, it is evident that this gradation does not only permit statements regarding the topography of the amyloid within the glomerulus, but also regarding the intensity and quantity of the amyloid deposition. In this connection, our gradation touches on that of Mackensen et al. (1977) which was already dealt with above.

We proceeded with the analysis in two steps:
a) Grading of the amyloidotic glomeruli of all renal biopsies in a pool without relation to the individual case (G 1 to G 4).
b) Grading of the glomerular amyloidosis of the individual cases in this way meant that the most frequently found Grade G was assigned to the respective case (C I to C IV). Figure 2a shows that the majority of the amyloidotically altered glomeruli evaluated in the pool is to be assigned to the grades G 2 and G 3. Grade G1 was found less, and grade G4 frequently. However, it is to be taken into account in the increased incidence of the grade G 4 that two biopsy cylinders with a pronounced amyloidosis contained an unusually large number (23 and 30) glomeruli compared to the other cylinders.
The frequency distribution in the gradation of the cases also corresponds to this (Fig. 2b). C IV is represented there with a similar rarity as C I, whereas grade C II and C III predominate.

From the frequency distribution of the pooled glomeruli on the one hand and of the individual cases on the other hand, the following overall statement can be made: glomerular amyloidosis of the kidneys is diagnosed in most cases in stage C II or C III by a renal biopsy occasioned as a rule by a (mostly large) proteinuria (Table 1). From the grading of the pooled glomeruli (Fig. 2a), it can be seen at the same time that there is a trend to a higher degree of severity, i.e. to deposition of amyloid in the peripheral portions of the loops. It is furthermore remarkable that the few cases diagnosed in the early stage (C I, mainly purely mesangial involvement) already displayed a large proteinuria (2.5 g, 4.2 g, 12 g and 12 g/24 h). This observation will be dealt with in more detail in the discussion of the electron microscopic findings (part B).

B. Electron Microscopic Findings in Glomerular Amyloidosis

Compared to light microscopy, the results of electron microscopy give further information with regard to

1. demonstration of smaller amounts of amyloid which may not be detectable by light microscopy,

2. structure of the capillary wall constituents, especially of the basement membrane and of the epithelium, the foot process fusion of which is regarded as a special feature of massive proteinuria.

The data reproduced in Table 2 relate to the totality of all micrographs obtained in the electron microscopic investigation without relation to individual cases. In this way, biological principles in the course of glomerular amyloidosis should be revealed. This also applies in view of the limitation that the electron micrographs only represent a limited portion of the loop convolution.

In agreement with earlier authors (Movat 1960; Suzuki 1963; Hinglais 1964; Shirahama 1967; Thoenes 1969; Jao 1972; Watanabe 1975) the electron microscopy results indicate that amyloid within the glomerulus is always deposited first in the mesangium: amyloid-containing mesangia with adjacent amyloid-*free* capillary loops were found repeatedly, but not the reverse situation (amyloid-containing loops without simultaneous amyloid deposition in the mesangium) (Table 2, 1.). It appears interesting that in glomeruli with mesangial amyloid deposition, two types of amyloid-free loops (type n and type b) can be observed. Loops with normal structure (type n) imply that amyloidosis of the mesangium by no means immediately gives rise to a more morphologically apparent alteration of the adjacent loops. It is probable even if it cannot be proved by morphology alone that such loops are also functionally intact.

More difficult to evaluate is the amyloid-free loop type b, which is characterized by the increased occurrence of broadened or fused epithelial foot processes and occasionally by a blurred delimitation of the subendothelial basement membrane. The relatively frequent occurrence (80 out of a total of 213 investigated loops = about 33%, Table 2) indicates that this is a loop lesion which precedes amyloid deposition (borderline). This hypothesis is also supported by the occasional observation of loops which display type b alterations *and* type a alterations (subendothelial amyloid deposits) side by side.

C. Relation Between Glomerular Amyloidosis and Proteinuria

The source of the proteinuria in amyloidosis is to be sought in raised permeability of glomerular capillary loops. The question arises as to whether the present investigations permit a statement concerning the structural substrate of this raised permeability. The assumption that the extent of the amyloidosis of the

Table 3. Results of electron microscopic findings in relation to case grades and proteinuria

