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Glomerulonephritis with Dense Deposits: A Variant of Membranoproliferative Glomerulonephritis or a Separate Morphological Entity?

Light, Electron Microscopic and Immunohistochemical Study of Eleven Cases

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Summary. Eleven cases of glomerulonephritis with dense deposits were selected on the basis of electron microscopic examination performed either on material treated according to conventional techniques (9 cases) or on previously paraffin-embedded material (2 cases). While uniform immunohistochemical patterns were observed, different features were shown by light microscopy: in only 3 cases were membranoproliferative or lobular patterns present, while in the others a varying degree of mesangial cell proliferation (moderate, mild or even very scanty with focal and segmental distribution) was detected.

The generally accepted statement that glomerulonephritis with dense deposits represents a subgroup of membranoproliferative glomerulonephritis therefore seems questionable. In addition to several clinical and serological data, these morphological features give further support to the hypothesis that glomerulonephritis with dense deposits is in all respects a peculiar and distinct form of glomerulonephritis.

Key words: Glomerulonephritis – Dense deposit disease – Electron microscopy.

Introduction

Dense deposit disease (DDD) was first described by Berger and Galle in 1963. Subsequently, several cases have been studied in detail by light and electron microscopy and immunofluorescence techniques (Hamburger et al., 1966; Bariéty et al., 1971; Mathew and Kincaid-Smith, 1971; Antoine and Faye, 1972;

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Habib et al., 1974, 1975; Galle and Mahieu, 1975; Vargas et al., 1976; Lamb et al., 1977; Davis et al., 1978).

DDD can be recognized by PAS or light green positive thickening of the glomerular, capsular and tubular basement membranes. Very recently, the fluorescent dye thioflavine T has been used for identification purposes (Churg et al., 1979). Nevertheless the most characteristic features are the ribbonlike dense deposits, shown by electron microscopy.

Morphologically, DDD has been considered as a variety of membranoproliferative glomerulonephritis (MPGN) (Bariéty et al., 1971; Burkholder, 1974; Bohle et al., 1974; Habib et al., 1974, 1975; West et al., 1976; Lamb et al., 1977; Jones, 1977; Davis et al., 1978). In contrast Zollinger and Mihatsch (1978) consider DDD as a specific entity, distinct from type I MPGN with subendothelial deposits, because of the morphology of the intramembranous material and the likelihood of a different aetiology and pathogenesis.

This latter suggestion is supported by a careful analysis of the morphological patterns of the cases reported by various authors. In fact, prominent mesangial cell proliferation and extensive circumferential cell interposition (i.e. the highly distinctive features of MPGN) were present only in some of the cases. Others displayed a more or less evident mesangial cell proliferation without extensive splitting of capillary basement membranes or lobular pattern (Morel-Maroger et al., 1972; Habib et al., 1975; Vargas et al., 1976; Davis et al., 1978). However, the immunohistochemical pattern of DDD is quite different from that described in type I MPGN (see for example Barbiano di Belgiojoso et al., 1976 and Zollinger and Mihatsch, 1978).

From the literature it seems that DDD can display various morphologic features, which hardly justify its classification as a subgroup of MPGN. For this reason we have reconsidered eleven cases of DDD diagnosed electron microscopically, paying particular attention to their light microscopic and immunofluorescent features.

Table 1. Clinical and light microscopic data

Patient		Age/Sex	Interval onset renal disease to biopsy	C_3 level	N° glomeruli	GBM thickening	BBM thickening
1.	B.G.	58/M	5 months	normal	30	+FS[b]	−
2.	R.E.	28/M	5 years	low	11	+++	+
3.	S.E.	40/F	13 years	low	20	+++	+
4.	T.G.	8/F	1 year	low	40	++	−
5.	G.E.	23/F	13 years	n.t.[a]	16	+++	+
6.	T.B.	32/M	not known	n.t.	120	+++	+
7.	C.A.	21/M	5 years	n.t.	9	+++	+
8.	S.B.	15/F	7 months	low	10	++	−
9.	A.M.	6/M	3 months	low	35	+	−
10.	B.N.	26/M	8 years	low	14	+FS	−
11a.	G.G.	27/M	10 months	low	13	+FS	−
11b.	G.G.	29/M	3 years	low	8	+FS	−

[a] n.t. = not tested

[b] FS = focally and segmentally distributed

Material and Methods

From our files we selected 12 biopsy specimens from 11 patients (6 were males and 5 females). The age at the onset of the nephropathy ranged from 6 to 58 years, while the period between the onset of symptoms and biopsy ranged from 3 months to 13 years. No patient showed clinical and/or serological evidence of systemic disease. Serum complement levels were tested in 8 patients: C_3 was reduced in 7, while C_4 was normal in all. One patient (n° 11) had two biopsies.

