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Basement Membrane Changes in Membranoproliferative Glomerulonephritis

II. Characterization of a Third Type by Silver Impregnation of Ultra Thin Sections*

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Summary. In a previous study on membranoproliferative glomerulonephritis (MPGN) we reported the preliminary result that a basement membrane (b.m.) lesion incompatible with the criteria of subendothelial deposits (type I) or intramembranous dense "deposits" (type II) can be recognized by silver impregnation (s.i.) of ultra-thin sections. This technique has been further evaluated and applied firstly to control cases with normal b.m., perimembranous GN, diffuse proliferative ("MPGN-like") lupus nephritis and, secondly to additional cases of idiopathic MPGN comprising 10 biopsies with doubtful findings as judged by electron microscopy with conventional impregnation. S.i. of ultra-thin sections proved to be a reliable method, of particular value in the visualization of fine structural details of b.m. changes in the field of MPGN. The light microscopic (l.m.) equivalent of the new lesion is defined. Accordingly, the series of 31 patients with idiopathic MPGN has been subdivided into three groups: Type I (19), type II (3), type III (9 patients). Type III is understood to be an intermediate type lesion, distinguished from type I by true membranous changes (discontinuity of the lamina densa) and from type II by the lack of the intramembranous electron-dense (argyrophilic) material. It resembles in part, however, perimembranous GN due to segmental spike formation and little proliferation.

The clinical course of the patients with the type III lesion did not significantly differ from that of the other groups. The details are given in short case reports. Serum C3 was persistently depressed in 6, initially depressed in 2 patients and normal in one. As in type II, a predominant or isolated presence of C3 can be seen by immunofluorescence microscopy. Therefore, type III is likely to be mistaken for type II on the basis of immunological and l.m. data, and for type I on the basis of e.m. with conventional impregnation. The resultant inconsistencies so far inherent in the dual subclassification concept of MPGN can probably be solved – at least in part – by the accep-

* Part I is identical with previous paper: D. Anders and W. Thoenes (1975), see references

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tance of the type III lesion as defined by its appearance in silver impregnated ultra-thin sections.

Both lesions, type II and type III, are understood to be conditions in which the notional difference between "deposits" and a substantial alteration of the b.m. is poorly defined.

Key words: Membranoproliferative glomerulonephritis – Ultrastructure of basement membrane changes – Silver impregnation in electron microscopy.

Introduction

Until now the morphological pattern of membranoproliferative glomerulonephritis (MPGN) has been subdivided into two different types: type I with subendothelial deposits and type II with so called intramembranous dense deposits or electrondense alteration of the basement membrane (b.m.) (Habib et al., 1973). In type I the b.m. is believed to remain essentially intact whereas in type II the b.m. material is itself characteristically involved in the disease process. As a rule, type II is associated with persistent depression of serum complement (C3) suggestive of a pathogenic mechanism with complement activation via the alternate pathway.

In a previous study we have reported on our findings in a series of 31 renal biopsies from patients with idiopathic MPGN (Anders and Thoenes, 1975). Our main interest was focussed on the light and electron microscopic characterization of the material causing glomerular capillary wall thickening. The light microscopic (l.m.) findings were classified according to the presence or absence of a lobular pattern, the electron microscopic (e.m.) findings according to the presence or absence of the intramembranous dense material. Our results differed from those of other groups in that 1. the type I lesion with an intact b.m. could not be demonstrated in a similar percentage to that given in the literature, 2. almost half of the hypocomplementemic cases examined by e.m. failed to show the classical electron-dense alteration of the b.m. Most of these cases were characterized by an irregular, more or less extensive, ill defined b.m. thickening which in uranyl and lead (U/Pb) impregnated sections did not allow a clear distinction between the b.m. proper and the abnormal material. Therefore, we hesitated to ascribe this medium dense alteration to the incorporation of deposits into the b.m. and wondered whether it represented a distention of the b.m. with loss of its original properties (such as electron-density and argyrophilia of the lamina densa). On l.m. examination these doubtful cases gave the impression of "dense deposits" with little or no apparent double contour formation. But neither the presence of electron-dense material nor well defined subendothelial deposits could be demonstrated by e.m. with conventional impregnation.

The only technique we found to yield conclusive results in investigating this particular problem was silver impregnation (s.i.) of ultra thin sections. Using this technique it was possible to delineate a so far undescribed lesion (Anders and Thoenes, 1975; Fig. 8) which cannot be described by the criteria of the types I or II, and therefore may have to be considered as a separate lesion, type III.

The present study, based on silver impregnated ultra-thin sections, was undertaken in order to 1. reexamine the diagnostic value of the technique, 2. obtain comparative findings from glomerular lesions with well defined deposits, 3. analyze additional cases of MPGN, with the newly defined lesion, and 4. relate the results to various inconsistencies so far inherent in the subclassification concept of MPGN.

Material and Methods

Silver impregnated ultra-thin sections were studied from 7 renal biopsies of our previous series (28 patients) and of 3 additional patients with idiopathic MGPN. Comparative findings were obtained from one renal biopsy with minor glomerular abnormalities and unaltered b.m. (case I), one with perimembranous GN (case II), and one with diffuse proliferative ("MPGN-like") lupus nephritis (case III). All biopsies were also examined by U/Pb-impregnation of ultra-thin sections. Short case reports are given in the appendix. The classification concept and the case numbers of our previous publication, namely Table 1, are maintained.

All sections were cut from glutaraldehyde and osmium tetroxide fixed Epon-embedded material using a Reichert microtome and glass knives. S.i. was performed according to the technique described by Movat (1961). No special modifications were used. Following oxydation by periodic acid (1%) the sections were incubated at a temperature of 60° C while swimming on methenamine silver solution. The time required for proper staining varied between 10 and 60 min. The procedure was watched stepwise under a stereo microscope. For transportation of the sections a modified Marinozzi ring was used. Thorough rinsing between the reactive steps was found to be important.

Results

This paper is concerned with (A) the technique and (B) the results of silver impregnation (s.i.) in the fine structural analysis of b.m. changes in MPGN.

A. Technique and Comparative Findings

Figure 1 is to reemphasize the validity of Movat's s.i. technique in the fine structural range and serves as a control for the following figures. It demonstrates the tinctorial properties of renal basement membranes in the normal state and the visualization of renal architecture in general. Argyrophilia is characteristic for all basement membranes including the mesangial branches of the glomerular b.m., the multilaminated b.m. of Bowman's capsule, and the tubular b.m. The presence and location of extremely thin basement membranes in small interstitial blood vessels becomes clear. The close relationship between basement membranes and reticulin fibers is evident.

This qualification is expressed since there may be some doubt whether the analysis of b.m. changes should be based on this technique. Clear contrast outweighs the restrictions of low resolution. Further advantages will be discussed later. It is one of the consequences of this study that the nature of b.m. changes in MPGN are now a new field for s.i. of ultra thin sections.