Light microscopy			Electron microscopy							
Case Grading	Biopsy Number B.-No.	Proteinuria g/24 h	Number of examinated Glomeruli	Mesangium		Loop type			Podocytes	
				without Amyloid	with	a	b	n	normal	altered
C-I	B 4756	4.2	4	3	7	3	10	19	19	13
C-I	B 5332	2.5	2	1	13	2	12	22	22	14
C-I	B 6863	12.0	2	9	11	3	23	7	7	26
Total		m.v. = 6.2	8	13	31	8	45	48	48	53
C-II	B 734	4.5	3	0	2	2	3	1	1	5
C-II	B 3042	5.4	2	0	6	9	0	1	1	9
C-II	B 3244	3.0	3	2	5	6	5	2	2	11
C-II	B 4504	3.5	1	0	14	8	4	7	9	10
Total		m.v. = 4.1	9	2	27	25	12	11	13	35
C-III	B 366	1.8	1	1	12	12	10	6	6	22
C-III	B 1126	7.5	3	0	15	17	6	0	1	22
C-III	B 1344	3.0	5	0	11	6	7	0	0	13
Total		m.v. = 4.1	9	1	38	35	23	6	7	57

glomerular capillary loops determines the extent of the glomerular filter defect and thus parallels the level of proteinuria is disproved by Table 1. Although from grade C I to grade C III (by definition) the amyloidosis of the glomerular capillary loops increases from less than 10% to a maximum of 100%, the level of the proteinuria and the variation of individual values is practically the same in the three groups (mean values: C I: 7.7 g/24 h; C II: 7.7 g/24 h; C III 8.0 g/24 h; differences not significant, see Table 3). Only C IV shows a higher mean value (15.6 g/24 h) due to one case B 1778) with the extreme value of 39 g/24 h among only a small number of cases. The electron microscopic investigation material (Table 3) also confirms this observation: Despite the increasing proportion of amyloid-containing loops (type a) from C I to C III, the mean proteinuria has the same level in the three groups.

Group C I is especially informative in this connection. These are cases with predominantly pure amyloidosis of the mesangium and consequently mainly amyloid-free capillary loops (only 8 out of 101 = ca. 8% of the electron microscopically investigated loops revealed presence of amyloid = loop type a, see Table 3). Nevertheless, two patients are found in this group who show a proteinuria of 12 g/24 h at the time of the renal biopsy. It is extremely improbable that the few amyloid-containing loops with a small amount of amyloid are the only cause of such a massive proteinuria. On the contrary, it must be assumed on the basis of these results that amyloid-free loops and particularly those of type b show a raised permeability of protein. This means in turn that the appearance of amyloid in the glomerulus, even if only in the mesangium (G-1) signalizes a pathological process which alters the permeability properties of the capillary loops, irrespective of whether light and electron microscopically demonstrable amyloid substance is present on the capillary loops.

The lack of correlation between the extent of the amyloid deposition in the loops and the degree of proteinuria indicates that the glomerular permeability disorder is associated with the amyloidosis in a different way than is previously assumed. It means that it is less a question of amyloid *deposition* than of amyloid *formation*, i.e. of the amyloidogenesis and the macromolecular and physicochemical processes at the filter associated with this.

D. Amyloidogenesis in the Glomerulus

There are three possible explanations for the genesis of amyloidosis in the glomerulus:

a) Amyloid is produced in the glomerulus itself with mediation of cells of the glomerular tissue, especially the mesangial cells (Shirahama and Cohen 1967).

b) Amyloid is supplied to the glomerulus in a ready-synthesized fibrillar form, and is preferentially deposited in the mesangium and in an increasing quantity also on the loops (Hinglais et al. 1964).

c) Fibrillar amyloid is formed in the glomerulus after amyloid precursors are supplied and increases as long as amyloid precursors are supplied from the body.

For the following reasons, we should like to give preference to thesis c:

1. According to knowledge available so far on the composition of amyloid substance, this consists a) of a fibrillar protein part, b) of the so-called P component and c) of a largely undefined matrix which contains as constituents mucopolysaccharides, fibrinogen and complement components (Wegelius 1975). If it is taken into account that in generalized amyloidosis (renal amyloidosis is always a submanifestation of generalized amyloidosis) the amyloid protein type AL consists of immunoglobulin light chains or their fragments (Glenner 1971) and that the protein type AA is formed from the serum type SAA (Rosenthal et al. 1976; Benditt and Erikson 1977) or SAAL (Linke 1978, 1979) by proteolysis, it can be regarded as substantiated that at least the lower molecular protein constituents reach the glomerulus via the blood.

2. It could be rendered probable (Schneider and Loos 1978) that the P-component constitutes a local tissue component which is found preferentially at sites at which amyloid is deposited in generalized amyloidosis. The nature of the P-component is not yet sufficiently known. However, it appears appropriate to look for an association with a special type of connective tissue proteoglycans.