DDD diagnosis was established by electronmicroscopy. In nine cases the ultrastructural investigation was performed on glutaraldehyde-fixed, Durcupan-embedded material. In two (n° 9, 10) previously paraffin-embedded material was used: thick (30 μ) sections were deparaffinized, fixed in OsO_4 and embedded in Durcupan ACM Fluka; although the ultrastructural images were poor, dense deposits were sufficiently recognizable to allow the diagnosis.

Specimens for light microscopy were fixed in Serra or Dubosq-Brazil fluid and embedded in paraffin. Sections were stained with haematoxylin and eosin, periodic acid-Schiff, Silver methenamine and Masson light green trichrome.

Immunofluorescence was performed in 8 cases. Small biopsy fragments were quickly frozen in liquid nitrogen or in dry ice. Cryostat sections (4–5 μ thick) were stained with fluorescein-labeled antisera to human IgG, IgA, IgM, C_3, C_4, C_{1q} and F (Behringwerke, Marburg).

Results

Light Microscopy. Light microscopic findings are summarized in Table 1. The most constant and characteristic histological feature was PAS-positive, eosinophilic thickening of the glomerular basement membranes. This finding was widespread in eight specimens (n° 2, 3, 4, 5, 6, 7, 8, 9), being prominent in seven and less marked in one (n° 9). In two patients (n° 3, 5) the marked and diffuse glomerular basement membrane thickening, together with focal segmental areas of sclerosis was the most evident change (Fig. 1). In the other four biopsies (n° 1, 10, 11a and b), the glomerular basement membrane thickening was mild, focal and segmental in character (Fig. 2). In nine biopsies (n° 1, 2, 3, 4, 5, 7, 8, 11a, b) ribbon-like deposits were clearly demonstrated by the Masson

TBM thickening	Endothelial cellularity	Mesangial cellularity	Mesangial sclerosis	GBM splitting	Glomerular exudation	Interstitial fibrosis	Tubular atrophy
−	+FS	+FS	+	−	+	++	++
+	+	++	++	−	−	+++	++
+	−	+	+++	−	−	+	++
−	−	+FS	+	−	−	−	−
−	−	+FS	++	−	−	+	+
−	−	++	+++	−	−	+	++
+	+	++	++	−	−	+	+
+	++	+++	++	++	+	−	+
−	−	+FS	+FS	−	−	−	−
−	++	+++	++	+	++	+	+
−	+FS	+	+	−	+	−	−
−	−	+	−	−	−	−	−

