Virchows Arch. A Path. Anat. and Histol. 369, 87—109 (1975)

Basement Membrane-Changes in Membranoproliferative Glomerulonephritis

A Light and Electron Microscopic Study

D. Anders and W. Thoenes

Institut für Cytobiologie und Cytopathologie der Universität Marburg/Lahn
Pathologisches Institut der Universität Mainz (Direktor: Prof. Dr. W. Thoenes)

Received July 5, 1975

Summary. This study is based on 31 renal biopsies from 28 patients with idiopathic membranoproliferative glomerulonephritis (MPGN). 18 cases were classified as "pure" MPGN, 10 as lobular GN. For light microscopy two staining procedures were found to be of particular value: Pearse trichrome (PAS+Orange G) and Jones-Chromotrope (methenamine silver + Chromotrope 2R). These techniques reveal conspicuous basement membrane (b.m.) lesions which are not observed in other types of GN and are characterized by thickening of the b.m. and a bright orange or red coloration, respectively, which can well be separated from normal (PAS-positive or argyrophilic) basement membranes.

Electron microscopy was performed in 13 cases in order 1. to analyze the fine structure of this lesion, 2. to match the results with the current subclassification concept which is essentially based on the discrimination of intramembranous dense (IMDD) and subendothelial deposits. The typical finding of a continuous intramembranous electron-dense material which proves to be argyrophilic in ultrathin sections was present in 3 (23%) of the cases.

In the remaining 10 cases the irregular and more pronounced thickening of the glomerular b.m. was mainly due to the accumulation of a medium dense fine granular material which in uranyl nitrate and lead citrate impregnated sections cannot clearly be separated from altered b.m. material. In these instances silver impregnation of ultra-thin sections reveals an abnormality which is characterized by 1. discontinuity of the lamina densa, 2. loss of argyrophilia and 3. circumscribed or diffuse, sometimes extreme thickening of the b.m.

On the one hand this frequently occurring type of lesion differs from IMDD in that the abnormal material is neither electron-dense nor argyrophilic. On the other hand it cannot easily be defined as "subendothelial deposit" in that the basement membrane itself is involved in the process, presumably representing a substantial transformation of the b.m. material.

The observations support the view that MPGN and lobular GN can be characterized as a pathohistological entity on the basis of these two types of b.m. lesion which, however, may not be appropriately described in terms of "deposits".

Introduction

Idiopathic membranoproliferative glomerulonephritis (MPGN) (Royer *et al.*, 1962; Habib *et al.*, 1973), also referred to as mixed membranous and proliferative (Burkholder *et al.*, 1970), mesangiocapillary (Churg and Duffy, 1972; Cameron *et al.*, 1973) or parietoproliferative GN (Bariéty *et al.*, 1971) has been of particular interest during recent years. Among other forms of chronic glomerulonephritis (GN) it is characterized by a specific histological pattern (Allen, 1955; Jones, 1957; Royer *et al.*, 1962; Meadows, 1973). Many authors now agree that MPGN and lobular GN represent one clinico-pathological entity (Cameron *et al.*, 1970; Bariéty

and Druet, 1971; Mandalenakis *et al.*, 1971; Michael *et al.*, 1971; Habib *et al.*, 1973). Gotoff (1965) and West (1965) recognized the close relationship between MPGN and persistent hypocomplementemia which in certain cases may precede the renal lesion (Peters, 1974). This association, however, has lateron proved to be an inconstant finding (West *et al.*, 1965; Cameron *et al.*, 1973). Clinical diagnosis of MPGN therefore cannot rely on serology (Gotoff *et al.*, 1965; Herdman *et al.*, 1970) but rests on renal biopsy (West *et al.*, 1973) with demonstration of the typical histological features: greatly enlarged glomeruli with thickening and double contour appearance of the basement membranes associated with marked hypercellularity of the glomerular tufts and an increase of mesangial matrix. Apart from this general appearance a considerable variety of histological phenomena associated with a predominant fixation of anti-C3 in the immunfluorescence microscopic pattern (Ogg *et al.*, 1968; Vallota *et al.*, 1971; Peters and Williams, 1974; Berger *et al.*, 1974; Verroust *et al.*, 1974) have lead the main working groups to stress different points of view. Habib and coworkers (1973, 1974) have offered a subclassification of idiopathic MPGN into different morphological types depending on the presence of "intramembranous dense" or "subendothelial deposits", either with "pure" MPGN or with lobular pattern (centrolobular sclerotic bodies). According to these authors a close correlation seems to exist between the dense deposit type and persistent hypocomplementemia.

The term "dense deposit" was introduced by Berger and his coworkers (Berger and Galle, 1963; Galle, 1962) and has subsequently been used by french authors mainly (Ganter and Berger, 1963; Faye *et al.*, 1971; Antoine and Faye, 1972) but lateron has found wide acceptance (McDonald, 1972; Jenis *et al.*, 1974) to describe a fine structural basement membrane lesion characterized by an electron-dense material lying in the middle of the glomerular b.m.. This peculiar b.m. lesion is said to be routinely demonstrated by light microscopic examination of paraffin sections with different staining techniques, Habib and coworkers (1973) preferably using a trichrome-light green stain.

It is the purpose of this paper to present the results of a different approach to the morphological characterization of the b.m. changes observed in MPGN. So far the exact origin and nature of IMDD are unknown. There is increasing evidence, however, that IMDD basically differ from what is generally described as an (immune-) deposit, e.g. in true membranous GN. The term "deposit" therefore may turn out to be inappropriate to describe this particular alteration of the b.m. in MPGN.

Our preliminary results included the observation that the presence of subendothelial deposits does not exclude the association with an altered b.m. material. We therefore decided to subclassify our cases according to the presence or absence of b.m. changes, with or without the occurence of an electrondense intramembranous material. Consequently this study is mainly concerned with the detailed characterization of glomerular b.m. changes. There is evidence based on the electron microscopic study of silver impregnated ultra-thin sections suggesting that in MPGN two types of b.m. lesions may exist: one is characterized by a silver-negative (non-argyrophilic) alteration of the b.m. material, the other one by the presence of an electron-dense intramembranous material which retains the silver-positive (argyrophilic) properties of the lamina densa.

Material and Methods

Selection of the Series. During the past 4 years the histological diagnosis of MPGN was established in 37 patients. Three of these had repeat biopsies. In all of the 40 biopsy specimens the following criteria had to be fulfilled: 1. Diffuse irregular thickening of the glomerular capillary wall with or without double contour appearance, 2. diffuse mesangial hypercellularity and increase of mesangial matrix or matrix like material, 3. glomerular enlargement due to these two. 4. The lobular pattern was considered eligible even in the absence of marked capillary wall thickening.

Nine of the 37 cases were excluded from this study because of proven or suspected systemic lupus erythematosus (4), Schönlein-Henoch's purpura (1), paraproteinemia (1), or due to the focal and segmental character of the histological lesion (2) and too small a number of glomeruli present in the biopsy specimen (1). The remaining 28 patients are listed in Table 1.

Age and Sex Distribution. Thirteen patients were under the care of pediatricians while 15 were adults, the age of the total group ranging between 7 and 55 years (average: 18 years). The male-to-female ratio was 15:13.

Clinical Data. The main characteristics of the clinical course in each patient are also listed in Table 1 (left). The presenting signs of renal disease varied between chance proteinuria (two patients with "pure" MPGN; 7%), insidious beginning of the nephrotic syndrome (65%), or an acute onset of the illness including bouts of gross hematuria (28%). During the further course the nephrotic syndrome was by far the most frequently occurring sign (93%) associated with hypertension in 14 (50%) and/or renal insufficiency in 7 (25%) of all cases. The duration of illness prior to the biopsy was extremely variable ranging from 1 month to 15 years.

Serum Complement. Measurements were obtained from 26 patients (93%) by the laboratories of the cooperating hospitals. During the last two years all of them have been using a standardized radioimmunodiffusion technique, and serum-C3-levels have been recorded to be low if they were below 80 mg per 100 ml. Serial measurements have been performed only in a few cases, but there are 14 patients (50%) in whom all of at least two measurements showed the serum-C3-level to be depressed. In the group of the 10 patients with lobular GN a normal C3-level was observed only once.

Morphological Laboratory Techniques

Light Microscopy. Biopsy material for light microscopy was fixed in 4% formaldehyde with phosphate buffer (pH 7, 4), embedded in paraffin, and cut to 3–4 μ sections. Routine staining included hematoxylin and eosin, PAS, Goldner's trichrome-light green, and Pearse' trichrome stain. (PAS + Orange C, also referred to as "tri-PAS"; Pearse, 1950, 1961). In addition Jones-Chromotrope-2R stain (Ehrenreich and Espinosa, 1971) was used as a technique which in paraffin sections combines methenamine silver impregnation of normal b.m., mesangial matrix, and matrix like material with a red coloration of silver-negative structures consisting of proteins or proteinaceous material such as "fibrinoid" or "hyalin".

Electron Microscopy. Material for electron microscopic study was fixed in phosphate buffered (pH 7.2) 3% glutaraldehyde solution during transportation, thereafter in phosphate-buffered (pH 7.4) 1% osmium tetroxid solution for 1–2 hours, embedded in Epon 812, and impregnated with uranyl nitrate and lead citrate or with methenamine silver (Movat, 1961).

Immunfluorescence Microscopy. Except for 6 cases from which the findings were kindly provided by the immunobiological laboratories of the cooperating hospitals, most of the frozen sections were examined by Dr. G. H. Thoenes (Laboratory of Immunobiology, I. Dep. of Internal Medicine, University of Munich) using anti-IgM, anti-IgG, anti-IgA, anti-C3 and anti-fibrinogen from the goat (Meloy, Springfield, Ill., USA) (G. H. Thoenes, 1973).