3. Even without taking into consideration the remaining not yet clarified amyloid components (mucopolysaccharide, fibrinogen, complement) it is thus to be expected that fibrillar amyloid is formed in the glomerulus by combination of a protein component foreign to the glomerulus supplied by the blood and a locally synthesized (proteoglycan?) component. The latter is so to speak incorporated into the fibrillar amyloid. Seen in this way, the fibrillar amyloid which can be demonstrated in the glomerulus is the morphologically recognizable end product of a process which is initiated outside the glomerulus, but which proceeds in the glomerulus. Because of its "consuming" intervention in the ground substances of the connective tissue including the basement membrane, it alters their physicochemical state.

Under the assumption that the proteoglycan suitable for amyloid formation is more abundantly pre-

sent in the mesangial matrix than in the basement membrane (lamina densa) (see Kanwar and Farquhar 1979), the mesangial predilection in the initial stage of amyloidogenesis would be explained. The topographical and substantial vicinity of the lamina rara interna to the mesangial matrix makes it comprehensible in addition that the spread of amyloidosis to the loop preferentially takes place subendothelially (Thoenes 1969; Beneke 1971). The extent to which the immunohistologically demonstrated binding of anti-C 3 (Fig. 3a) points to an importance of the complement for amyloidogenesis which cannot yet be assessed at present.

What has been said above, indicates the changing state of the glomerular filter as the basis of proteinuria is linked to a continuous *process of amyloidformation.* Here, two subprocesses must be distinguished: I. *Amyloidogenesis,* which comprises the macromolecular processes as a prior condition of fibrillar amyloid and the resulting physicochemical alterations in the state of the basement membrane or of the "gel filter", and II. the *manifestation of amyloid,* i.e. the morphological demonstrability of the fibrillar amyloid. From this is derived the following: In the course of amyloidosis (whether it is related to the body or to the tissue unit concerned), amyloidogenesis naturally precedes manifestation of amyloid. However, the two processes later superimpose and proceed in parallel, as a rule to the same extent until the patient dies in consequence of a generalized amyloidosis (Fig. 7a).

If for any reason (e.g. spontaneous or therapeutically induced subsidence of an underlying disease), the amyloidogenesis comes to a stop, the situation in Fig. 7b would result: After the amyloidogenesis has ceased, the deposition of fibrillar amyloid once formed continues to exist in the capillary wall, since the amyloid substance is largely resistant to breakdown mechanisms, but the interferences in the physicochemical state of the glomerular capillary filter which are notoriously associated with amyloidogenesis are arrested.

Two so far incomprehensible observations can be plausibly explained on this basis: 1. the observations of Dikman (1977) and Gafni (1978), who were able to observe a complete regression of the proteinuria after successful treatment of a chronic burn disease and of familial Mediterranean fever patients, although severe amyloid deposits in the glomeruli continued to be found in biopsies (corresponding to phase y in Fig. 7b). 2. In our observation material not only cases of grades C II to C IV but also those of grades C I are in some cases highly proteinuric, although in the latter case the proteinuric glomerular capillary loops are practically free of amyloid. According to our conception, these glomerular capillary loops are in the state of amyloidogenesis (corresponding to phase x in Fig. 7a).

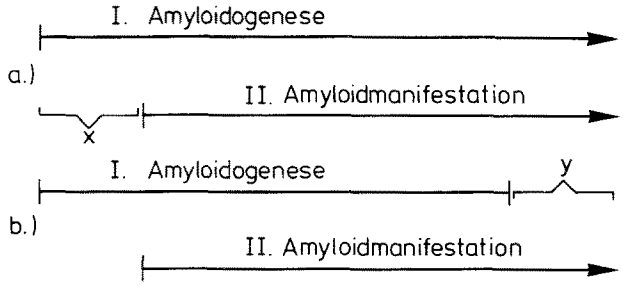

Fig. 7. Scheme for time relation of amyloidogenesis and amyloid manifestation. **a** Amyloidogenesis precedes amyloid manifestation (*x*), the two are combined with each other later. **b** In inactivation of the underlying disease, the manifestation of amyloid may last longer than amyloidogenesis (*y*)

Theoretically, it would be required that there are amyloid patients who do not yet show any amyloid in the glomeruli but nevertheless suffer from proteinuria, because the glomeruli are in the phase of glomerular amyloidogenesis (before the glomerular manifestation of amyloid). One of our cases of grade C I (B 6863 in Tables 1 and 2) approaches its postulate very closely, since only very small mesangial amyloid depots were found in 3 out of 27 otherwise light microscopically normal glomeruli with a proteinuria of 12 g/24 h.

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