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Kidney Tubule Basement Membrane Alterations in Type II Membranoproliferative Glomerulonephritis

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Summary. Fourteen kidney biopsy specimens from nine patients with type II membranoproliferative glomerulonephritis (MPGN) were examined by electron microscopy for tubular basement membrane (TBM) alterations. In all biopsies, laminal densities, characteristic for type II MPGN, were present in the glomerular basement membranes.

The TBM alterations observed included: 1) the presence of laminal, and/or discrete, and/or aggregated densities; 2) focal thickening; 3) multilamination; and, 4) vesicular structures. Laminal densities occurred in 6 of the 9 cases examined. All biopsies had TBM densities representative of at least one of the three forms.

The occurrence of electron densities in or near the TBM in type II MPGN may have diagnostic value. In those biopsies where tissue is insufficient for immunofluorescence microscopy and where glomeruli are not found on electron microscopy, an electron microscopic search for densities associated with TBMs would be warranted. Although TBM-associated densities are not pathognomonic for type II MPGN, the observation of such densities, especially laminal densities, would be useful in complementing light microscopic and clinical findings.

Key words: Type II membranoproliferative glomerulonephritis – Dense deposit disease – Kidney tubule basement membrane – Kidney tubules.

Introduction

Electron microscopic examination of kidney biopsies has revealed zones of increased electron density within the renal tubular basement membrane (TBM) in renal allografts (Galle et al., 1971; Galle and Mahieu, 1975; Habib et al., 1975; Turner et al., 1976), lupus nephritis (Klassen et al., 1972; Andres and

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McCluskey, 1975; Brentjens et al., 1975), and type II membranoproliferative glomerulonephritis (MPGN) – "dense deposit disease" (Berger and Galle, 1963; Galle et al., 1971; Jenis et al., 1974; Galle and Mahieu, 1975; Habib et al., 1975; Turner et al., 1976).

The molecular nature of the electron densities is uncertain, though there is evidence that, in patients with lupus nephritis, the electron densities are formed from antigen-antibody complexes (Andres and McCluskey, 1975; Brentjens et al., 1975). Based on associated tubular and interstitial changes, both groups of investigators have suggested a pathogenetic significance of TBM electron densities for lupus nephritis.

The significance of TBM electron densities in type II MPGN is unclear. Evidence from recent studies of glomeruli in type II MPGN (Galle and Mahieu, 1975) has been interpreted to suggest that glomerular basement membrane electron densities represent a modification of the biochemical composition of the basement membrane rather than deposits. This suggestion, presumably, would also apply to the electron densities of tubular basement membranes.

In the study reported here, 14 biopsy specimens from 9 patients previously found to have type II MPGN by routine electron microscopic examination of glomeruli were re-examined to determine the incidence and form of TBM electron densities and what, if any, tubular and/or interstitial changes were associated with the presence of TBM electron densities.

Materials and Methods

The specimens of renal tissue examined in this study were obtained by percutaneous needle biopsy or at the time of nephrectomy and were submitted for routine study by light and electron microscopy. The biopsy specimens were collected over a period of four years.

The tissue specimens evaluated in this report were obtained from: both the original kidney and the functionally normal, 9-month-old transplant of one patient (case # 1); from three patients, each of whom had undergone two or more successive biopsies; and from five patients, each of whom had undergone a single biopsy. The patients' sex, age, clinical symptoms and pathologic diagnosis are given in Table 1.

For electron microscopy, samples of tissue from each biopsy were fixed either with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, or with 1.5% formaldehyde (made from paraformaldehyde) and 1.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. Post-fixation was carried out with 2% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4. Following fixation, the tissues were dehydrated and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips EM-200 electron microscope. One micron thick sections of Epon-embedded tissue were stained with toluidine blue and examined by light microscopy.

Results

Electron microscopic observations are limited to proximal convoluted tubules and to the interstitium immediately adjacent to the tubules.

In this article, the space between the base of the tubular epithelial cells and the interstitial connective tissue cells and/or fibrils is termed the TBM space. The TBM space may contain only the TBM or it may contain additional

Table 1. Patient Data

Patient number	Sex	Age (yrs)	Clinical	Light microscopic pathologic diagnosis[a]
1	F	21	Terminal renal failure of unknown origin. Received kidney transplant and 9 months later the remaining kidney and allograft kidney were biopsied during repair of a ureteral obstruction	Chronic MPGN
2	F	10	Gross hematuria, proteinuria and consistently depressed complement levels	Chronic MPGN
3	M	11	Developed hematuria subsequent to pneumonia and was found to have depressed complement	MPGN
4	M	12	Hematuria, proteinuria and depressed complement levels	MPGN
5	F	13	History of nephrotic syndrome and depressed complement levels	MPGN
6	F	18	Slowly decreasing renal function with depressed complement levels	MPGN
7	M	18	Proteinuria and decreased renal function	Focal sclerosis
8	M	23	Several year history of hematuria and proteinuria	Proliferative glomerulonephritis
9	M	11	Hematuria in patient with Downs' syndrome	Acute and chronic MPGN

[a] All cases were diagnosed as type II MPGN by electron microscopic examination of glomeruli

structures and materials. The TBM may be a single, thick, moderately electron dense cylindrical belt (lamina) that surrounds the tubule or it may consist of several separated or connecting belts of variable thickness. Some electron densities were as thick as the TBM and extended for considerable lengths (laminal densities), some appeared as small, discrete densities, and still others had the appearance of closely packed discrete densities (aggregated densities). The electron microscopic findings are summarized in Table 2.