Results

The main morphological findings obtained from the individual biopsies are listed in Table 1. Representative cases were chosen to demonstrate the light and electron microscopic details in Figs. 1–9 according to the above mentioned sub-

Table 1 summarizes the individual clinical and morphological data. The main morphological
Cases 1—18 represent the histological pattern

	Clinical data							All patients (28 cases)				
	isolated proteinuria	nephrotic syndrome	gross hematuria	erythrocyturia	hypertension	renal insufficiency	serum-C3/β_{1C} (n = normal, l = low)	patient nr.	biopsy nr. (r = repeat biopsy)	male/female	age (years)	duration of illness before biopsy (y = years, m = months)
%	7	93	28	32	50	25	54					
n	2	26	8	9	14	7	>2× low: 14					
"pure" MPGN		+					ll	1	108	f	16	4 y
	+			+			ll	2	167	m	18	1 y
		+		+	+	+	lll	3	250	f	41	12 y
		+					l	4	272 r	m	19	10 y
		+		+	+	+	○	5	297	m	31	6 m
		+	+		+		ll	6	363	f	12	1 m
		+		+	+		n	7	372	m	9	3 m
		+		+	+		llnn	8	389	m	9	3 m
		+					nnnnn	9	430 r	f	32	3 y
		+			+		l	10	863	f	23	15 y
		+	+		+		ll	11	875	m	16	4 y
		+			+	+	l	12	907	m	36	1 m
		+			+		llnnn	13	999	f	34	5 y
		+			+		ll	14	1128	m	9	3 m
	+		+				l	15	1323	f	55	1 m
		+	+		+	+	ll	16	1465	m	11	3 y
		+	+				n l	17	1507	f	14	3 m
		+		+	+		l	18	1641	f	14	2 y
lobular pattern		+	+		+	+	lll	19	441 r	m	13	3 y
		+	+				l	20	500	f	38	1 m
		+			+		○	21	607	f	28	5 y
		+		+		+	lll	22	785	m	33	1 y
		+		+			ll	23	839	f	7	6 m
		+				+	lll	24	924	m	17	6 y
		+	+				ll	25	1206	m	20	?
		+		+			ll	26	1385	m	9	2 m
		+					n	27	1564	f	32	1 m
		+					ll	28	1795	m	15	2 m

[a] In these two cases light microscopy was kindly performed by Prof. R. Habib's laboratory

classification which may be achieved by three diagnostic steps: 1. the distinction of "pure" MPGN and lobular GN, 2. the light microscopic characterization of b.m. changes, namely with silver impregnation, and 3. the electron microscopic examination with respect to the presence or absence of the electron-dense intramembranous material.

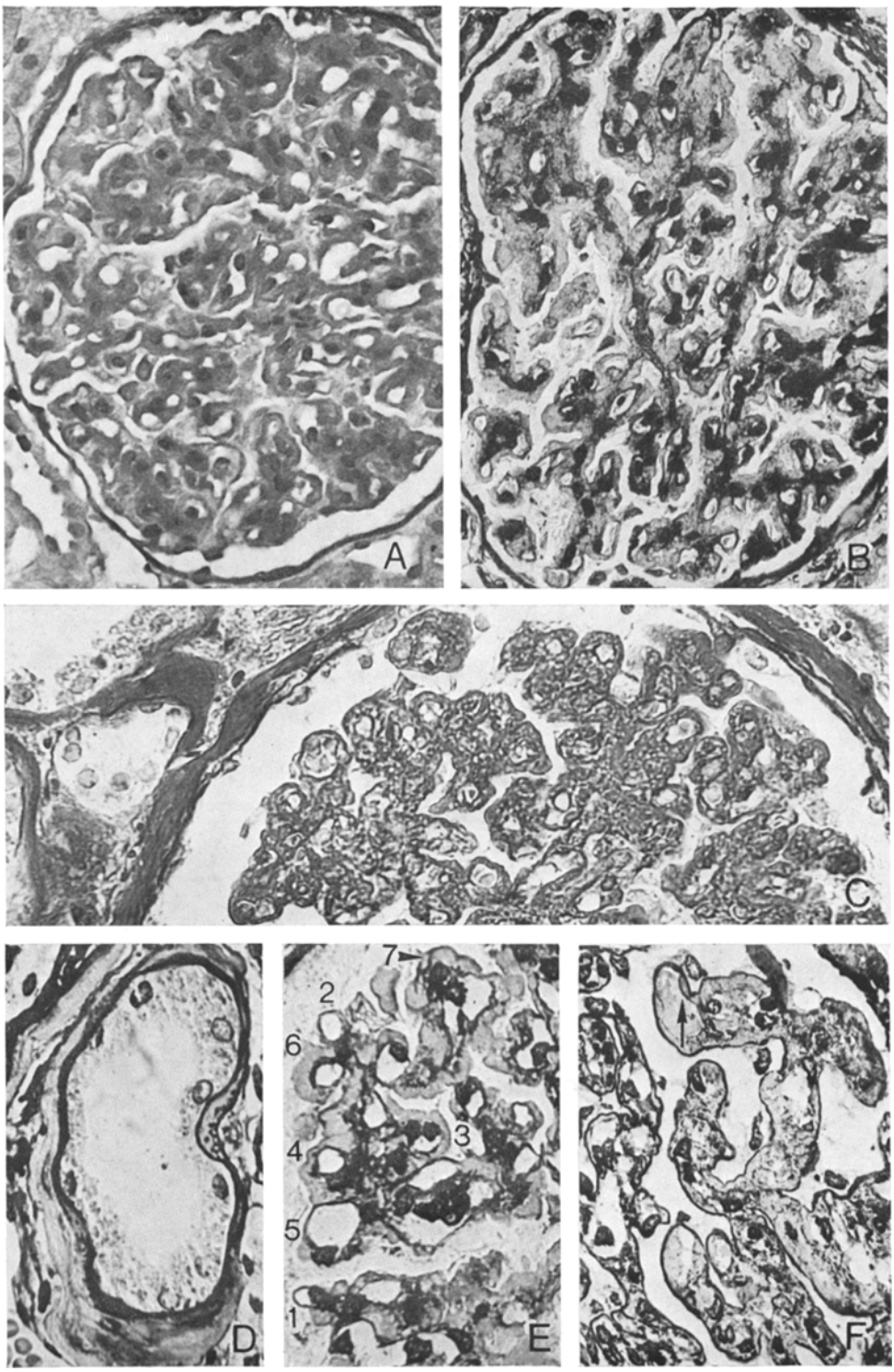
criteria on which the selection of this series was based (see text) are not listed here. of "pure" MPGN, cases 19–28 the lobular type

Light microscopy (26 cases)				Electron microscopy (13 cases)						Ifl microscopy (16 cases)			
										significant fixation of			
										Anti-Ig			Anti-
Pearse trichrome: Orangeophilia of b.m.	J. Chr. 2R.: loss of argyrophilia	related changes of tubul./Bowman's b.m.	exsudative component	mesangial interposition	thickening of glomerular b.m.	subendothelial deposits	intramembranous dense material	intramembr. defects	mesangial fibrillar mass	G	M	A	C3
73	65	15	15	100	100	77	23	46					44 (isol.)
19	17	4	4	13	13	10	3	6	5				7
+	+			+	+	+							
+	+												
+	+												
+	○			+	+	+							
○	○		+										
+	○												
+	+									+	+	○	+
n.d.[a]		+		+	+		+	+					
+	+												
+	+	+		+	+	+		+		+	+	+	+
+	+			+	+	+	+	+		○	○	○	+
○	○		+										
+	+			+	+	+		+		○	○	○	+
+	+												
○	+									○	○	○	+
+	+			+	+	+				○	○	○	+
+	+									+	+	○	+
n.d.[a]			+	+	+	+				○	○	○	+
+	○			+	+	+			+	○	○	○	+
○	+	+								+	+	○	+
○	○									+	+	○	+
+	+			+	+	+		+	+				
○	+	+		+	+		+		+				
+	+			+	+				+	○	+	+	+
+	○		+	+	+	+		+	+	+	○	+	+
+	+									○	○	○	+
+	○									+	+	○	+
○	○									+	○	○	+

at Paris in cooperation with the Universitäts-Kinderklinik Heidelberg (Prof. K. Schärer).

Light Microscopy

Eighteen cases (64%) were classified as "pure" MPGN whereas 10 cases (36%) presented a lobular pattern as defined not only by the lobulated aspect of the glomerular capillary tuft but mainly by the presence of centrolobular sclerotic nodules (Fig. 3). This distinction was not unequivocally clear in two cases (6 and


A
B
C
D
7
2
6
3
4
5
1
E
F

17) which support the concept of "pure" MPGN and lobular GN being variants closely related to each other. The special histological findings observed in b.m. with the aid of Pearse trichrome and Jones-Chromotrope stains will later be discussed in detail. A conspicuous uptake of Orange G (Pearse) by certain b.m. portions was observed in 19 (73%) of the cases (Figs. 1A, C, 2A, B). Due to methenamine silver Jones-Chromotrope provides the possibility of a more exact localization of any b.m. lesions; partial or complete loss of argyrophilia was observed in 17 (65%) of the cases (Figs. 1B, E, F, 2C, D, 3C).

Related tubular and/or capsular b.m. changes with the same staining characteristics (silver-negative, Orange-G- and Chromotrope-positive; Fig. 1C, D) were found in 4 cases (15%). Tubular b.m. changes which usually include considerable thickening of the b.m. were considered to represent a significant finding only in the absence of tubular atrophy.

A conspicuous increase of polymorphonuclear granulocytes in the glomerular capillary lumens (in Table 1 referred to as "exsudative component") was obserced in 4 other cases (15%).

Epithelial crescents mostly occuring in a focal pattern were observed in 5 cases (20%). They were considered to reflect a more severe degree and segmental accentuation rather than a different type of the lesion.

Electron Microscopy

Valuable electron microscopic results were obtained from 13 of the 28 patients. The following fine structural characteristics of MPGN could be demonstrated (Table 1):

1. Interposition of mesangial cytoplasmic extensions and layers of a fine fibrillar matrix like material between the glomerular b.m. and endothelial cells (Figs. 4–7) were observed to a greater or lesser extent in all cases (100%). This phenomenon was always associated with mesangial hypercellularity and marked increase of the mesangial matrix (Figs. 6A, 7A), or of a fine fibrillar matrix-like material, respectively, which in lobular GN (Fig. 9B) accumulates to the point of sclerotic nodule formation.

2. Fine structural changes involving the glomerular b.m. itself may consist in:

a) Mild or moderate continuous thickening of the b.m. due to the presence of a "linear" homogeneously electron-dense material as observed in 3 cases (23%; Figs. 5, 7).