Figure 2 presents the rationale for s.i. in the field of idiopathic MPGN compared to well defined glomerular lesions. Perimembranous glomeruloneph-

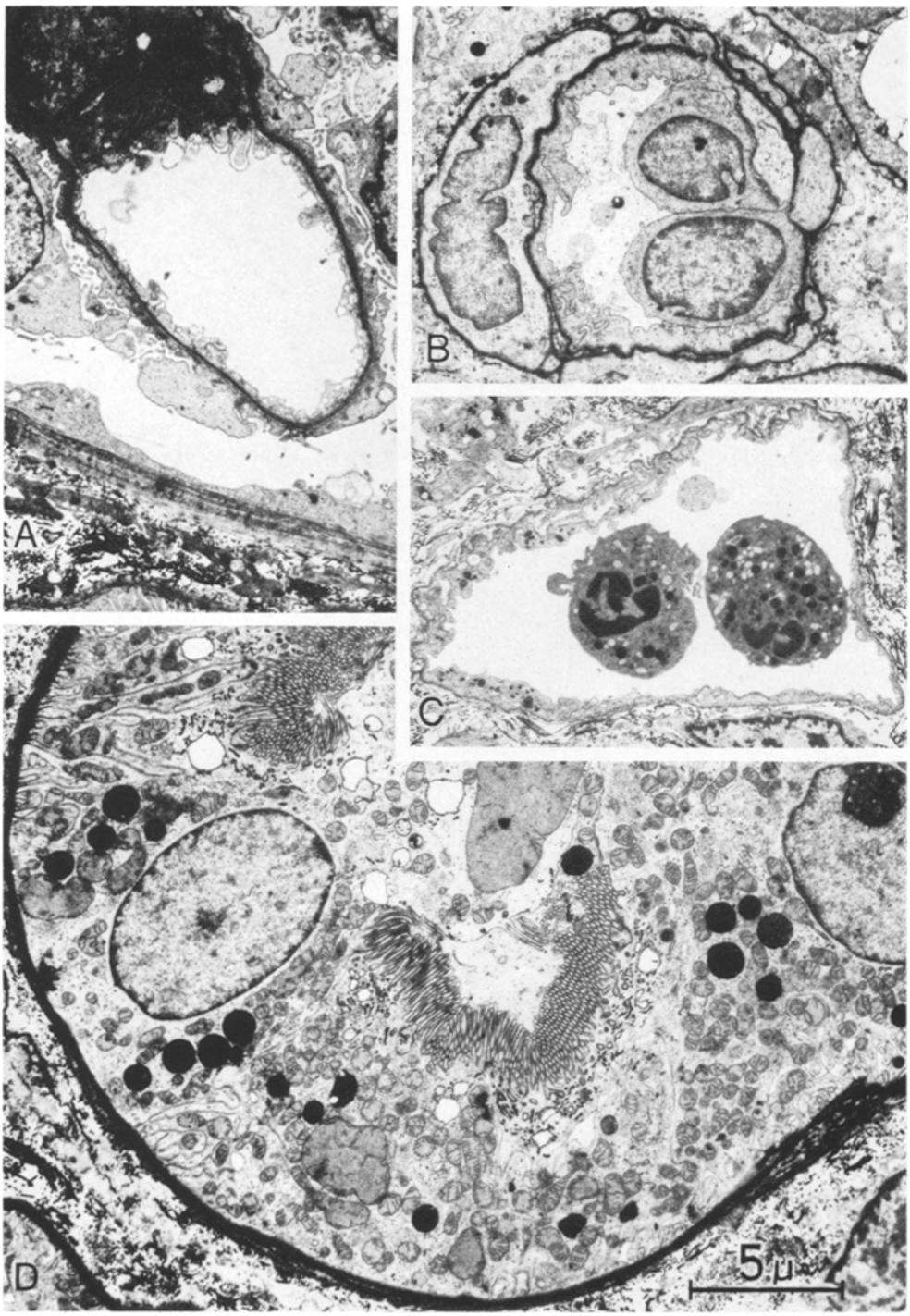

Fig. 1A–D. Renal fine structure and normal basement membranes with silver impregnation (all 4500 ×). **A** Unaltered glomerular capillary loop and Bowman's capsule (case I). **B** and **C** Interstitial vessels, presumably vas efferens (**B**) and peritubular capillary (**C**; case 29). **D** Proximal tubule (case 30); proper silver impregnation allows extremely clear delineation of fine structural details such as cell membranes and organelles including enlarged and matrix enriched mitochondria: proof of a reliable technique

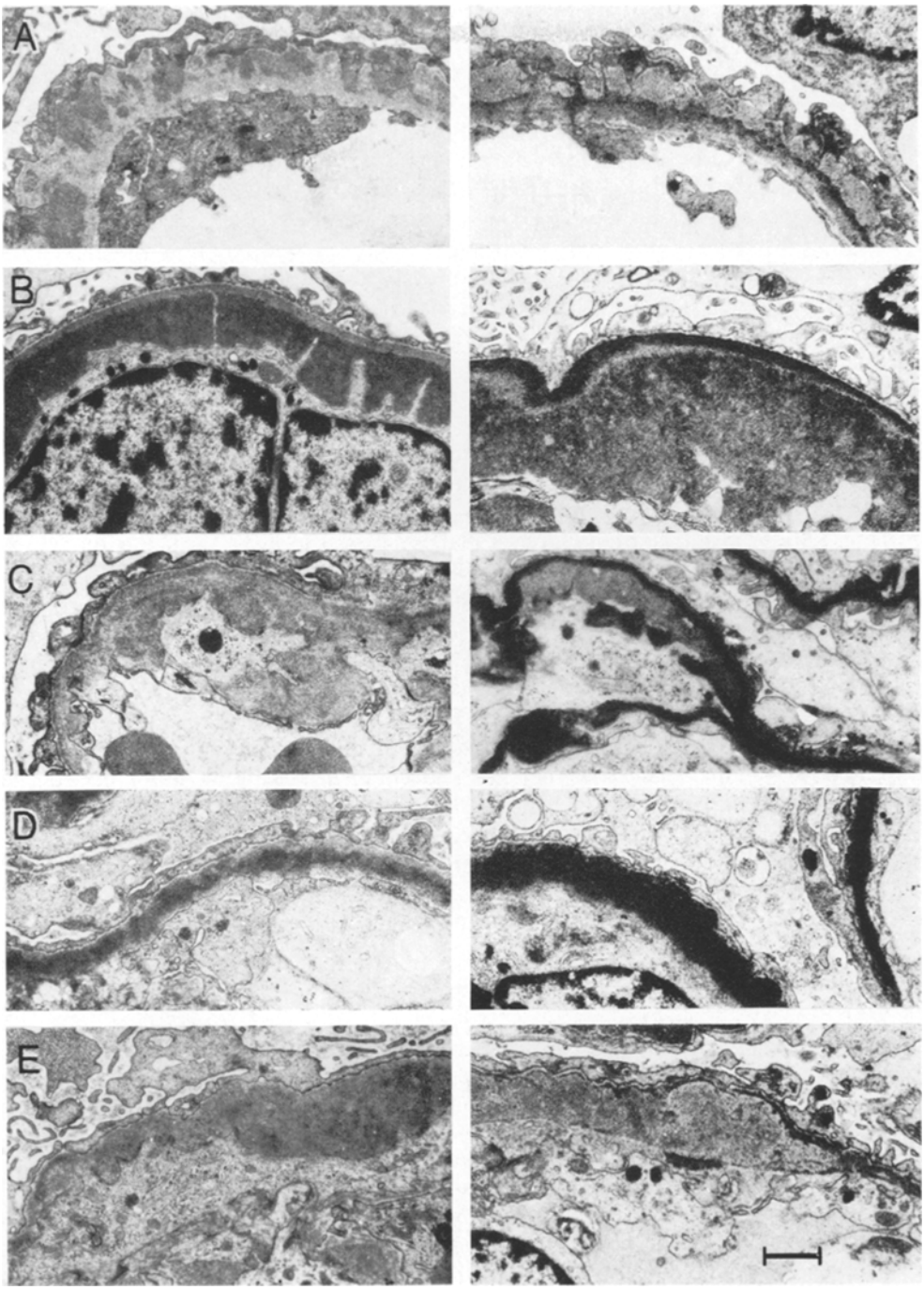

Fig. 2A–E. Comparison of glomerular lesions with well defined deposits to three types of MPGN. Uranyl and lead (left) and silver impregnation (right, all 7500×). **A** Perimembranous GN; case II. **B** Lupus nephritis with electron-dense subendothelial deposits; case III. **C** Idiopathic MPGN, type I, with subendothelial deposits; case 30. **D** MPGN, type II (lobular pattern), with electron-dense alteration of the b.m. and preservation of argyrophilia; case 23. (**E**) MPGN, type III, with medium dense alteration of the b.m. and essential loss of argyrophilia; case 16; for light microscopic appearance see Figure 3C and D. Silver impregnation is despensible in (**A**) and (**B**), informative in (**C**) and (**D**), diagnostic in (**E**). For details see text

ritis (GN) is characterized by an undisturbed continuity of the lamina densa which forms "spikes" between the fairly regular arrangement of electron-dense. silver-negative subepithelial deposits. S.i. does not add essential information to that obtained by U/Pb-impregnation (Fig. 2A).