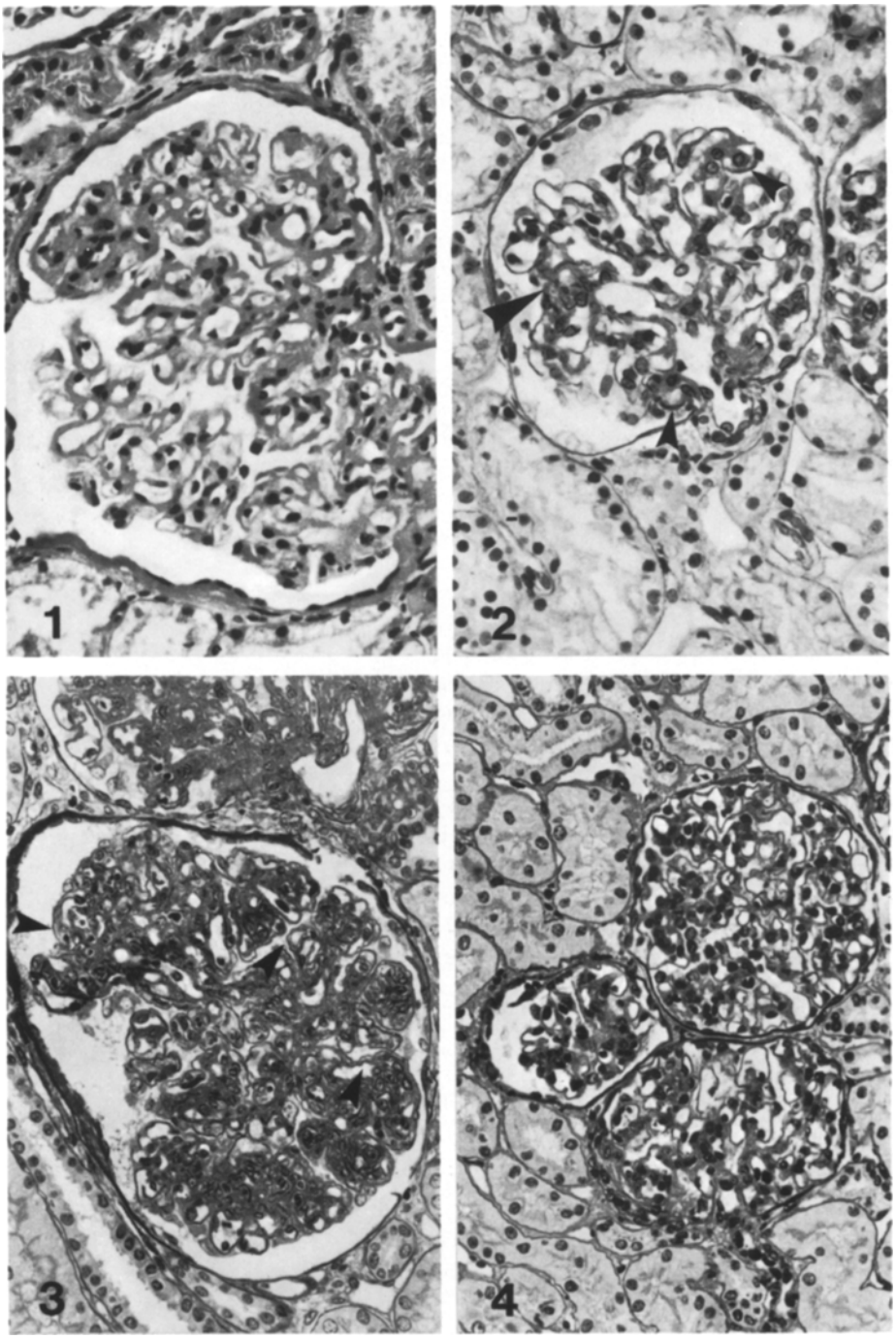


Fig. 1. Case 5. Glomerulus with marked and diffuse basement membrane thickening and very mild and segmental mesangial proliferation. PAS ×250

Fig. 2. Case 1. Glomerulus with segmental thickening of the basement membranes (*small arrows*). Cell proliferation is scarce and focal (*large arrow*). PAS ×150

Fig. 3. Case 8. Glomerulus showing membranoproliferative pattern. Endothelial and mesangial cell proliferation is severe. Basement membrane splitting and mesangial cell interposition are evident (*arrows*) PAS ×210

Fig. 4. Case 2. Glomeruli with diffuse mild endothelial and moderate mesangial cell proliferation. The basement membranes show light but diffuse thickening. PAS ×110

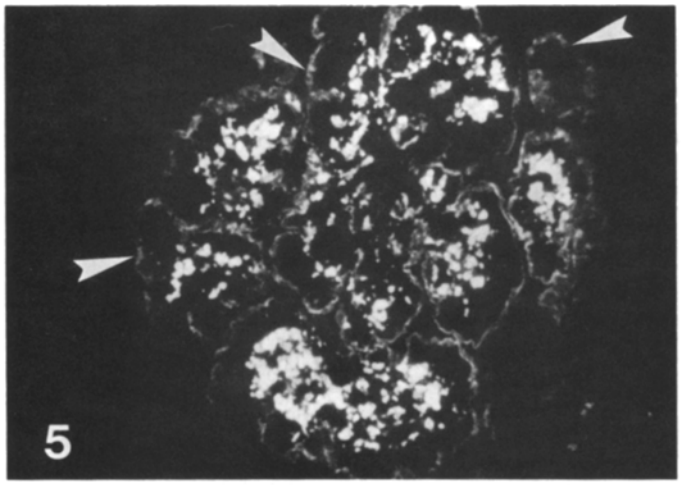
trichrome stain. Similar thickening and deposits, although focal and segmental, were present in the Bowman's capsule in five biopsies (n° 2, 3, 5, 6, 7) and in the tubular basement membranes in four (n° 2, 3, 7, 8).