Fig. 1A–F. Light microscopic findings in "pure" MPGN (A) The main characteristics of MPGN: diffuse thickening of capillary walls, narrowing of capillary lumina, mesangial hypercellularity; case 11 (Pearse, ×400). (B) Same type of lesion, Jones-Chromotrope; non-argyrophilic b.m. material (grey; original: red) can well be separated from argyrophilic material, e.g. mesangial matrix (black). Note capsular involvement (lower right); case 13 (×400). (C) Detail from glomerulus exhibiting "splitting" and Orange-G-positive thickening of capillary walls. Capsular and tubular b.m. involvement; case 10 (Pearse, ×500). (D) Non-argyrophilic thickening and double contour appearance of tubular b.m.; case 10. (E) Demonstration of the various b.m. changes which may simultaneously occur: normal b.m. (1), partial (2) or complete (3) loss of argyrophilia, associated with thin subepithelial and subendothelial argyrophilic layers (4), hump-like subepithelial "deposit" (5), aspect of circumferential subepithelial (6) and subendothelial (7) deposition of non-argyrophilic material; case 10. (F) Subendothelial deposits (arrow) in the absence of b.m. changes; case 12. (D–F) Jones-Chromotrope (×600)

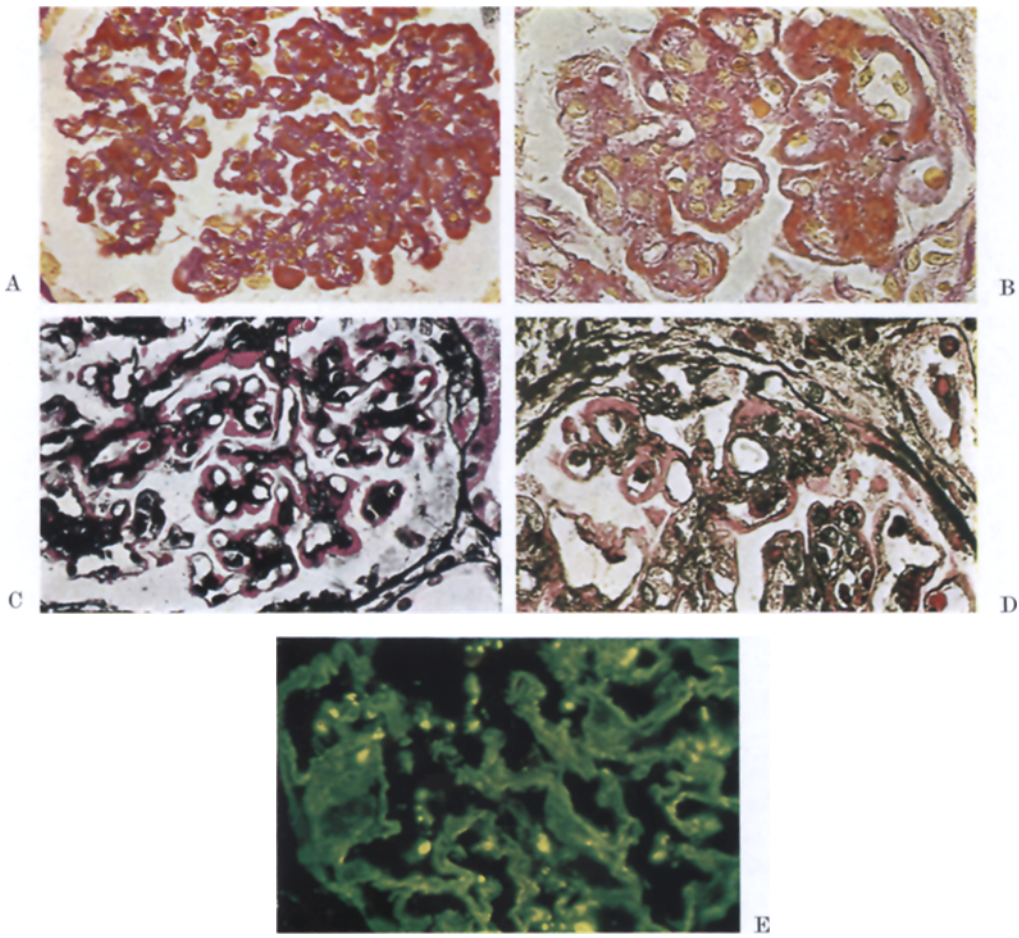


Fig. 2A–E. Membranoproliferative GN (pure). (A) and (B) Pearse trichrome stain: thickening of PAS-positive capillary walls exhibiting not only splitting, but also conspicuous orangeophilia of the glomerular basement membrane. (C) and (D) Jones-Chromotrope 2 R-stain: In place of orangeophilia in Pearse trichrome stain non-argyrophilic basement membrane material can be demonstrated, which is well separated from argyrophilic material, e.g. mesangial matrix. Compare the normally thin argyrophilic basement membrane portion in (C), lower left. (E) Immunohistologically coarse granular deposits of C3′ are shown in the thickened glomerular basement membrane and in the mesangium (Fig. E Courtesy of Dr. G. H. Thoenes, München)

Fig. 3A–E. Light microscopic findings in lobular GN; (A and E) with electron-dense intramembranous material and "single contour" appearance (for details see Fig. 7); (B–D) without electron-dense intramembranous material but with "double contour" appearance of the glomerular b.m. (A) Clear delineation of the thin b.m. (case 23) as compared to B (case 24). Otherwise similar aspect (Pearse, ×300). (C) Argyrophilic centrolobular nodules and occasional double contour appearance of capillary loops (arrow); case 22 (Jones-Chromotrope, ×400).

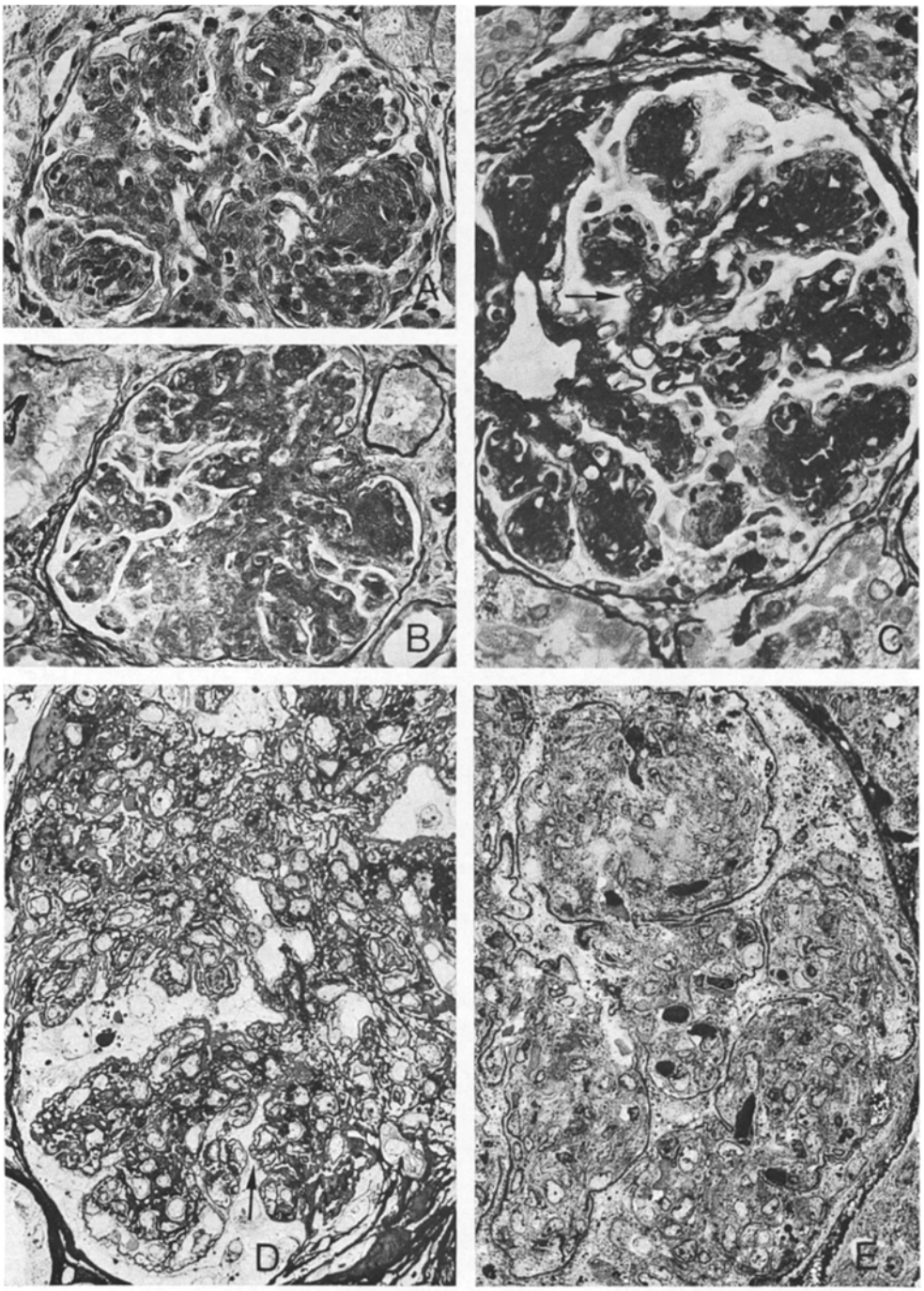
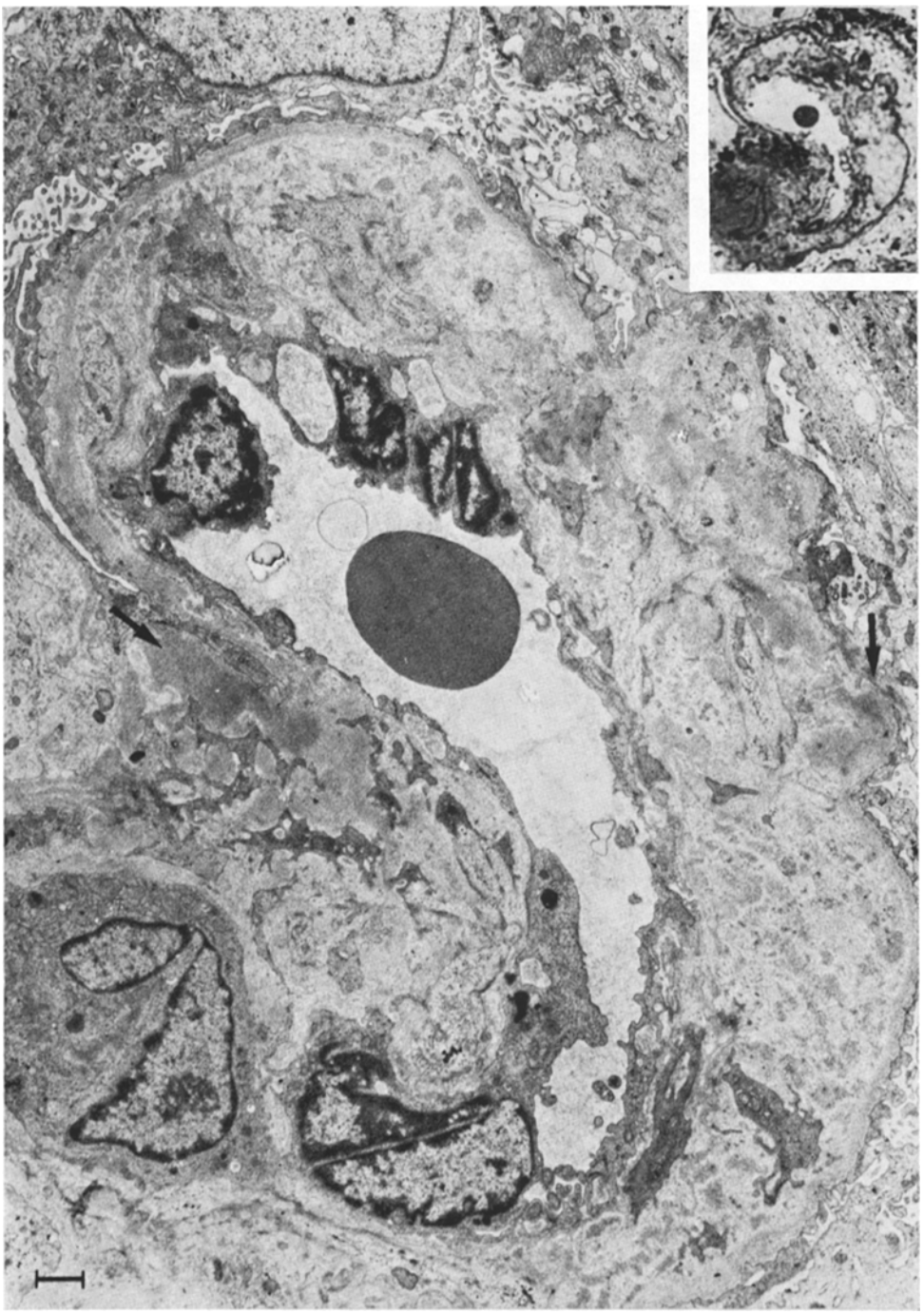