The same is true for diffuse proliferative "MPGN-like" lupus nephritis with electron-dense subendothelial deposits. S.i. is not needed for a better discrimination of the deposit and the well preserved b.m., a constellation reminiscent of MPGN, type I. However, with respect to the lesions described below it demonstrates that following silver impregnation the b.m. becomes unequivocally darker than the adjacent deposit (Fig. 2B right) which exhibits the higher degree of electron-density with U/Pb-impregnation (Fig. 2B left).

Figures 2C–E demonstrate the staining characteristics of the three types of b.m. changes in idiopathic MPGN. The validity of s.i., becomes evident: In type I (Fig. 2C) the intact b.m., subendothelial deposits, the mode of mesangial interposition with double or even triple contour formation are easily recognized in contradistinction to the grey-in-grey of U/Pb-impregnation. In type II (Fig. 2D) the intramembranous dense material proves to be argyrophilic, thereby demonstrating a tinctorial, and suggesting a substantial, relationship between the abnormal material and the lamina densa.

In both lesions – type I and type II – s.i. is most helpful though not essential for the correct diagnosis. As a rule both types can be distinguished in U/Pb-impregnated sections with sufficient accuracy. However, the lesion shown in Figure 2E (left) might well be interpreted as an intermediate or mixed state between the types I and II, or either type assuming poor technique. Therefore, Figure 2E (right) is to show that s.i. is essential in separating this type from the others. The material which in U/Pb-impregnated sections (left) forms an abnormal b.m. with fairly uniform, medium dense thickening may accumulate to greater thickness than that of type II. Mesangial interposition and double contour formation are present (left). S.i. reveals that the lesion clearly differs from type I in that the b.m. is not intact (the thin subepithelial argyrophilic layer cannot be considered as a normal b.m.), and it differs from type II in that the major part of the b.m. forming material is neither electron-dense nor argyrophilic.

B. Analysis of the Cases

This study supplements our previous study on 28 patients with idiopathic MPGN. The biopsies of three new patients were added. The total series of 31 patients can now be subdivided into three groups on the basis of silver impregnated ultra thin sections according to the three examples of b.m. changes given in Figure 2C–E: 19 patients with type I, 3 patients with type II, and 9 patients with type III, the newly defined lesion on which our main interest was now focussed. Among 10 doubtful cases studied by s.i. of ultra thin sections 5 were found to present this lesion (cases no. 1, 10, 11, 16, 29) as the only or predominant fine structural characteristic. The light microscopic equivalent of this lesion as seen with Pearse trichrome and Jones Chromotrope-2R stain

of paraffin sections was recognized in 4 other biopsies (cases no. 2, 3, 13, 14) from which material for proper electron microscopic examination was not available.

Light Microscopy. Figure 3 gives a survey of the histological characteristics of MPGN, type III [1]. Glomerular enlargement is moderate or missing in relation to the low degree of proliferation as judged by mesangial hypercellularity and mesangial interposition (double contour formation). The prevailing characteristic is a diffuse though irregular capillary wall thickening due to the accumulation of an "orangeophilic" silver-negative material in the place of the b.m. Single glomerular capillary loops may assume a hump-backed appearance associated with segmental spike formation (Fig. 3A) similar to that seen in perimembranous GN though in a localized, sometimes atypical (Fig. 3B) manner. Single or serial deposits may either appear as humps or may be firmly interspersed into the b.m. (Fig. 3B) thereby interrupting the regular continuity of the argyrophilic b.m. Depending on the extension of the non-argyrophilic material major or minor parts of the capillary walls loose their original properties becoming increasingly silver-negative and thickened. This type of thickening tends to be lucid rather than dense with our staining techniques, and bumpy rather than linear in character (Fig. 3C) as compared to MPGN type II. With silver stain the mesangial regions may be prominent (Fig. 3D) suggesting a moderate degree of mesangial activation and a tendency towards lobulation; the classical pattern of lobular GN, however, could not convincingly be demonstrated in association with the type III lesion. With PAS or Pearse stain alone the type III may resemble type I (Fig. 3E); the true character of the b.m. lesion becomes evident with silver stain revealing irregular, in places extreme non-argyrophilic Chromotrope 2R-positive thickening of capillary loops; the silver-negative parts may in places be lined by thin argyrophilic layers on either side (Fig. 3F). It should be stated, however, that the l.m. appearance does not always allow a definitive subclassification. The histological differential diagnosis may be MPGN, type I, type II, or perimembranous GN.

Electron Microscopy of the altered capillary walls does not reveal a linear, electron-dense b.m.-like material as a prevailing constituent of the thickened portions. Figure 4A demonstrates the irregular shape, medium density, and fairly uniform composition of the abnormal material with U/Pb-impregnation. The details of the underlying process, however, can only be visualized by s.i. (Fig. 4B). With this technique it becomes clear why in parts the lesion may appear as subendothelial deposit (c) with preservation of the b.m. The abnormal material, however, tends to accumulate towards the mesangial area with the b.m. gradually loosing its original structure: it looks fragmented, split, and dissolved to thin laminated argyrophilic remnants, embedded in the non-argyrophilic material or covering it from either side. Thus the appearance of subendothelial deposits changing to subepithelial deposits may be created. The characteristic feature of type III, however, seems to be the definitive incorporation of

[1] For colour photographs see Figure 2 of our previous publication (Anders and Thoenes, 1975)