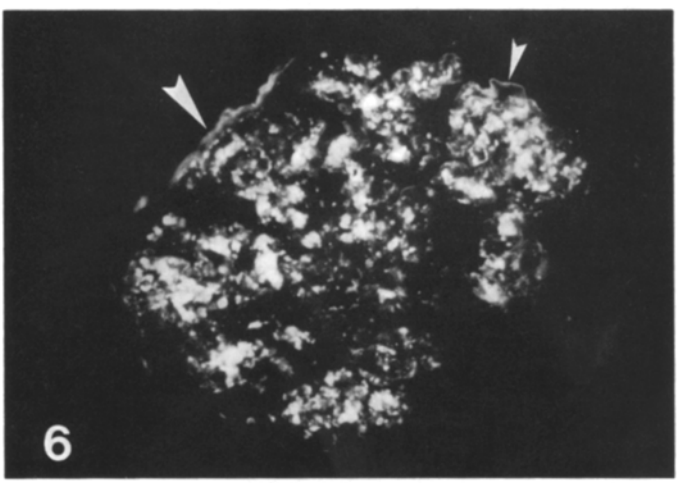
The degree of glomerular proliferation was highly variable. Endothelial proliferation was present in six specimens, being focal and/or segmental in two (n° 1, 11a) and diffuse in the others (n° 2, 7, 8, 10); in cases 2 and 7 it was mild, while in cases 8 and 10 it was so extensive as to affect the patency of the glomerular capillaries (Fig. 3). Mesangial hypercellularity, even though present in all the cases, showed various degrees of severity. It was mild in seven biopsies (n° 1, 3, 4, 5, 9, 11a, b), being segmental and focal in four of them (n° 1, 4, 5, 9) (Figs. 1, 2); moreover, four of six cases showed no endothelial proliferation, while in case 1 and in the first biopsy of case 11 it was mild and segmental. Diffuse mesangial hypercellularity was present in five specimens, being moderate in patients 2, 6, 7 (Fig. 4) and severe in patients 8 and 10 (Fig. 3); in the latter, glomerular basement membrane reduplication and circumferential mesangial cell interposition were clearly evident, giving the typical membranoproliferative pattern (Fig. 3). In patient 6, owing to the prominent nodular mesangial sclerosis, lobular features were easily recognizable. Apart from the abovementioned cases (n° 8, 10), mesangial proliferation was confined to the glomerular stalk and mesangial cell interposition could not be detected, in spite of a careful search even with silver staining.

Glomerular exudation was present in four patients, being mild in three (n° 1, 8, 11a) and moderate in one (n° 10). Different degrees of extraglomerular changes (interstitial fibrosis and inflammation, tubular atrophy) were present in most cases, roughly correlated with the severity of the glomerular changes.

Immunofluorescence. Data are summarized in Table 2. All cases displayed diffuse and generalized C_3 deposits with various patterns. All cases showed prominent lumpy mesangial deposits (Figs. 5, 6); C_3 deposits were seen along the peripheral capillary walls in specimens 1, 2, 3, 4, 8, 10, 11a (Figs. 5, 6) either as granular or as interrupted linear segments. In addition, either granular or linear C_3 deposits were detected along Bowman's capsule in two cases (n° 1, 8) (Fig. 6) and along tubular basement membranes in four others (n° 2, 8, 9, 11). C_3 alone was found in cases 4, 9 and 11. IgM granular deposits, mainly along the glomerular basement membranes, were identified in four instances (n° 1, 2, 8, 10), showing an irregular distribution in three cases (Fig. 7) and a clearly focal segmental pattern in one (n° 1). IgM staining was from mild to moderate, but always less evident than that of C_3. Globular mesangial deposits of IgM were present in case 10. IgG and IgA were detected only in one case (n° 10 and 2 respectively). Slight C_{1q} and C_4 deposits were observed in three (n° 2, 3, 8) and two (n° 2, 8) specimens respectively.