Fig. 3 A—E

(D) (case 24) and (E) (case 23): the two subtypes of lobular GN can well be separated in silver impregnated semithin sections of the Epon-embedded material (×500)



b) Marked or extreme thickening of the b.m. due to the circumscribed or continuous accumulation of a fine granular medium dense material has been observed in 12 cases (i.e. in all cases except case 23, Fig. 7). Using uranyl nitrate and lead citrate impregnation it may be impossible to clearly separate "deposits" from altered b.m. material; both components may add up to a fairly homogeneous layer which surpasses the original b.m. many times in thickness.

3. Different types of inhomogeneous or coarsely granular substances accumulated in the subendothelial space (subendothelial deposits in the presence of a clearly separated b.m.) can be demonstrated in 10 (77%) of the cases (Fig. 4).

The changes listed as (1) are well known fine structural characteristics by which the diagnosis of MPGN can be confirmed. Our main interest was directed towards the b.m. changes listed as (2a) and (b). The discrimination of substances which either cause (2) or imitate (3) b.m. thickening seems to be a controversial subject and will later be discussed in detail. The only way of separating intramembranous changes from subendothelial deposits is by silver impregnation of ultra thin sections. As a result it can be stated that essentially two types of b.m. changes exist: One is characterized by the presence of the intramembranous electron-dense material which is argyrophilic (Figs. 5, 7). The other type is characterized by a discontinuity of the lamina densa, by a circumscribed or circumferential loss of argyrophilia, and by considerable thickening of the b.m. due to the accumulation of a much less electron-dense fine granular material (Fig. 8). This material most likely represents the fine structural equivalent of the non-argyrophilic, Orange-G- and Chromotrope-2R-positive portions of b.m. as observed by light microscopy.

The exact characterization of the b.m. changes in MPGN is complicated by two facts: 1. The argyrophilic electron-dense material and the non-argyrophilic medium-dense material may simultaneously occur at least in some cases (e.g. cases 8, 11) and may show a continuous transition to each other (Fig. 9A). The only case in which no mixture of the electron-dense material with any less dense material did occur is case 23, the biopsy of a 6-year-old girl with lobular GN and persistent hypocomplementemia. 2. The non-argyrophilic type of b.m. changes may be associated with the occurrence or the aspect of subendothelial deposits (e.g. case 16, Fig. 8E). There may be a continuous transition from the argyrophilic to the non-argyrophilic material which demonstrates that a clear distinction of these two lesions may be difficult. This situation can further be exemplified by case 10 which in light microscopy shows intramembranous changes not only of the glomerular but also of the tubular and capsular basement membranes (Figs. 1C–D) but in the electronmicrograph (Fig. 4) exhibits well established subendothelial deposits.

In 6 (46%) of the cases an additional fine structural b.m. alteration was observed which seems to be a common part of the b.m. changes in MPGN.

Fig. 4. "Pure" MPGN (case 10, see Figs. 1C–E). Marked thickening of the glomerular capillary wall mainly due to subendothelial deposits and mesangial interposition. Electron-dense intramembranous material (arrows) does only occasionally occur (Uranyl/lead, ×6000). Inset: Aspect of subendothelial deposits and double contour appearance in silver impregnated semithin section of the same capillary loop (×1000)

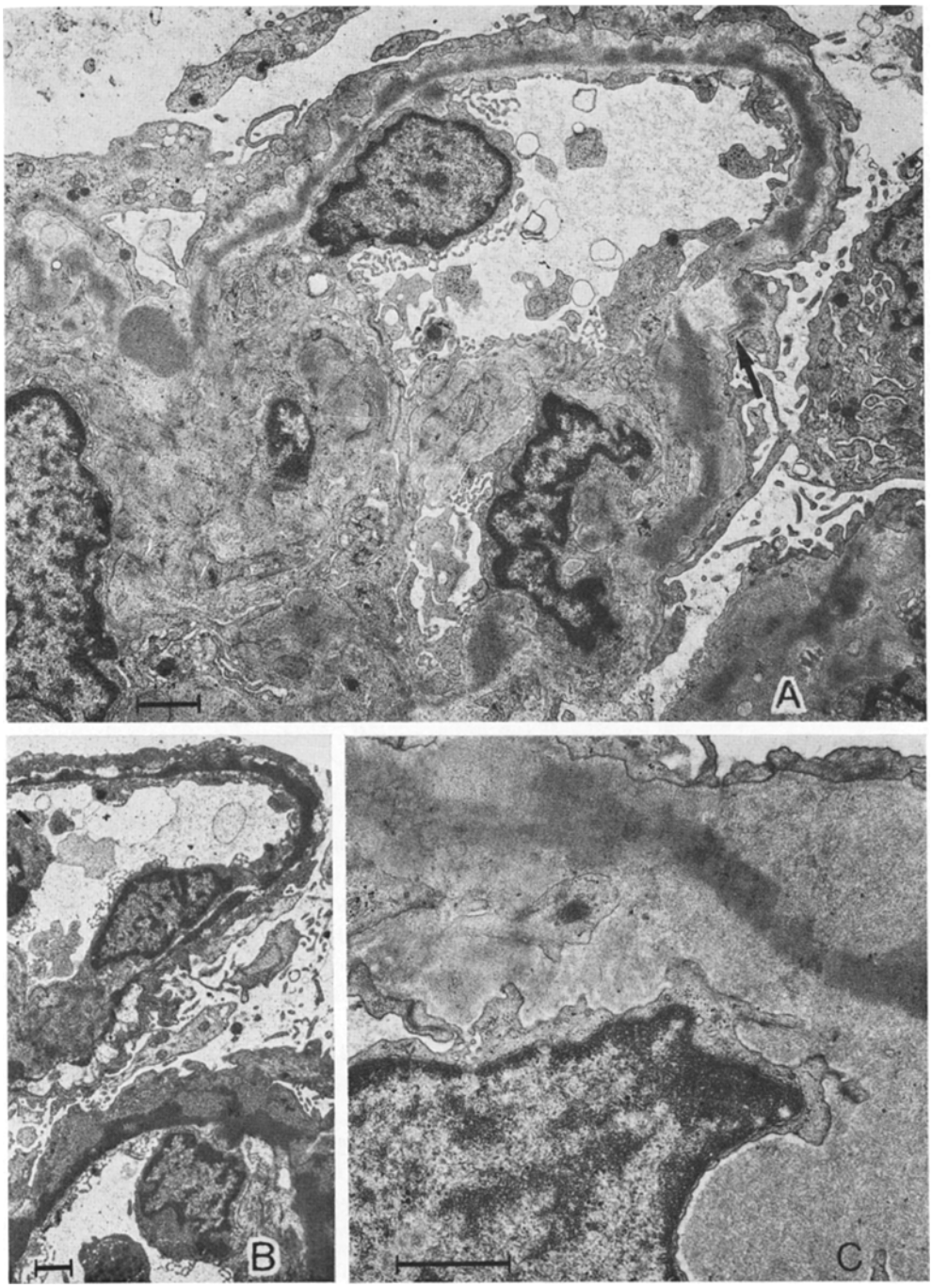


Fig. 5A–C. "Pure" MPGN with electron-dense intramembranous material (case 8). (A) The midportion of the b.m. is irregularly thickened but remains linear in character except for occasional interruptions ("intramembranous defects", arrow; uranyl/lead, ×7500). (B) Similar capillary loop with silver impregnation: The intramembranous material proves to be

In Table 1 it is listed as "intramembranous defects" and consists in a discontinuity or circumscribed substantial loosening of the altered b.m. material, namely of the electron-dense type (Fig. 9C).