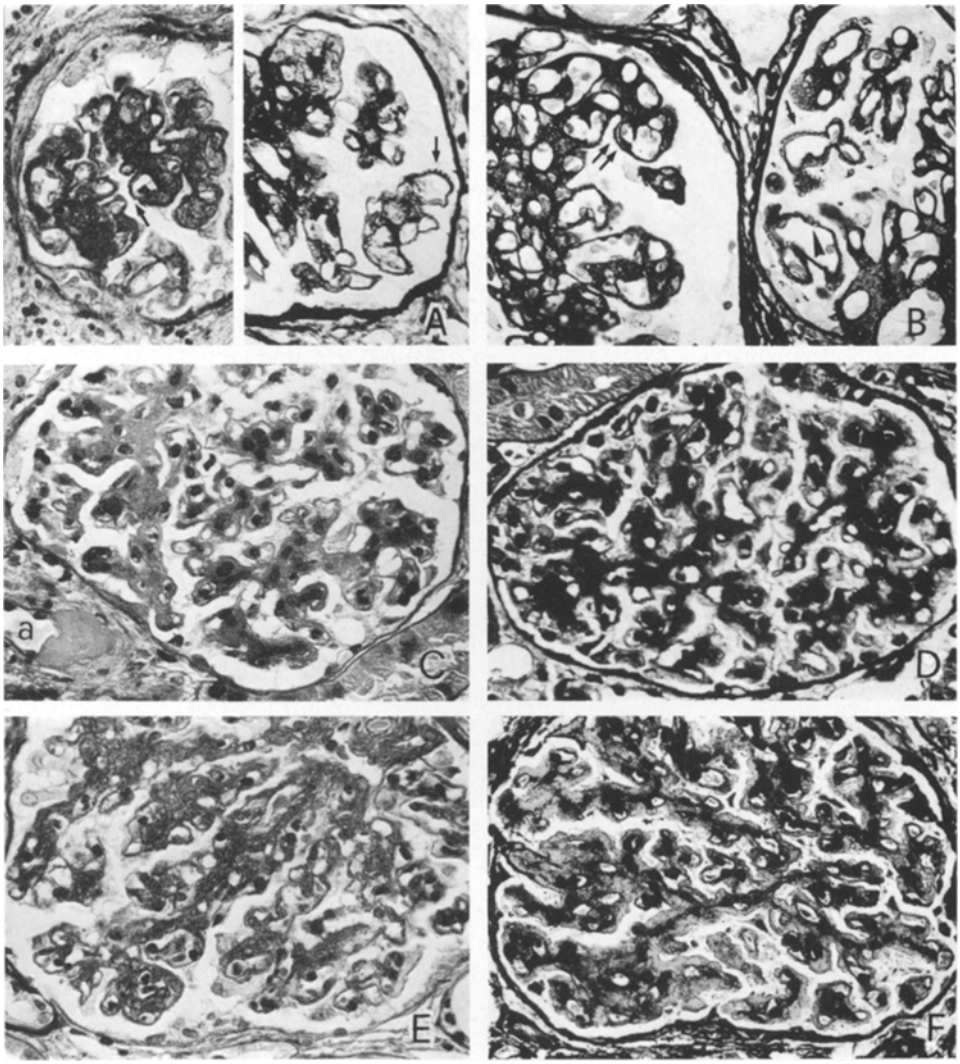

Fig. 3A–F. The histological pattern of ("pure") MPGN, type III, is characterized by diffuse but irregular thickening of glomerular capillary walls, little or no apparent proliferation, double contour formation or glomerular enlargement thereby presenting a puzzling combination with features of MPGN, type I, type II, and perimembranous GN. **A** Aspect of segmental subepithelial deposits and classical spike formation (arrows; case 3). **B** Two neighbouring glomeruli exhibit both, double contour formation suggestive of MPGN, type I (double arrow), and hump-backed capillary loops reminiscent of (atypical) perimembranous GN (arrow); light microscopic silver impregnation may sometimes be sufficient to reveal the atypical, interspersed character of "subepithelial deposits" (arrow head; case 2, persistently hypocomplementemic). **C** and **D** B.m. thickening may be extreme occurring in a very irregular distribution together with loss of argyrophilia. Almost complete lack of mesangial proliferation. Marked patchy hyalinosis of adjacent arteriole (a; case 16; for electron microscopy see Figures. 2E and 4). **E** Glomerulus suggestive of MPGN, type I; **F**, however, illustrates definitive b.m. involvement due to abundant accumulation of the non-argyrophilic (grey; original: pink) material characteristic of type III (case 13). Tri-PAS (Pearse; left) and silver (Jones-Chromotope-2R; right), all ×320. For colour prints see our previous publication, Figure 2

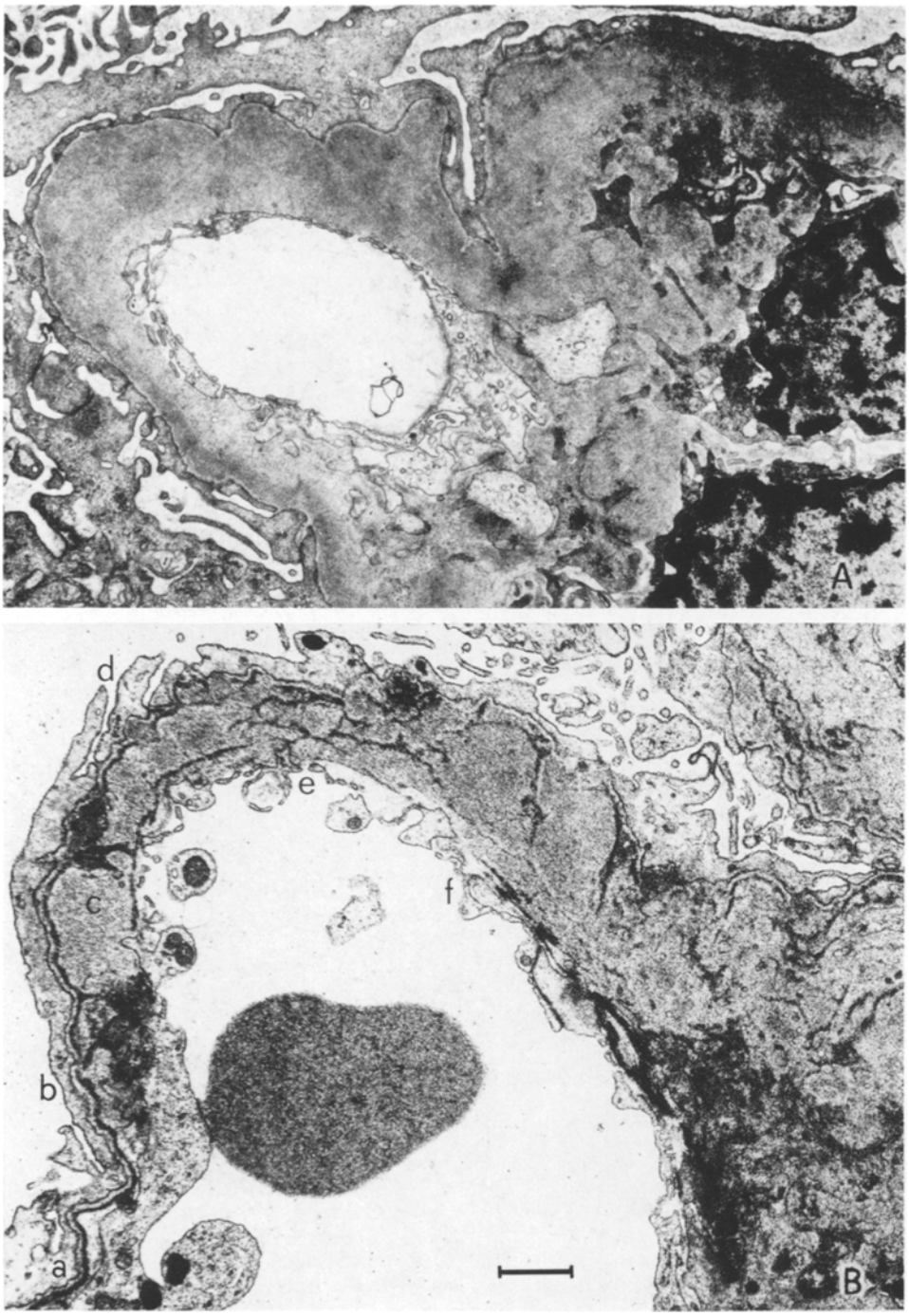

Fig. 4A and B. MPGN, type III. U/Pb-impregnation (**A**) gives the impression of a diffuse, ill defined b.m. thickening due to a medium dense, homogeneous material in association with mesangial interposition (upper right); there is no way of discriminating the abnormal material from the lamina densa other than (**B**): S.i. reveals the particular type of b.m. involvement with several stages (from lower left): Intact lamina densa (*a*), disrupted and distorted argyrophilic material (*b*), aspect of subendothelial deposits (*c*), subepithelial and subendothelial argyrophilic layer (*d*), multifold layering (*e*), and complete loss of the argyrophilic, linear structure or replacement of the lamina dense, respectively, by accumulating masses of a non-argyrophilic material in places reminiscent of subepithelial deposits (*f*). Case 16 (× 10,000); also see Figure 2E, for light microscopy Figure 3C and D