Electron Microscopy. Intramembranous ribbon-like electron dense material was observed in all instances. With one exception (case 11), it occupied long segments of the lamina densa region of the glomerular basement membranes and less extensively the mesangial stalk (Figs. 8, 9); in case 11 intramembranous deposits were short, segmental and did not extend to the mesangial stalk. However in the second biopsy of this case (performed two years later), segmental deposits





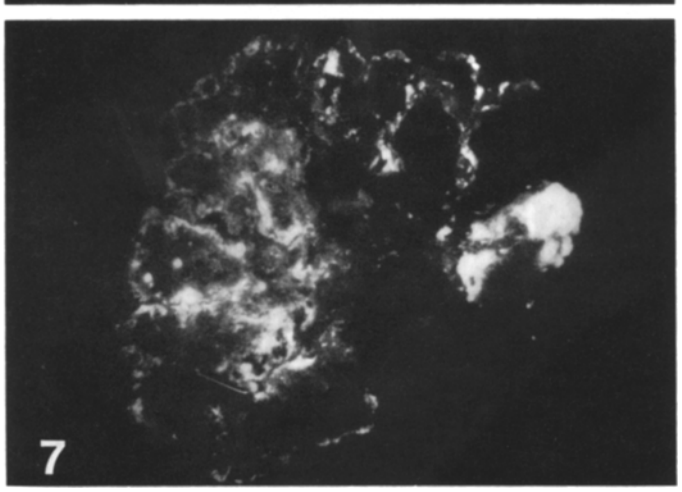
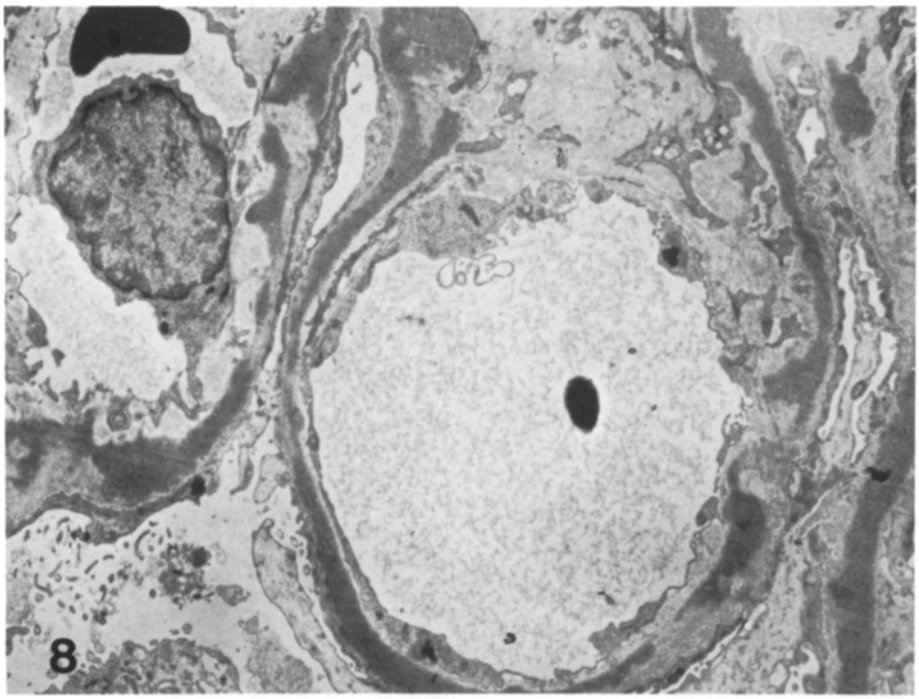
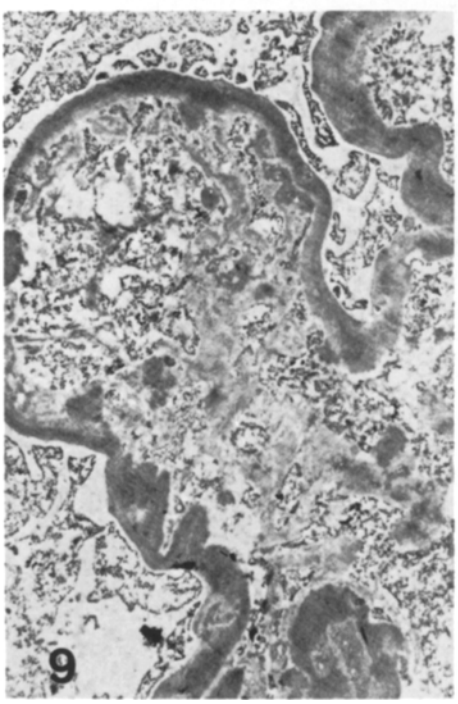