The electron microscopic results obtained in lobular GN include the demonstration of a fine fibrillar material which accumulates in the mesangial stalks and differs from the normal aspect of mesangial matrix. These fibrils are clearly visible (Fig. 9B) and constitute a very compact formation as compared to the sparsely occurring filaments of normal mesangial matrix. There seems to be no doubt that this fibrillar material forms the (argyrophilic) "sclerotic nodules" as observed by light microscopy (Fig. 3 and Table 1). In electron micrographs these fibrils can be followed towards the subendothelial space where they decrease in thickness and density (Fig. 9B), and participate in the formation of the matrix like layers also referred to as "false b.m." or "double contour appearance" of the b.m.

In our series this fibrillar material was observed in all cases of lobular GN and in no case of "pure" MPGN. The two doubtful cases in which the acceptance of "sclerotic nodules" was felt to be debatable (cases 6 and 17) probably could have been classified more precisely as "pure" MPGN or lobular GN on the basis of this finding if electron microscopy would have been available.

Immunofluorescence Microscopy

Immunofluorescence microscopy was done in 16 of the 28 cases. Seven of these (44%; Table 1) could be characterized by the isolated fixation of anti-C3. The other ones showed different combinations in the fixation of anti-IgG and/or anti-IgM, with (7 cases) or without (1 case) simultaneous demonstration of C3. IgA (present in 3 cases) was always associated with IgG, IgM or C3. These data are given here without further details just to indicate the general agreement with similar studies but not to draw any conclusions for which the series would be too small.

Discussion

During recent years MPGN has emerged as an intermediate type lesion between membranous and lobular GN. When silver impregnation was introduced to renal histology by Jones (1957) true (peri-, extra-) membranous GN could be recognized as an own entity as it became clear that b.m. thickening in this condition resulted from subepithelial deposition of immune complexes and "spike" formation of the b.m.. A similarly clear characterization of the b.m. lesion in MPGN has so far not been achieved. On the other hand there was increasing evidence based on clinical, histological and immunofluorescence findings that MPGN is closely related to lobular GN (Cameron *et al.*, 1970; Bariéty and Druet, 1971; Mandalenakis *et al.*, 1971; Habib *et al.*, 1973).

strongly argyrophilic suggesting some relationship with the lamina densa (×4500). (C) The abnormal electron-dense material appears to be embedded in a fine granular medium dense material which is not easily defined in terms of "deposits". Note mesangial interposition. (Uranyl/lead, ×16000)

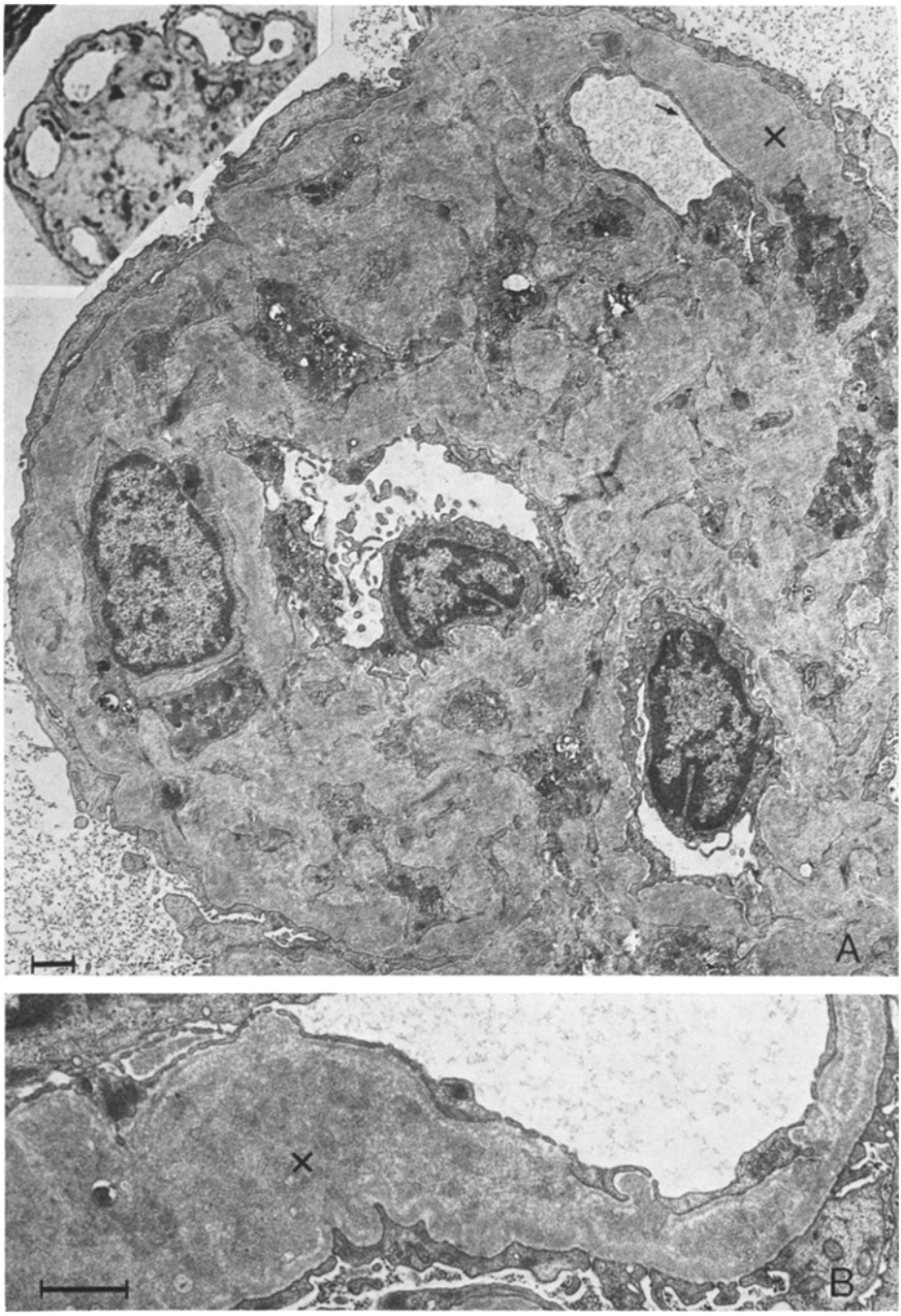


Fig. 6A and B. Lobular GN without apparent electron-dense intramembranous material. Binephrectomy specimen of a persistently hypocomplementemic boy (case 19). Inset: Double contour appearance of the glomerular capillary walls and centrolobular sclerotic nodule formation (Methenamine silver, ×640). (A) Three capillary lumina are extremely narrowed by the deposition of a finely granular, medium dense material (*x*) and circumferential mesangial interposition (×6000). (B) Detail; aspect of "subendothelial deposits" (Uranyl/lead, ×12300)

This view can be supported by some of our own observations. There are two cases included in our series (Nr. 6 and 17) which are classified as MPGN but could as well have been classified as lobular GN depending whether or not the centrolobular accumulation of matrix-like material is felt to constitute a nodule. As has been shown by others (Habib *et al.*, 1973; Bohle *et al.*, 1974) the extension of this centrolobular material may also vary during the course of the disease as confirmed by repeat biopsies. "Pure" MPGN and lobular GN are therefore grouped together in this study.

In lobular GN a different appearance ("double" or "single contour") of the glomerular b.m. can be observed which has led Bohle and his coworkers (Gärtner and Bohle, 1973) to separate the "lobular variant of MPGN" (with "splitting" of the glomerular b.m.) from "true lobular GN" (with single contour appearance of the b.m.) (Figs. 3 D, E). If this discrimination is to be explained in terms of "deposits" the former most likely represents the "subendothelial" type, the latter the "dense deposit" type. This distinction, however, cannot be based on the view that "dense deposits" are silver-negative. The contrary may be true, e.g. in semithin sections as shown in our case 23 (Figs. 3E, 7).