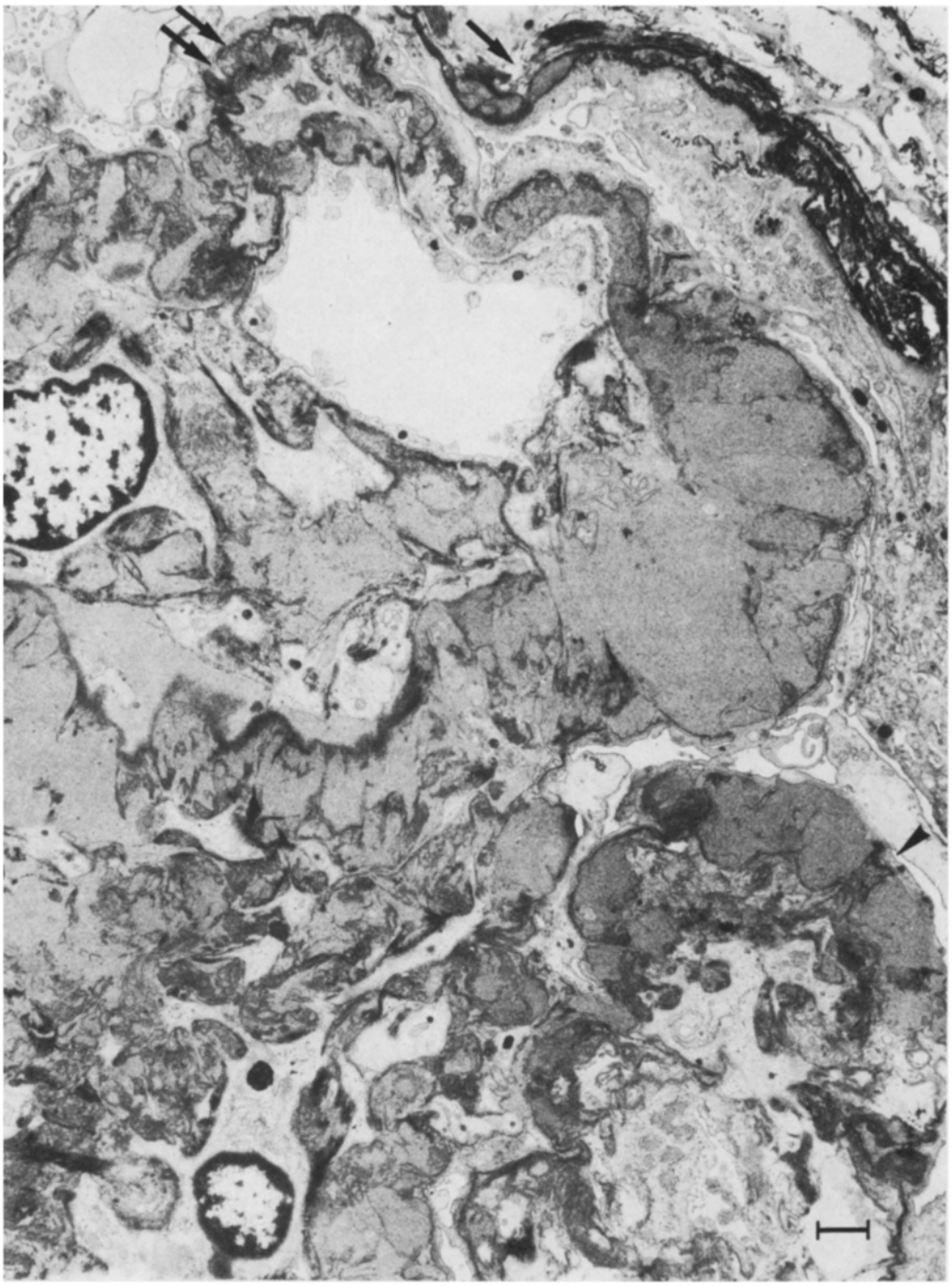

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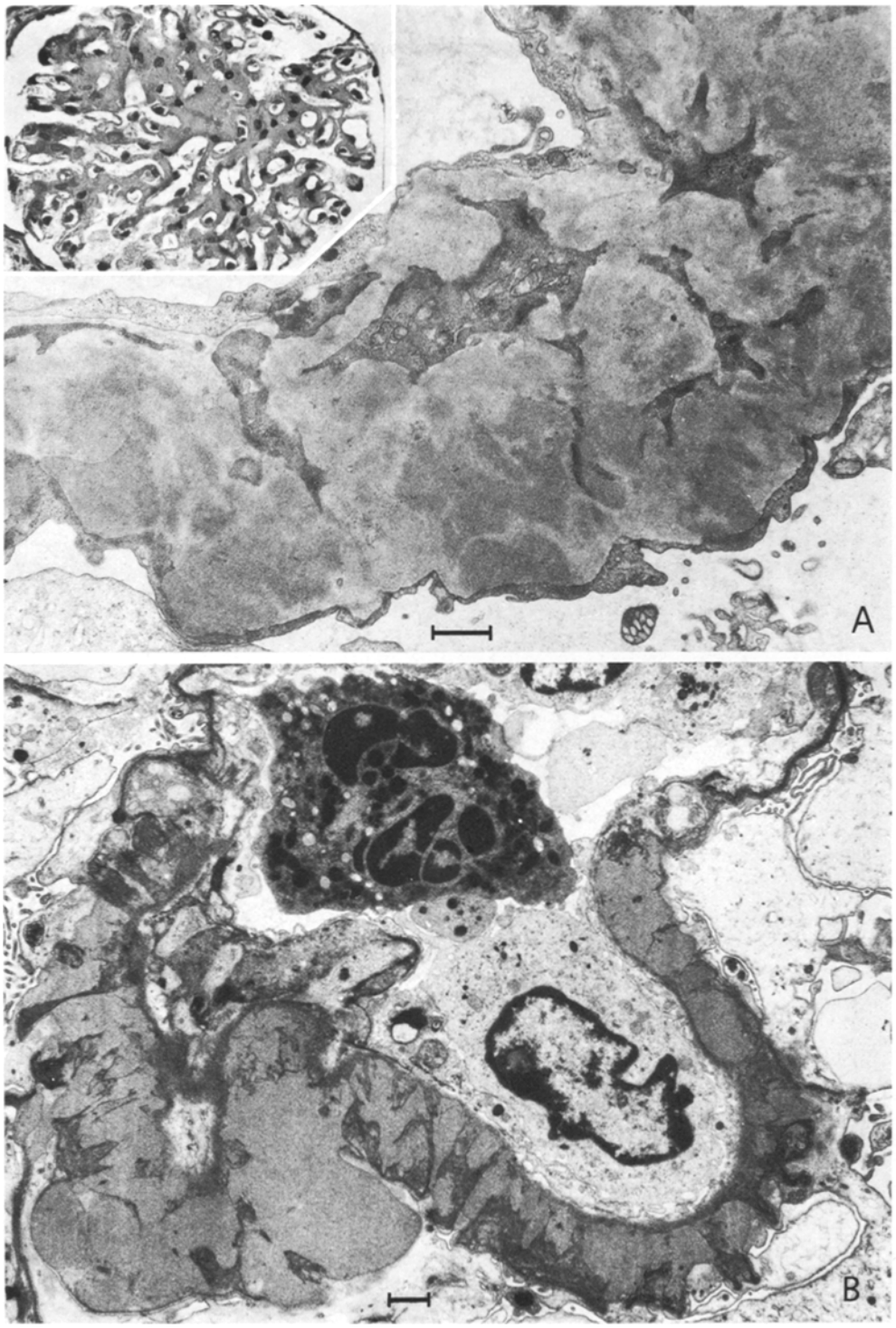
Fig. 5. MPGN, type III. Advanced stage of b.m. lesion with accumulation of a granular, medium dense, non-argyrophilic material in place of the b.m., lined only by a very thin subepithelial b.m.-like layer. Mesangial interposition is present though not very conspicuous (*double arrow*). Note involvement of the b.m. of Bowman's capsule (*arrow*). Single spike formation gives the aspect of adjacent subepithelial deposits (*arrow head*). Case 29, silver impregnation (×7500)