Fig. 5. Case 8. Glomerulus with coarse and bright granular mesangial deposits. Arrows point out small granular deposits in the capillary basement membranes. Fluorescent anti-human C_3. ×230

Fig. 6. Case 1. Glomerulus with mesangial granular deposits. The small arrow points out pseudolinear deposits in the capillary wall. Deposits are present in the Bowman's capsule as well (*large arrow*). Fluorescent anti-human C_3. ×200

Fig. 7. Case 8. Granular deposit of IgM are evident, mainly in the basement membranes, with irregular distribution. Fluorescent anti-human IgM. ×230





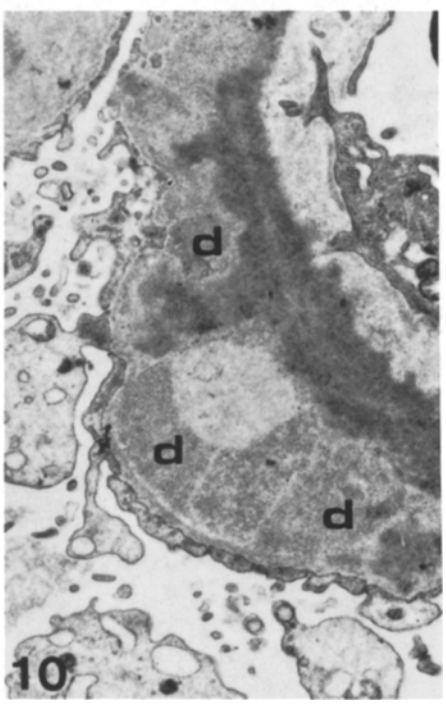


Fig. 8. Case 5. Ribbon-like deposits are evident in the basement membranes and in the mesangium. It is worth stressing the absence of endothelial proliferation. ×3,800

Fig. 9. Case 9. Section obtained from previously paraffin-embedded material. Dense deposits are evident in the basement membranes. ×3,900

Fig. 10. Case 5. Besides ribbon-like deposits, other deposits (*d*), less electron dense, are evident in the outer side of the basement membrane. ×9,000

Table 2. Immunofluorescence and electron microscopic findings

Immunofluorescence					
Patient		IgG	IgM	IgA	C_3
1	B.G.	–	GBM + SF granular	–	GBM ++ linear MES +++ lumpy BBM + linear
2	R.E.	–	GBM + + granular	GBM + + granular	GBM ++ linear MES +++ lumpy TBM + linear
3	S.E.	–	–	–	GBM ++ linear MES +++ lumpy
4	T.G.	–	–	–	GBM ++ linear MES +++ lumpy
5	G.E.	n.t.	n.t.	n.t.	n.t.
6	T.B.	n.t.	n.t.	n.t.	n.t.
7	C.A.	n.t.	n.t.	n.t.	n.t.
8	S.B.	–	GBM + granular	–	GBM + granular MES +++ lumpy BBM + linear TBM + linear
9	A.M.	–	–	–	MES ++ lumpy TBM + linear
10	B.N.	GBM + granular MES + + granular	GBM + granular MES + lumpy	–	GBM ++ granular MES +++ lumpy
11a	G.G.	–	–	–	GBM + linear MES +++ lumpy TBM ++ linear
11b	G.G.	n.t.	n.t.	n.t.	n.t.

GBM = glomerular basement membrane; BBM = Bowman's capsule basement membrane; TBM = tubular basement membrane; MES = mesangium; SF = segmentally and focally distributed; n.t. = not tested

were more evident and numerous. Small electron dense deposits, often in continuity with and less dense than the ribbon-like structures could be seen in the inner or the outer side of the basement membrane in cases 5 and 7 (Fig. 10). They were tentatively interpreted as partially degraded components of older intramembranous deposits. Large subepithelial, coarsely granular deposits ("humps") were present in the first biopsy of case 11.