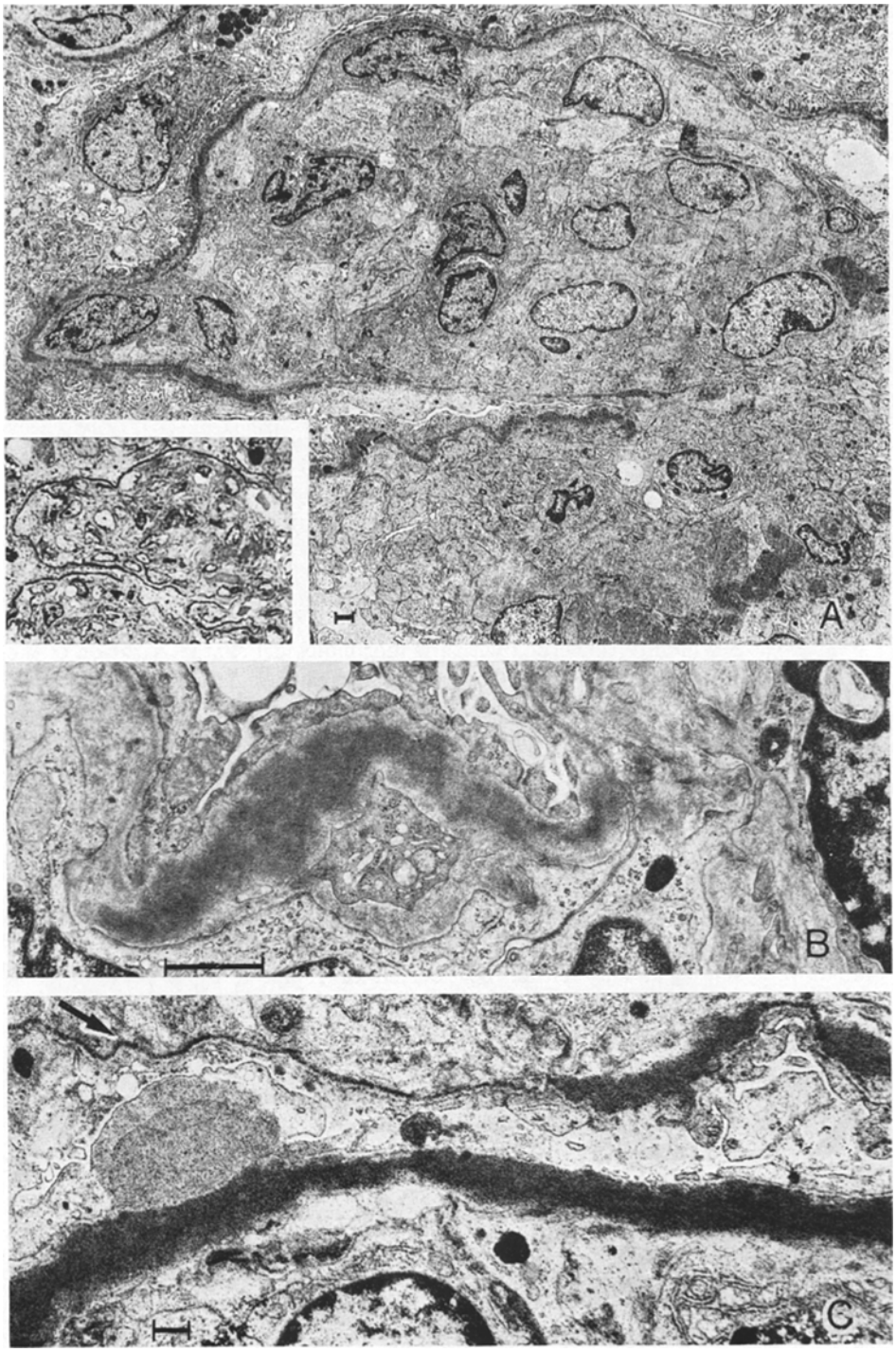
As far as age and sex distribution, clinical signs, morphological characteristics, and immunofluorescence patterns are concerned (Table 1) this series has been selected in accordance with the literature (Royer *et al.*, 1962; Bariéty *et al.*, 1971; Mandalenakis *et al.*, 1971; Holland and Bennett, 1972; Habib *et al.*, 1973). Namely there is a high frequency of persistent hypocomplementemia as well as a high percentage of isolated or predominant anti-C3fixation in the immunofluorescence pattern.

Light Microscopic Findings

The study is mainly concerned with the morphological characterization of the glomerular b.m. lesion in MPGN. Our special interest originated from the observation that the histological staining techniques of Pearse-trichrome and Jones-Chromotrope-2R proved to be of particular value in the visualization of certain b.m. lesions which appeared to be common in MPGN. These changes are characterized by a lucid orange coloration of the b.m. material (Fig. 2A, B), or by a loss of argyrophilia, respectively, with the black stain being replaced by a red Chromotrope-2R coloration (Fig. 2C, D). These changes are most striking if thickening of the b.m. is present (Fig. 1), but they also do occur in thin b.m. In general both, Orange-G and Chromotrope-2R stain proteins and proteinaceous material. Consequently, one can conclude that in MPGN the original (PAS-positive, argyrophilic) b.m. material may either be masked or replaced by a proteinaceous ("hyaline") material. A more exact localization of argyrophilic and non-argyrophilic portions of the b.m. can be achieved by light microscopic examination of silver impregnated semithin sections of the Epon embedded material. Using a combination of all three techniques it becomes evident that the b.m. lesion may either consist in a complete loss of argyrophilia or in a thin argyrophilic layer accompanying the non-argyrophilic portion on the endothelial side, on the epithelial side, or on either side (Fig. 1E).

If an argyrophilic layer accompanies the non-argyrophilic portion of the b.m. on the epithelial side the altered b.m. material cannot clearly be separated from the subendothelial space. Both, subendothelial deposition and intramembranous changes may contribute to the characteristic capillary wall thickening in MPGN.

The subclassification proposal of Habib and coworkers (1973, 1974) is essentially based on the discrimination of "subendothelial" and "intramembranous


A
B
C

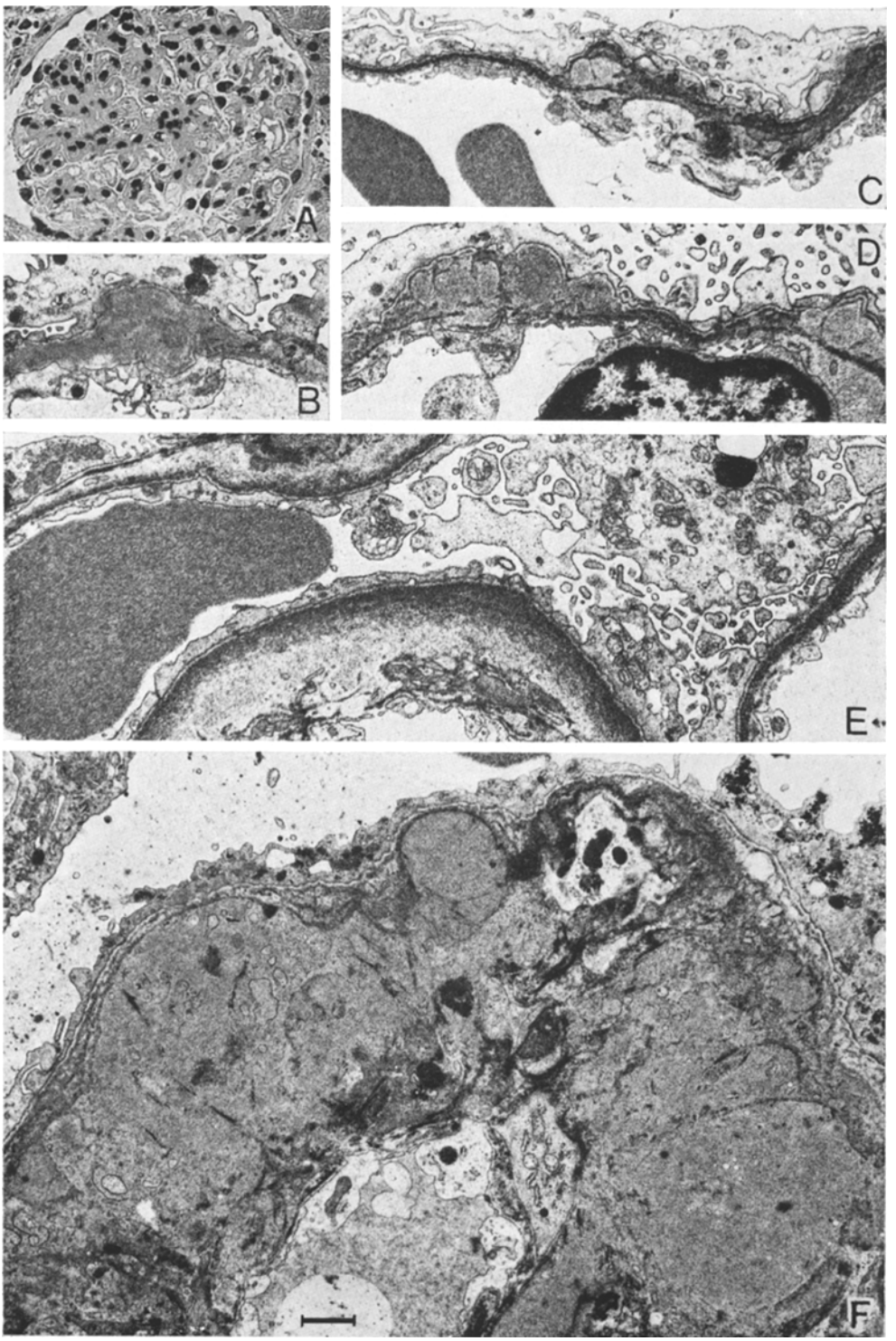
dense" deposits. In an attempt to classify the own observations according to this proposal our interest was focussed on the distinction of the altered b.m. material and any other material accumulated in the subendothelial space. With respect to light microscopy the premise should be made that the fixation and staining technique used by the french group (Dubosq-Brasil, trichrome-light green) differs from ours. The light microscopic fixation and staining methods used in this study do not provide a means to demonstrate a dense material in paraffin sections; they detect b.m. changes characterized by orangeophilia and loss of argyrophilia. Both, however, represent increased lucidity rather than increased density of the material concerned. These lesions can be compared to "intramembranous dense deposits" only in that they can also be described as "thickened by a non-argyrophilic" (Habib *et al.*, 1973) substance. To our experience, however, this non-argyrophilic distention of the glomerular b.m. occurs in MPGN with a much higher incidence (65–73%, Table 1) than has been reported for intramembranous dense deposits (28%, Habib *et al.*, 1973).

Therefore, in order to compare our own observations to those of others with respect 1. to the *dense* character of any b.m. material and 2. to a distinction between the abnormal b.m. material and any substances filling the subendothelial space we have to rely on electron microscopy.

Electron Microscopic Findings

As mentioned above only three (23%) of the 13 pts. studied presented clear evidence for an electron dense intramembranous material as part of slight or moderate b.m. thickening. In two cases the lesion was associated with "pure" MPGN (Fig. 5) and in one case with the lobular pattern (Fig. 7). In pure MPGN the dense material appeared to be embedded in a fine granular medium dense material (Fig. 5C) whereas in the lobular type the (moderate) b.m. thickening was predominantly due to the presence of the electron dense material, and the medium dense component was almost completely lacking (Figs. 7A, B). The intramembranous dense material is always linear in character and contributes much less to the b.m. thickening than does the fine granular medium dense material which may irregularly protrude towards the subepithelial and subendothelial side of the b.m. (Figs. 5C, 9A). These protrusions can be distinguished from true deposits, e.g. from subepithelial humps which are known to occur in MPGN but which are not found in a comparably systemic distribution and usually can well be separated from the b.m. material (Fig. 7C).