Fig. 6A and B. MGPN, type III. Same glomerulus as Figure 5. Based on the light microscopic appearance (inset: Pearse, ×240) the case was originally classified as perimembranous GN due to the virtual lack of proliferation and smooth appearance of capillary loops. Mesangial interposition,



however, resulting in fairly extensive double contour formation associated with stories of subepithelial deposits becomes evident by electron microscopy (**A**; U/Pb, ×8400). A better understanding of the b.m. lesion and the atypical mode of spike formation is achieved by s.i. (**B**; ×6000). The continuity of the original b.m. is not always preserved. In places the "spikes" seem to be closer related to the subepithelial argyrophilic layer (see Figure. 3B, single arrow) than to the original b.m. There is no "wash out" of deposits nor classical dome formation. For comparison to true perimembranous GN see Figure 2A

the abnormal material into the capillary wall thereby replacing its original structure (Fig. 5). Within a single glomerulus the mode of deposition may vary between a continous (Fig. 5) and a more circumscribed type (Fig. 6) with the appearance of single (hump-like) or serial subepithelial deposits with argyrophilic spike formation comparable to, though not identical with, that seen in perimembranous GN (Fig. 2A). In MPGN, type III spikes (Fig. 6B) are less regular in distribution, usually occurring in a segmental pattern or in single capillary loops. The deposits seem to be less commonly included into the capillary wall by dome formation but tend to remain "open" towards the (broadened) epithelial foot processes. "Wash out" effects resulting in a necklace appearance of the capillary loop as seen in the late stage of perimembranous GN have not so far been observed.

Immunofluorescence Microscopy. Findings were obtained from the cooperating laboratories in 4 of the 9 cases now classified as MPGN, type III. Three biopsies (cases 11, 13, 16) showed an isolated fixation of anti-C3, whereas in the fourth IgM, IgG, and IgA were present along the glomerular capillary loops together with mesangial IgM and C3.

Clinical Data. Short case reports with details of the clinical picture are given in the appendix. The group consists of 5 males and 4 females. At onset of the disease 5 patients where less than 15 years of age. Symptoms and signs associated with the type III lesion did not differ in any particular from those of the other patients with idiopathic MPGN. Except for one patient with asymptomatic proteinuria (case no. 2) all patients experienced at least one episode of the nephrotic syndrome; otherwise persistent proteinuria and microscopic hematuria were the constant findings. An apparently acute onset of renal disease with the nephritic syndrome was observed in two patients (cases no. 10, 11); a third (no. 16) experienced bouts of gross hematuria during the later stage of the disease. Abacterial leucocyturia was conspicuous in case 14. There was no evidence of antecedent streptococcal infection in any case. Five patients were hypertensive. All adults (cases no. 3, 10, 13, 29) developed chronic renal failure, two of them requiring maintenance dialysis after a follow-up period of 15 years.

Serum Complement (C3) was persistently depressed in six patients, initially depressed for several months in two (cases no. 13, 14), and normal throughout the observation period in one patient (no. 29).

Discussion

From the technical point of view the application of silver impregnation (s.i.) to renal tissue has been a major factor in the morphological understanding of the glomerular lesion now generally referred to as membranoproliferative glomerulonephritis (MPGN). Jones (1957) introduced a modified Gömöri technique into renal pathology and (1963) gave the classical description of double

contour formation of the glomerular capillary wall which up to now has been regarded as one of the main characteristics of the disease. The phenomenon is based on the argyrophilic properties of both the glomerular b.m. and the fine fibrillar "b.m.-like" material produced by mesangial cells along their cytoplasmic processes which, in turn, are interposed between the b.m. and the endothelium. This particular mode of proliferation is generally understood to be a mesangial reaction to deposits which are to be removed from the subendothelial space, leaving behind a more or less intact b.m. To be correct "mesangiocapillary GN" would be the preferable name for this classical pattern (type I) as it does not include true membranous changes, an assumption, however, which is still open to criticism. The association of true membranous changes with a variable degree of mesangial proliferation is generally recognized in MPGN, type II. Another mode of true b.m. involvement has been delineated in our previous study (Anders and Thoenes, 1975). For the lack of any better name it is now called "type III". The unique interrelationship between depot formation and substantial involvement of the b.m. in the MPGN types II and III has opened a new field for the application of s.i. techniques.

Movat (1961) was the first to use s.i. in electron microscopy. Following the description of the method his working group published several papers on the glomerular fine structure in the nephrotic syndrome (Movat, Steiner et al., 1961), in "pure nephrosis" (Steiner, Slater and Movat, 1961), and in acute glomerulonephritis (Movat, Steiner et al., 1962). The authors describe "argentophilia as distinct from osmiophilia", they use the term "Quellung" (=distention) of the b.m. in contradistinction to deposits located outside of the b.m., and they give examples of intramembranous dense deposits in cases which "progressed from the acute to the subacute stage." These findings regain interest from the view point of our present understanding of MPGN.

The value of silver impregnated ultra thin sections has later been reestablished by only a few groups (Habib et al., 1973; Strife, McAdams et al., 1974). In general, however, s.i. methods are said to be hazardous or of little value due to staining artefacts, poor reproducibility of the results, and the low degree of resolution which can be achieved under the electron microscope (original magnification not beyond $\times 4000$). Also, l.m. studies with s.i. of the type II lesion give equivocal results depending on technical factors such as fixation, embedding, and/or thickness of cutting. On the other hand, our preliminary results (Anders and Thoenes, 1975) suggested that s.i. of ultra thin sections might be the only way to settle the question of whether or not there is true membranous change in MPGN. On the basis of the e.m. control studies (Figs. 1, 2) it can now be stated that s.i. of ultra-thin sections gives uniformly good results for the visualization of fine structural details, in particular the b.m. changes under discussion. The restriction to low power resolution is no serious disadvantage as 1. the enhanced contrast compensates for low magnification, 2. wide field electron microscopy is particularly useful in the search for scattered deposits, and 3. previous studies had shown that high power resolution of U/Pb-impregnated sections did not contribute to understanding of the b.m. changes.

To date, the true nature of the type II lesion ("intramembranous dense

deposits" or "electron-dense alteration of the b.m.") had been debatable in terms of argyrophilia. The different laboratory methods of different groups gave differing results. Movat et al. (1961, 1962) were not in a position to classify their findings. Using protargol impregnation they described dark deposits, "some apparently within the b.m.", and concluded that the abnormal material might become "more b.m.-like through ageing and polymerization". The original description of "dense deposits" by Berger and Galle (1962) reflects the impression of an altered b.m. material ("altération singulière") with loss of argyrophilia in paraffin sections ("large bande bistre bordée en dedans et en dehors d'un mince liséré noir"). Also, from a single working group different views have emerged at different times:

"The dense substance which impregnates the b.m. in long ribboned strands reveals an intense argyrophilia which is homogeneous and resembles that of the normal b.m." (Habib et al., 1972, Fig. 24). "With silver stains... the b.m. proper was thickened by a non-argyrophilic deposit involving most of the peripheral capillary loops lying between the epithelial and the endothelial layers" (Habib et al., 1973, p. 198). But again: "When impregnated by silver methenamine this material exhibited the same strong affinity for silver as the lamina densa" (Habib et al., 1975, p. 210).