			Electron Microscopy	
C_{19}	C_4	F	Dense deposits localization	Other deposits localization
–	–	–	GBM MES	–
MES+SF lumpy	MES+SF lumpy	–	GBM MES TBM	–
GBM+granular	–	–	GBM MES TBM	–
–	–	–	GBM MES BBM	–
n.t.	n.t.	n.t.	GBM MES TBM	GBM small discontinuous
n.t.	n.t.	n.t.	GBM MES	–
n.t.	n.t.	n.t.	GBM MES	GBM small discontinuous
GBM+granular	GBM+granular	–	GBM MES	–
–	–	–	GBM MES	–
–	–	–	GBM MES TBM	–
–	–	–	GBM discontinuous	humps
n.t.	n.t.	n.t.	GBM discontinuous	–

Deposits were observed in the tubular basement membranes in four cases (n° 2, 3, 5, 10) and in the Bowman's capsule in one (n° 4). The lack or paucity of the endothelial and/or mesangial proliferation, as shown by light microscopy, was confirmed in cases 3 and 5 (Fig. 8). The presence of prominent circumferential mesangial cell interposition, observed under light microscopy in case 8 and 10, was confirmed in the former, while in the latter the material (obtained

from paraffin embedding) was not adequate for assesment. Mesangial cell interposition was observed only occasionally in some cases, being limited to occasional capillary walls, particularly in sclerotic areas.

Discussion

The presence of peculiar ribbon-like electron dense deposits in the renal basement membranes is an unquestioned morphological feature for the diagnosis of DDD. Even though it is generally accepted that dense deposits can be identified by light microscopy, in some cases with few and very thin deposits, the diagnosis can be made only by means of electron microscopy (McCluskey, 1977; Churg et al., 1979). However, as stated by Churg et al. (1979) "in many glomerular diseases, basement membranes often acquire a degree of refractility that may lead to overdiagnosis of dense deposits". Furthermore, in our experience light microscopy alone is inadequate for diagnosis (Mazzucco et al., 1979). For this study the most restrictive diagnostic criterion has been chosen: only cases with ultrastructurally identified dense deposits have been selected and a careful evaluation of light microscopic and immunofluorescence patterns was performed.

Apart from the constant finding of thickened, chromophilic, and refractile basement membranes, our cases of DDD displayed quite different light microscopic patterns. Only three patients showed membranoproliferative (cases 8, 10) or lobular (case 6) changes. These data are in disagreement with those usually reported in the literature; in effect, most cases of DDD were reported to show membranoproliferative patterns (Bohle et al., 1974; Vargas et al., 1976). In contrast, in six of our patients (seven biopsies) the most prominent alteration was the mesangial stalk cell proliferation without extension to the capillary wall; it was moderate and diffuse in two (n° 2, 7), slight and irregularly distributed or clearly focal and segmental in character in the other four (biopsies 1, 4, 9, 11a, b). Similar proliferative changes involving the mesangial stalk only have been reported occasionally (Morel-Maroger et al., 1972; Habib et al., 1975; Vargas et al., 1976; Davis et al., 1978); however, it is worth stressing that in part of our cases these lesions were focally and/or segmentally distributed. The severity of glomerular cell proliferation has been related to the duration of the disease, but conflicting interpretations have been suggested: according to Germuth and Rodriguez (1973) and to Jenis et al. (1974), glomerular hypercellularity is increasing with time, while Lamb et al. (1977) observed a more marked mesangial cell proliferation in biopsy specimens obtained early in the course of the disease. On the other hand, decreased proliferative changes and greater evidence of capillary wall thickening have been reported in the later phases of the disease in patients studied with serial biopsies (Habib et al., 1974; McCluskey, 1977). In our material glomerular cell proliferation seemed to be more severe in cases lasting some years than in the more recent ones, where it was mild or focal and segmental. In the two long lasting cases however (13 years) (n° 3, 5) proliferative changes were absent or inconspicuous; because of prominent basement membrane tickening and segmental sclerotic areas, light microscopic changes in these cases were somehow reminiscent of those of advanced membranous glomerulonephritis. Nevertheless the latter diagnosis could be ruled out from the immunofluorescence and electron microscopic data. Even though

uncommon, similar changes have also been observed occasionally by others (Antoine and Faye, 1972; Pirani, 1979). In addition, the recurrence of dense deposits without glomerular cell proliferation has been described in transplanted kidneys (Turner et al., 1976; Droz et al., 1979).