Fig. 7A–C. Lobular GN with electron-dense intramembranous material (case 23, see Figs. 3A, E). (A) The dense material forms a slightly thickened single contour b.m. surrounding hypercellular lobules (uranyl/lead, ×2400). Inset: Same lobule; silver impregnation of semithin section shows the intramembranous material to be argyrophilic giving the appearance of a normal b.m. (×400). (B) Local thickening of the b.m. due to the dense material and occasional mesangial interposition are evident only in electron micrographs (uranyl/lead, ×14000). (C) Silver impregnation of ultrathin sections reveals considerable thickening of the b.m. as compared to normally (?) thin portions of the lamina densa (arrow). Subepithelial deposits can clearly be separated from intramembranous changes (×5500)

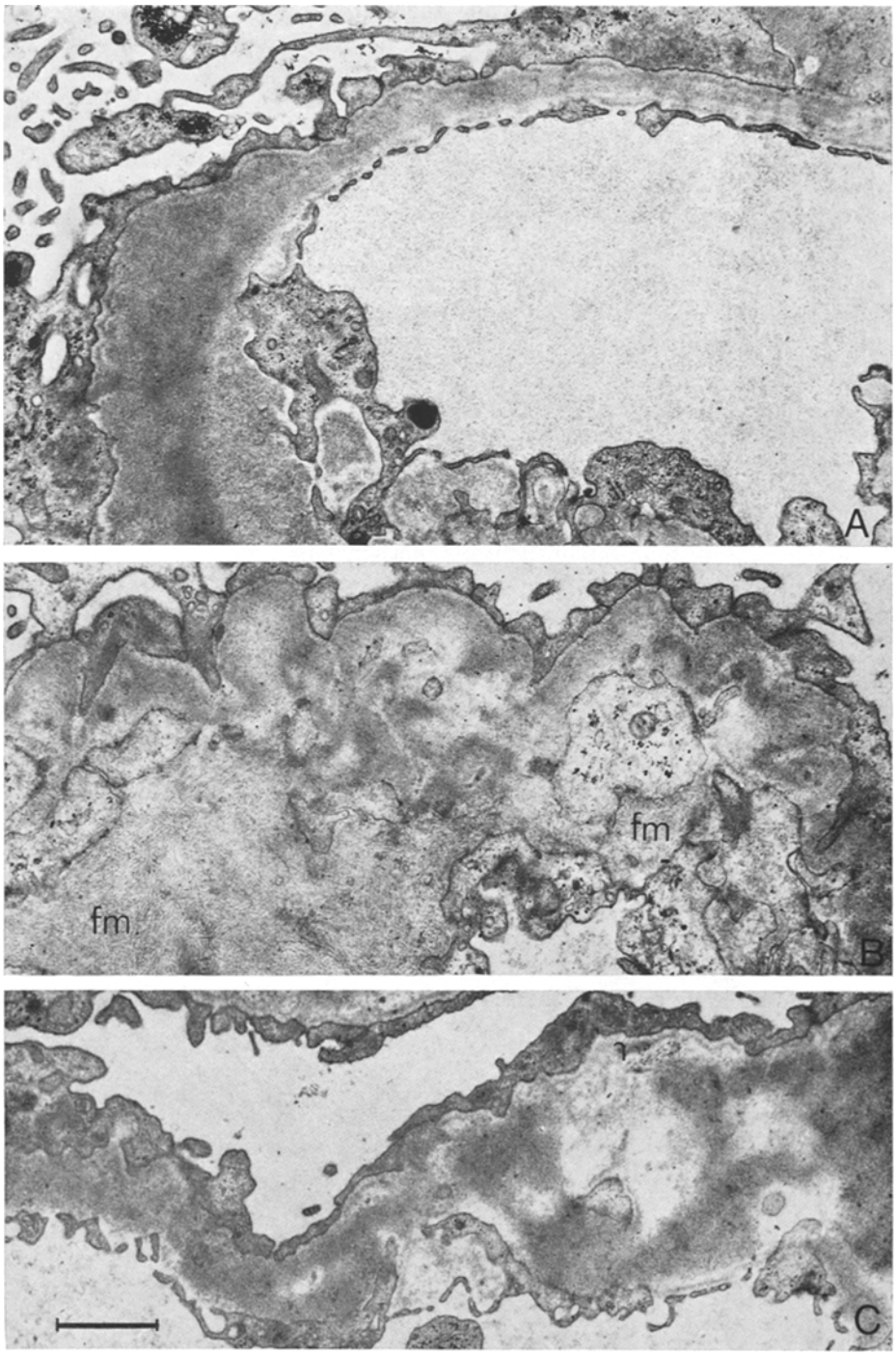

A
B
C
D
E
F

It is felt that the fine granular medium dense material described is equivalent with the orangeophilic, non-argyrophilic thickened parts of the b.m. as observed by light microscopy. Even though this material has lost the staining characteristics and fine structural aspect of normal b.m. it is most likely that it represents or is derived from the b.m. as it may be the only noncellular constituent of the capillary wall in those b.m. which are characterized by a complete loss of argyrophilia (Figs. 1E, 8F). Even by electron microscopy it may be difficult if not impossible to clearly distinguish this fine granular b.m. material from any similar material or deposit in the subendothelial space. As has also been stated by Habib and coworkers (1973) uranyl nitrate and lead citrate impregnation of ultra thin sections does not always allow a clear separation of some types of subendothelial deposits which "seem to be incorporated into the b.m.".

One rather has to rely on silver impregnation of ultra thin sections. Using this technique is should be possible to distinguish intramembranous changes from subendothelial deposits. This is true as long as the b.m. remains intact, and argyrophilia of the b.m. material is preserved as can indeed be found in MPGN. To our experience, however, argyrophilia of the b.m. as an important prerequisite of this distinction may not be preserved even in very small b.m. lesions which presumably represent the early stage of the process. This type of lesion can well be observed in the absence of electron-dense intramembranous material.

One single glomerulus (Fig. 8) may exhibit the whole spectrum of b.m. changes ranging from the normal appearing, perfectly thin and argyrophilic basement membrane (C, left) to severe alterations in extremely thickened non-argyrophilic b.m. (F). Most interesting part of the spectrum is the early lesion (B, C) which can be described as a local non-argyrophilic distention of the b.m. with the abnormal material extending to the epithelial as well as to the endothelial side. The lesion includes narrowing and breaks of the argyrophilic lamina densa (D) which lateron may be completely lost or somehow replaced by the abnormal material. It is our impression that this type of lesion cannot easily be interpreted as an (immune-) deposit. It rather seems to represent a transformation of the b.m. material which may begin with a circumscribed lesion, may gradually spread and finally result in circumferential non-argyrophilic thickening of the b.m. In the advanced stage (Fig. 8F) there is no way of localizing any material in terms of "subendothelial",

Fig. 8A–F. The development of non-argyrophilic transformation of the b.m. material in the capillary loops of one single glomerulus in "pure" MPGN (case 16). (A) Light microscopic appearance (hematoxylin and eosin, ×320). (B) Localized distention of the b.m.; no details are visible within the medium dense material (uranyl/lead, ×7800). (C) Silver impregnation reveals a thin layer of the lamina densa which can be followed through the non-argyrophilic distention; a thin subepithelial and a subendothelial argyrophilic layer are also present. (D) Further increase of the non-argyrophilic material associated with discontinuity of the lamina densa (left) which appears to be embedded in the former (right; compare Fig. 4C). (E) Other capillary loops give the impression of subendothelial deposits; note the continuous transition between argyrophilic and non-argyrophilic material. The red blood cell is located in the urinary space. (F) Extreme thickening of the capillary wall. Except for a thin subepithelial and subendothelial argyrophilic layer and minor "intramembranous" remnants the whole of the b.m. consists of an abnormal non-argyrophilic medium dense material. Note general loss of foot processes (C–E: methenamine silver, ×7800)


A
fm
fm
B
C

"subepithelial" or "intramembranous"; the b.m. itself appears to be substantially transformed as a whole. This process cannot easily be understood without considering an abnormality in the turnover (Kurtz and Feldman, 1962; Walker, 1973) of the b.m. material as indicated by biochemical analysis (Mahieu, 1972). It is our impression that this transformation of the b.m.—whether or not it involves immune complexes, complement components or properdin—represents a frequently occurring and probably essential morphological alteration in MPGN.

Not too long ago this lesion has been equated up with IMDD (Habib *et al.*, 1973; Bohle *et al.*, 1974) but according to the frequency of occurrence and the staining characteristics it seems to bear a closer relationship to what has so far not quite convincingly been described as "subendothelial deposits". This non-argyrophilic transformation of the b.m. described above will have to be classified more precisely whereas MPGN associated with IMDD can be considered as a well defined entity.

Conclusion

Intramembranous dense deposits (IMDD) are a well defined basement membrane lesion (Berger and Galle, 1963) which to our experience can safely be diagnosed by electron microscopical examination. The nature of this peculiar lesion which occurs in about 20–30% of all cases of idiopathic MPGN remains to be determined.

Cases of MPGN lacking IMDD demonstrate b.m. alterations which are generally referred to as "subendothelial deposits". Our observations suggest that this term may not be sufficient to describe the essential difference between the two lesions which indeed may represent two distinct fine structural entities. In about 70–80% idiopathic MPGN is characterized by the occurrence of a non-argyrophilic b.m. thickening which in places may give the aspect of subendothelial deposits but generally should better be interpreted as a transformation of the basement membrane combined with incorporation of proteinaceous material.

We wish to acknowledge the cooperation of all clinicians who provided the clinical data and the biopsy material on which the observations are based, namely Dr. F. Bläker (Univ.-Kinderklinik Hamburg), Dr. V. Dittrich (Med. Univ.-Klinik Innsbruck), Dr. D. Gekle (Univ.-Kinderklinik Würzburg), Drs. U. Gessler and W. Schulz (IV. Med. Klinik Nürnberg), Dr. D. Klaus (Med. Univ.-Poliklinik Marburg/L.), Dr. Luz (Marienkrankenhaus Amberg/Opf.), Dr. C. Mietens (Kinderklinik Bochum), Dr. Nessler (Städt. Kinderklinik Karlsruhe), Dr. H. Olbing (Kinderklinik Ges.-Hochschule Essen), Dr. E. Ritz (Med. Univ.-Klinik Heidelberg), Dr. K. Schärer (Univ.-Kinderklinik Heidelberg), Dr. F. Schindera (Univ.-Kinderklinik Freiburg), Dr. E. Straub (Univ.-Kinderklinik Mainz). We also acknowledge the skillfull technical assistence of Mrs. B. Agricola, Miss M. Sippel, and Mrs. Wenz.