The answer seems to be that these discrepancies do not simply reflect technical differences. They rather indicate that in MPGN two similar varieties of true membranous changes exist. Relying on s.i. of ultra thin sections as the most subtle technique of differentiation the discrepancies can be eliminated by the acceptance of a third type which is neither identical with type I nor type II but shares certain morphological features of either: Type III resembles type I in that the intramembranous electron-dense and strongly argyrophilic material of type II is missing, and it resembles type II in that the b.m. does not remain intact as presumed in type I. Therefore, type III takes an intermediate position between the types I and II thereby supporting a unifying concept of MPGN. Without s.i. of ultra thin sections type III is likely to be mistaken for type II or perimenbranous GN on the basis of the l.m. appearance, and for type I on the basis of the e.m. appearance with U/Pb-impregnation.

The essential morphological differences are summarized in Table 1. The three MPGN types are not considered to be strictly isolated entities. Some observations rather suggest that the morphological characteristics constitute a spectrum of changes with the typical cases standing out, and where intermediate or mixed lesions may also occur. Case 10, e.g. exhibits non-argyrophilic b.m. thickening associated with prominent subendothelial deposits, a mixed immunofluorescence microscopic pattern, and persistent serum C3 depression; it therefore can be understood as an intermediate lesion between the types I and III. Another example, case 11, displays overlapping features of the types II and III in that some capillary loops contain intramembranous strands of greater electron-density (Fig. 9A of our previous publication) than usually seen in the pure type III lesion.

A subclassification concept of MPGN with "at least three different ultrastructural and several immunofluorescence patterns" was proposed by Burkholder (1974). This concept, however, differs from the view presented in this paper. His type I (with electron-dense alteration of the b.m.) corresponds to our type II and vice versa. His type III is described as a mixed variety closely related to his type II (with prevalence of subendothelial deposits, mesangial

Table 1. The essential morphological differences between the three MPGN types

		Type		
		I	II	III
Light microscopy	Glomerular enlargement, mesangial hypercellularity, double contour formation	++	(+)	(+)
	Lobular pattern	+	++	0
	Capillary wall thickening	++	(+)	+
	"Spike" formation	0	(+)	+
Electron microscopy	True b.m. involvement	0	+	+
	Argyrophilia of b.m. continuity of lamina densa	+ (preserved)	++ (pronounced)	0 (lost)
Immuno-fluorescence microscopy	Capillary wall	coarsely granular IgG, IgM	isolated linear C_3+	predominant C_3++
	Mesangium	C_{1q}, C_4, C_3++ , (Prop.)	isolated granular C_3++	predominant C_3++

interposition and 'tram track' alteration of the capillary wall) but characterized by additional features of membranous GN such as subepithelial deposits and focal spike formation; the pattern has therefore been named "mixed membranous and proliferative GN" (Burkholder, Hyman et al., 1972). The authors also describe subendothelial and intramembranous deposits as characteristic of both type I and type II. Thus the distinction between mixed deposits and true b.m. changes does not remain clear, presumably due to the previously mentioned disadvantages of conventional impregnation techniques.

The lesion presented here as "type III" resembles type II in that it is hard to describe in terms of deposits with a defined localization. Type III basically differs from lesions with "mixed" deposits. The abnormal material replacing parts of the b.m. may be the result or remainder of true (immune) deposits but it is less electron-dense and less granular than classical deposits. It seems to be fairly resistent to break down, if not irremovable. While accumulating it is covered again and again by layers of (argyrophilic) b.m. material thereby becoming even less accessible and firmly incorporated into the capillary wall which, as a result, steadily increases in thickness. In contradistinction to type II the abnormality of the (non-argyrophilic) material is evident whereas in type II the surplus material is more b.m.-like as shown by s.i. and by chemical analysis (Galle and Mahieu, 1975). Therefore, type III cases are likely to be classified as type I. The low grade electron-density in the abnormal type III material signals a composition different from both lamina densa and classical immune deposits which are comparatively electron-dense with U/Pb-impregnation, presumably due to their contents of antigen (e.g. in LE, Fig. 2B), immune globulin,

or both. The absence of immunoglobulins in type III is also suggested by the predominant or exclusive presence of C3 in the immunofluorescence pattern.

This conclusion cannot be drawn firmly from our small series. There is indirect evidence, however, that the type III lesion has been observed in other studies concerned with MPGN. If the clinico-pathological interrelationship of the disease is to be based on a dual system with type I being essentially normocomplementemic and type II being hypocomplementemic, type III appears as a more or less hidden subclassification problem. Of 43 patients with "dense deposits" (type II) studied by Antoine and Faye (1972) five were examined for serum complement levels, and all were normal; the morphological classification was mainly based on light microscopy with J. Berger, P. Galle and other experienced pathologists as consultants. Conversely, Habib et al. (1973) reported on at least 4 children with subendothelial deposits (type I) and persistently low serum C3 levels which in 2 cases were related to a predominant presence of β1c in the immunofluorescence pattern. A similar group of 8 patients with constantly diminished serum C3 levels has recently been distinguished by Loirat and Levy (1976) from a major group of 34 children with subendothelial deposits; of 21 studied by immunofluorescence microscopy the isolated presence of C3 and properdin was noted in 7. Among 19 patients with "dense 'deposits'" observed at Guy's Hospital, London (Vargas et al., 1976), only 11 had a consistently low plasma concentration of C3.

For several reasons the significance of the serum C3 level should not be overestimated. If persistent serum C3 depression is present it is an important characteristic of MPGN. Hypocomplementemia, however, is not an accurate measure of the actual degree of complement activation or extent of renal disease, nor does normocomplementemia exclude complement activation. Also, Cameron et al. (1973) have shown that the serum C3 level is of little clinical significance as compared to the presence or absence of the nephrotic syndrome.

Renal morphology has usually proved to be the most reliable technique for the diagnosis and subclassification of MPGN. The inconsistencies described above as inherent in the current dual subclassification concept can probably be overcome – at least in part – by the acceptance of the type III lesion as defined by its appearance in silver impregnated ultra thin sections. This approach has recently also been proposed by McAdams (1976) and McDonald et al. (1976) and Strife et al. (1977). The authors describe the same type of b.m. lesion, hard to distinguish from the types I or II without s.i. of ultra thin sections. The fine structural characteristic of a "disrupted" b.m. is correlated with the clinical and immunological data of seven patients including four in whom rebiopsies were performed. The term "disruption of the b.m." might suggest an acute destructive event; to our impression, however, the abnormal material is slowly incorporated into the capillary wall as a firm constituent owing to its substantial stability.

One might object to further complication of the subclassification of MPGN by introducing a third type. It seems more important, however, to gain further insight into the pathogenesis of MPGN. This can probably be achieved by detailed analysis of the b.m. changes. The existence of the type III lesion as interpreted in this study favours the working hypothesis that the nature of this form of chronic glomerulonephritis may depend on the inability of the organism to eliminate an abnormal product from the glomerular capillary wall. This may be due either to a persistent formation or an abnormal composition

of the material or to a deficiency of the break-down mechanism, e.g. mesangial cell function. In any event the main characteristic of both MPGN types II and III, seems to be that irremovable material, bearing an immunological relationship to complement, tends to be firmly incorporated into the glomerular capillary wall. As a result the notional difference between "deposits" and a substantial alteration of the basement membrane – with or without preservation of argyrophilia – cannot be clearly defined.

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Appendix: Short Case Reports

A. Control Cases

Case I (B 1033; Fig. 1A). 11-y-old girl. Biopsy 5 months following onset of steroid-resistent nephrotic syndrome. Clinical data at the time of biopsy: Proteinuria 12–22 g/d, microscopic hematuria 50–300/µl, serum creatinine 1.0 mg%, total serum protein 5.0 g%, albumin 35%, α_2-46%, γ-globulin 4%. *Histological Diagnosis.* Slight mesangial proliferation.