Immunofluorescence findings were more homogeneous. In all cases C_3 deposits were present and appeared both as bright coarse granules in the mesangium and as small granules or weak pseudolinear segments along the capillary walls. In addition to the glomerular tuft localization, C_3 localized along Bowman's capsule and tubular basement membranes as well. These findings agree with those of most authors (Vargas et al., 1976; Barbiano di Belgiojoso et al., 1976; Droz et al., 1977). In agreement with several reports (Bariéty et al., 1971; Burkholder, 1974; Davis et al., 1978) early acting complement components and immunoglobulis (with IgM prevalence) have been detected only in some of our cases, being always more irregularly distributed and clearly less evident than C_3.

It seems, therefore, that DDD shows quite typical immunofluorescence patterns (with prevailing and somehow characteristic C_3 mesangial and parietal deposits), different from those of the type I MPGN. In the latter, besides C_3, prominent granular parietal deposits of early acting complement fractions (C_{1q} and C_4) and immunoglobulins are usually detected (Burkholder, 1974; Habib et al., 1974; Zollinger and Mihatsch, 1978). Those cases of type I MPGN with prevailing or isolated C_3 staining are usually easily distinguishable from DDD because of the granular pattern of the deposits, more evident along the capillary wall and from the absence of deposits along Bowman's capsule and the tubular basement membranes (Barbiano et al., 1976). Further evidence of peculiar immunofluorescence patterns present in DDD (and not in type I MPGN) has been published by Kim et al. (1979) who observed the presence of C_3 along the margin of dense deposits in the glomerular basement membranes and in the mesangium, giving a double linear (railroad tracks) and circular (mesangial rings) appearance.

With few exceptions (Bohle et al., 1974; Davis et al., 1978) most studies have indicated that DDD and type I MPGN are clinically different: in particular the former shows greater incidence of nephrotic syndrome, higher likelihood of recurrence in allografts, more unfavorable outcome and, in some cases, association with partial lipodystrophy (Cameron et al., 1973; Habib et al., 1973; 1974, 1975; Ooi et al., 1976). Furthermore, a higher percentage of patients with DDD have circulating C_3 nephritic factor and more severe and persistent hypocomplementemia with characteristic abnormalities of the complement profile (Droz et al., 1977).

The pathogenesis of the two forms is also likely to be different. While it is generally accepted that type I MPGN is an immunologically mediated immune complex disease (Jones, 1977), the pathogenesis of DDD is debated. Even though an immune complex pathogenesis has been suggested (Jenis et al., 1974; Chesney et al., 1976), no definite supportive evidence has been yet obtained. In fact, none of the known immune complex diseases in experimental animals displays the morphological features of DDD (Davis et al., 1978). Moreover studies of Kim et al. (1979) show that dense deposits do not react with antiimmunoglobulins and anticomplement antisera, being neither immunocom-

plexes nor complement components that have become trapped within the basement membrane.

Galle and Mahieu (1975) demonstrated decreased cystine and increased sialic acid residues levels in analysis of isolated glomerular basement membranes from kidneys affected by DDD. These authors have concluded that the electron dense material is not a deposit, but represents a modification of the basement membrane itself, being the renal manifestation of a systemic disease. New data favouring this hypothesis have been recently reported: Ormos et al. (1979) have observed a case of DDD with dense deposits both in the kidneys and in the splenic sinuses basement membranes.

In conclusion, the bulk of the observations from several authors suggest that DDD and type I MPGN are separate entities with distinct clinical features and, at least in part, different pathogenetic mechanisms. As far as morphology is concerned, it must be pointed out that: 1) typical membranoproliferative patterns are present in only a number of cases and cell proliferation may be slight or absent, in either recent onset or long standing cases. 2) DDD immunofluorescence features are singular and clearly distinguishable from those of type I MPGN. 3) The only specific (and therefore diagnostic) morphological feature of DDD is the presence of ribbon-like electron-dense deposits.

In our opinion DDD and type I MPGN must be separated from the morphological point of view as well and therefore considered in all respect as two distinct nosological entities.

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