Fig. 9A–C. Further details of b.m. changes. (A) Continuous transition of the fine granular medium dense b.m. material (upper right) to the homogeneously electron-dense material (lower left); "pure" MPGN, case 11. (B) In lobular GN a fibrillar matrix material (*fm*) forms the centrolobular sclerotic nodule (lower left) and can be followed towards the capillary wall (right) in subendothelial position (case 24). (C) Loosening zones may be part of the b.m. changes associated with the electron-dense intramembranous material (case 8, compare Fig. 5). (A–C) Uranyl/lead (×14000)

References

Allen, A. C.: The clinicopathologic meaning of the nephrotic syndrome. Amer. J. Med. **18**, 277–314 (1955)

Antoine, B., Faye, C.: The clinical course associated with dense deposits in the Kidney basement membranes. Kidney Internat. **1**, 420–427 (1972)

Bariéty, J., Druet, Ph.: Les glomérulonéphrites pariétoprolifératives (GNPP). Etude histopathologique en microscopie optique, électronique et en immunhistochimie de 49 cas. Path. et Biol. **19**, 259–283 (1971)

Berger, J., Galle, P.: Dépôts denses au sein des basales du rein. Presse méd. **71**, 2351–2354 (1963)

Berger, J., Noel, L. H., Yaneva, H.: Les dépôts de complément dans le rein. Act. Néphrol. Hôp. Necker, 87–100. Paris: Flammarion 1974

Bohle, A., H. V. Gärtner, H. Fischbach, K. D. Bock, H. H. Edel, U. Frotscher, R. Kluthe, W. Mönninghoff, F. Scheler: The morphological and clinical features of membranoproliferative glomerulonephritis in adults. Virchows Arch. Abt. A **363**, 213–224 (1974)

Burkholder, P. M., Marchand, A. and Krueger, R. P.: Mixed membranous and proliferative glomerulonephritis. Lab. Invest. **23**, 459–479 (1970)

Cameron, J. S., Glasgow, E. F., Ogg, C. S., and White, R. H. R.: Membranoproliferative glomerulonephritis and persistent hypocomplementemia. Brit. Med. **4**, 7–14 (1970)

Cameron, J. S., Ogg, C. S., Turner, D. R., Weller, R. O., White, R. H. R., Glasgow, E. F., Peters, D. K., Martin, A.: Mesangiocapillary glomerulonephritis and persistent hypocomplementemia. In: Glomerulonephritis I (eds. Kincaid-Smith, P., Matthew, T. H. and Becker, E. L.). New York: Wiley & Sons 1973

Cameron, J. S., Ogg, C. S., White, R. H. R., Glasgow, E. F.: The clinical features and prognosis of patients with normocomplementemic mesangiocapillary glomerulonephritis. Clin. nephrol. **1**, 8–13 (1973)

Churg, J., Duffy, J. L.: Classification of glomerulonephritis based on morphology. In: Glomerulonephritis I (eds. Kincaid-Smith, P., Matthew, T. H., and Becker, E. L.), p. 43. New York: J. Wiley & Sons 1972

Ehrenreich, Th., Espinosa, T.: Chromotrope silver methenamine stain of glomerular lesions. Amer. J. clin. Path. **56**, 448–458 (1971)

Faye, C., Giromini, M., Antoine, B.: Les néphropathies avec dépots denses dans les membranes basales. J. Urol. Néphrol. **77**, 406–417 (1971)

Galle, P.: Mise en évidence au microscope électronique d'une lésion singulière des membranes basales du rein et de la substance hyaline. Thèse Paris (1962)

Gärtner, H. V., A. Bohle: Zur Frage der Existenz der lobulären Glomerulonephritis. Verh. dtsch. Ges. Path. **57**, 455 (1973)

Ganter, P., Berger, J.: Néphropathie associant une glomérulonéphrite proliferative et des dépôts au sein des basales. II. Etude histochimique de trois cas. J. Urol. Néphrol. **69**, 96–98 (1963)

Gotoff, S. P., Fellers, F. X., Vawter, G. F., Janeway, C. A., Rosen, F. S.: The B1C globulin in childhood nephrotic syndrome. Laboratory diagnosis of progressive glomerulonephritis. New Engl. J. Med. **271**, 524–529 (1965)

Habib, R., Kleinknecht, C., Gubler, M. C., Levy, M.: Idiopathic membranoproliferative glomerulonephritis in children. Report of 105 cases. Clin. Nephrol. **1**, 194–214 (1973)

Habib, R., Loirat, C., Gubler, M. C., Levy, M.: Démembrement des GN membrano-prolifératives (analyse morphologique et étude des taux plasmatiques des fractions C_3 et C_4 du complément). Act. Néphrol. Hôp. Necker: 157–184. Paris: Flammarion 1974

Herdman, R. C., Pickering, R. J., Michael, A. F., Vernier, R. L., Fish, A. J., Gewurz, H., Good, R. A.: Chronic glomerulonephritis associated with low serum complement activity (chronic hypocomplementemic glomerulonephritis). Medicine (Baltimore) **49**, 207–226 (1970)

Hirbec, G., Gouygou, Ch., Lagrue, G.: Une nouvelle coloration histologique: L'héxaméthylènetétramine argentique-chromotrope. Ses résultats en pathologie rénale. Sem. Hôp. Paris **50**, 523–530 (1974)

Holland, N. H., Bennett, N. M.: Hypocomplementemic (membrano-proliferative) glomerulonephritis. Amer. J. Dis. Child. **123**, 439–445 (1972)

Jenis, E. H., Sandler, P., Hill, G. S., Knieser, M. R., Jensen, G. E., Roskes, S. D.: Glomerulonephritis with basement membrane dense deposits. Arch. Path. **97**, 84–91 (1974)

Jones, D. B.: Nephrotic glomerulonephritis. Amer. J. Path. **33**, 313–329 (1957)

Kurtz, S. M., Feldman, J. D.: Experimental studies on the formation of glomerular basement membrane. J. Ultrastruct. Res. **6**, 19–27 (1962)

Mahieu, P.: Biochemical structure of glomerular basement membrane in chronic glomerulonephritis. I. Lobular and membrano-proliferative GN. Kidney Internat. **1**, 115–123 (1972)

Mandalenakis, N., Mendoza, N., Pirani, C. L., Pollak, V. E.: Lobular glomerulonephritis and membrano-proliferative gln. A clinical and pathologic study based on renal biopsies. Medicine (Baltimore) **50**, 319–355 (1971)

McDonald, M. K.: Dense deposit disease: A subgroup of membrano-proliferative glomerulonephritis, identified by electron microscopy. In: Glomerulonephritis I (Kincaid-Smith, P., Matthew, and Becker, E. L. eds.), p. 515–530. New York: J. Wiley & Sons 1972

Meadows, R.: Renal Histopathology. London: Oxford Univ. Press 1973

Michael, A. F., Westberg, N. G., Fish, A. J., Vernier, R.: Studies on chronic membranoproliferative glomerulonephritis with hypocomplementemia. J. exp. Med. **134**, 208–227 (1971)

Movat, H. Z.: Silver impregnation methods for electron microscopy. Amer. J. clin. Path. **35**, 528–537 (1961)

Ogg, C. S., Cameron, J. S., and White, R. H.: The C3 component of complement (β1C-globulin) in patients with heavy proteinuria. Lancet **1968 II**, 78–81

Pearse, A. G. E.: Differential stain for the human and animal anterior hypophysis. Stain Technol. **25**, 95–102 (1950)

Pearse, A. G. E.: Histochemistry, theoretical and applied. 2nd ed., p. 831. London: Churchill 1961

Peters, D. K., Williams, D. G.: Complement and mesangiocapillary glomerulonephritis. Role of complement deficiency in the pathogenesis of nephritis (Editorial). Nephron **13**, 189–197 (1974)

Royer, P., Habib, R., Vermeil, G., Mathieu, H., Alizon, M.: Les glomérulonéphrites prolongées de l'enfant. Ann. Pédiat. **38**, 173–187 (1962)

Thoenes, G. H.: Die Immunhistologie der Glomerulonephritis. Klin. Wschr. **51**, 739–747 (1973)

Thoenes, W.: Pathomorphologische Prinzipien und Formen der Glomerulonephritis. Mschr. Kinderheilk. **122**, 728–740 (1974)

Vallota, E. H., Forristal, J., Davis, N. C., West, C. D.: The C_3 nephritic factor and membranoproliferative nephritis: Correlation of serum levels of the nephritic factor with C3 levels, with therapy, and with progression of the disease. J. Pediat. **80**, 947–959 (1972)

Vallota, E. H., Forristal, J., Spitzer, R. E., Davis, N. C., West, C. D.: Continuing C_3 breakdown after bilateral nephrectomy in patients with membrano-proliferative glomerulonephritis. J. clin. Invest. **50**, 552–558 (1971)

Verroust, P. J., Wilson, C. B., Cooper, N. R., Eddington, T. S., Dixon, F. J.: Glomerular complement components in human glomerulonephritis. J. clin. Invest. **53**, 77–84 (1974)

Walker, F.: The origin, turnover and removal of glomerular basement membrane. J. Path. **110**, 233–244 (1973)

West, C. D.: Membranoproliferative hypocomplementemic glomerulonephritis. Nephron **11**, 134–146 (1973)

West, C. D., McAdams, A. J., McConville, J. M., Davis, N. O., Holland, N. H.: Hypocomplementemic and normocomplementemic persistent (chronic) glomerulonephritis; clinical and pathological characteristics. J. Pediat. **67**, 1089–1112 (1965)

Dr. D. Anders
Zentrum f. Kinderheilkunde
D-6300 Giessen
Feulgenstr. 12
Federal Republic of Germany

Prof. Dr. W. Thoenes
Pathologisches Institut
der Universität
D-6500 Mainz
Langenbeckstr. 1
Federal Republic of Germany