Case II (B 1609; Fig. 2A). 8-y-old girl. Renal biopsy 8 months after onset of the nephrotic syndrome with facial edema, persistent proteinuria up to 4 g/d, and microscopic hematuria. Au-SH-Ag positive acute hepatitis proven by liver biopsy 4 weeks prior to renal biopsy. BP 110/70 mm Hg, proteinuria 0.5 g/d, microscopic hematuria. Total serum protein 6.2 g%, cholesterol 503 mg%, creatinine 1.0 mg%, LE preparation neg. *Histological Diagnosis.* Perimembranous GN; immunefluorescence: generalized nodular deposits of IgG, Au-SH-Ag, C_{1q}, C_4 and C_3 along glomerular capillary walls.

Case III (B 990; Fig. 2B). 46-y-old female. Biopsy 3 weeks after onset of rapidly progressive GN, nephrotic syndrome and renal insufficiency associated with myositis. BP 170/90 mm Hg. Proteinuria 3.0 g/d. Antinuclear antibodies + +, depression of serum complement, serum cholesterol 330 mg%, creatinine 3.4 mg%. *Histological Diagnosis.* Diffuse proliferative GN compatible with LE; immunefluorescence: massive glomerular deposition of IgG (+ +), IgM (+ +), IgA (+) and complement (+ +) in a segmental and lobular pattern with mixed granular and linear distribution.

B. MPGN, Type I

Case 30 (B 3273; Fig. 1B, D; 2C). 12-y-old boy. Present (3.) biopsy 2 years after diagnosis of proteinuria and microscopic hematuria following streptococcal pharyngitis with ASO 625 U. and normal C_3 during the acute phase. At the time of biopsy: Febrile episode with sinusitis, abdominal pain, nausea. BP 120/80 mm Hg. Trace proteinuria, microscopic hematuria 20/µl, ASO 250, C_3 normal.

C. MPGN, Type II

Case 23 (B 839; Fig. 2D). 7-y-old girl. Biopsy (lobular GN) 6 months following insidious onset of slight periorbital edema, proteinuria (2.5–4 g/d), microscopic hematuria (80/µl), abacterial leucocyturia (100/µl), and slight hypertension (140/80 mm Hg). Serum protein 4.3 g%, creatinine 1.0 mg%. Persistent depression of serum-C3 on five determinations during 2 years. No treatment, no deterioration.

D. MPGN, Type III

Case 1 (B 108). 14-y-old girl. First biopsy 4 years following discovery of proteinuria (4–6 g/d) irresponsive to long term treatment with steroids and penicillin. Six episodes of the nephrotic syndrome. Microscopic hematuria, serum protein 4.4 g%, creatinine 0.4 mg%, C3 persistently depressed. Repeat biopsy one year later following a six months course of cyclophosphamide with

slight improvement of proteinuria, normal renal function, essentially unchanged hypocomplementemia, no edema, BP normal, serum protein 5.2 g%, cholesterol 420 mg%. ASO and LE-prep. negative.

Case 2 (B 167; Fig. 3B). 18-y-old male patient. Biopsy 1 year following detection of asymptomatic proteinuria and microscopic hematuria. BP and renal function normal. Serum C3 depressed on two occasions during an observation period of 14 months.

Case 3 (B 250; Fig. 3A). 41-y-old female patient. Proteinuria and edema during pregnancies 12 and 7 years prior to biopsy. General deterioration with the nephrotic syndrome, hypertension (200/100 mm Hg), allergic reactions to several antibiotics, and acute renal failure following blood transfusion. Peritoneal dialysis. Recovery to a serum creatinine of 1.9 mg% at the time of biopsy. Persistent proteinuria (5 g/d), microscopic hematuria. C3 depressed on two occasions, normal on control, again depressed 2 years later. LE-preparations repeatedly negative, and no symptoms of SLE following cessation of steroid and azathioprine treatment. Maintenance dialysis 3 years following biopsy.

Case 10 (B 863). 24-y-old female patient. Onset of acute GN and the nephrotic syndrome at the age of 9 following repeated episodes of tonsillitis. Inefficient steroid treatment at the age of 15 and 22. Persistent proteinuria (3.8 g/d), microscopic hematuria, serum albumen 2.2 g%, creatinine 1.8 mg%. C3 normal, one year later depressed. ASO negative. Maintenance dialysis $1^1/_2$ years following biopsy. *IFM*: IgM, IgG, IgA along the glomerular b.m., mesangial IgM and C3

Case 11 (B 875). 18-y-old male patient. Present (2.) biopsy 4 years following onset of acute GN and the nephrotic syndrome without apparent antecedent streptococcal infection. Previous biopsy: "Intracapillary GN with focal lobulation". Inefficient combined treatment with steroids, azathioprine and cyclophosphamide. Persistent proteinuria (1.3 g/d), microscopic hematuria, and marked C3 depression (15–30 mg%) on four occasions during 3 years. Renal function normal, slight hypertension (145/90 mm Hg). *IFM*: Isolated diffuse fine granular deposition of C3 along the glomerular capillary walls reminiscent of perimenbranous GN.

Case 13 (B 999; Fig. 3E, F). 34-y-old female. Biopsy 5 years following onset of the nephrotic syndrome and hypertension. Serum C3 initially depressed on two occasions, lateron normal on three determinations during two years before installation of maintenance dialysis. *IFM*: Isolated deposition of C3 along the glomerular capillary loops.

Case 14 (B 1128). 9-y-old boy. Biopsy 1 year following detection of urinary abnormalities and 3 months following onset of steroid-resistent nephrotic syndrome. Slight orbital and tibial edema, proteinuria 4–6 g/d, microscopic hematuria, abacterial leucocyturia (90/µl), serum albumen 1.1 g%, cholesterol 630 mg%, renal function and BP normal. C3 initially depressed but returning to normal and remaining so on three occasions during 2 years. No response of proteinuria to continued alternate day prednisone therapy and a two months course of cyclophosphamide.

Case 16 (B 1465, Figs. 2E, 3C and D, 4). 11-y-old boy. Detection of asymptomatic proteinuria (less than 2 g/d), microscopic hematuria (800/µl) and C3-depression on occasion of surgery for unilateral cryptorchidism at the age of 9. First renal biopsy at that time: "Severe postacute GN". Occasional bouts of gross hematuria and persistent proteinuria irresponsive to a 4 weeks leucopenic course of cyclophosphamide. Ten months later laboratory diagnosis of the nephrotic syndrome: Serum albumen 3 g%, cholesterol 311 mg%. Present (2.) biopsy 3 years following recognition of renal disease. General deterioration with pallor, edema, hypertension, serum creatinine 3.5 mg%, marked depression of C3. *IFM*: Isolated granular deposition of C3 in the mesangial regions of single capillary loops. Follow-up: Normalization of serum creatinine, BP 150/85 mm Hg, persistent proteinuria (2 g/d) and microscopic hematuria irresponsive to another course of cyclophosphamide (100 mg/d for three months).

Case 29 (B 284). 50-y-old male. Present (2.) biopsy 4 years after discovery of proteinuria. Clinical diagnosis: Chronic nephrotic GN with renal insufficiency. Previous histological diagnosis: Chronic membranous GN. Clinical data: BP 120/80 mm Hg, proteinuria 4–8 g/d, microscopic hematuria 3/µl, total serum protein 6,5 g%, Albumen 51,4%. Serum creatinin 2.4 mg%, LE preparations (+), $\emptyset$, $\emptyset$, $\emptyset$, C_3 normal.

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