Less dense flocculent material, fine granules and ill-defined small vesicular structures were present in the TBM space of all cases. The vesicular structures were often located around the periphery of the discrete densities.

Descriptions of light microscopic findings are based on examination of one micron-thick, plastic-embedded sections that were stained with toluidine blue.

Case # 1 (Endogenous and Allograft Kidneys)

Electron microscopic examination of the endogenous kidney in areas of tubular atrophy and interstitial fibrosis revealed the presence of discrete and aggregated

Table 2. Basement membrane findings

Case #	Biopsy	Types of densities observed in tubular basement membrane spaces		
		Laminal	Discrete	Aggregated
1	Own kidney	−	+	+
	Allograft	+	+	+
2	1st	+	−	+
	2nd[a]	+	−	−
	3rd[b]	+	+	+
3	1st	−	+	−
	2nd[c]	−	+	+
4	1st	−	+	+
	2nd[d]	*	+	*
5	−	−	+	+
6	−	+	−	+
7	−	−	+	−
8	−	+	+	−
9	−	*	+	*

* Nature of density, whether laminal or aggregated, is uncertain
[a] Taken 21 months after first biopsy
[b] Taken 24 months after first biopsy
[c] Taken 24 months after first biopsy
[d] Taken 3 months after first biopsy

electron densities (Fig. 1). Discrete electron densities were also found in the TBM spaces of less altered tubules (Fig. 2). Small, electron lucent vesicular structures, approximately 0.2 μ in diameter, were located along the periphery of many of the discrete electron densities (Fig. 2).

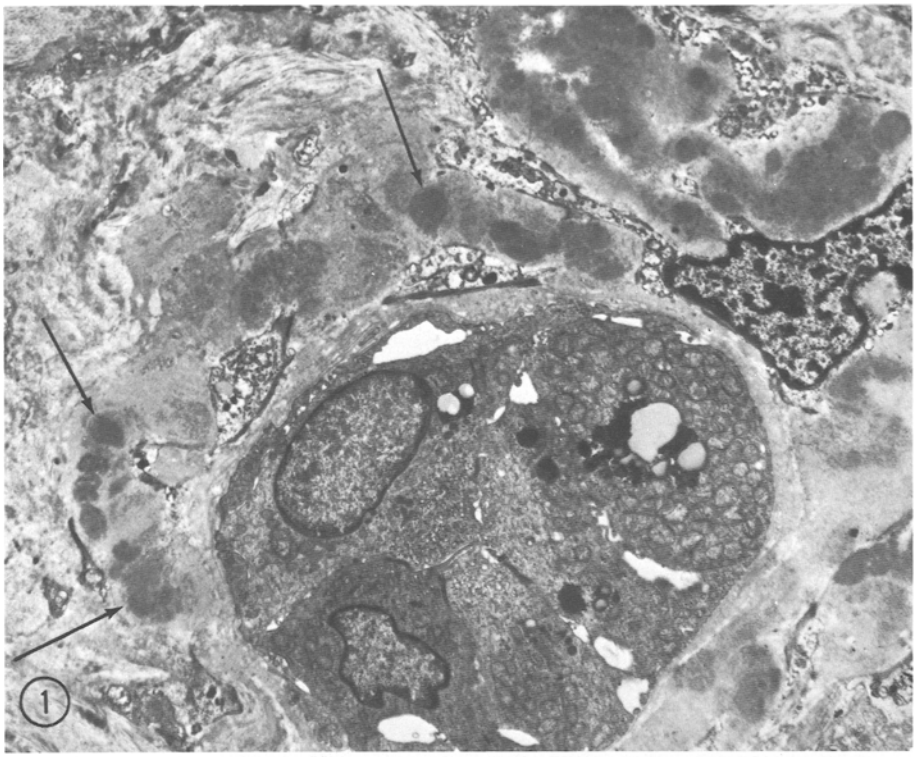
Electron densities were also present in the TBM space of the allograft kidney and were associated with local thickenings of the TBM space (Fig. 3). Here, laminal, discrete and aggregated electron densities occurred close to each other and often merged together.

Case # 2 (Three Biopsies)

In the first biopsy, light microscopy showed that, except for focal atrophy, the tubules appeared normal. Minimal, focal lymphocytic infiltrates were pre-

Fig. 1. A fibrotic interstitial area from the host kidney from case # 1. In this area there is an atrophic tubule with a greatly thickened TBM space which contains numerous discrete electron densities (*arrows*). At the upper right a portion of a TBM space is seen. Here, the electron densities are discrete and aggregated. ×4,600

Fig. 2. A portion of atrophic tubule from the same biopsy as Fig. 1. Electron densities (*D*) are present in a TBM space which contains also a multilaminated, irregular TBM. Small, circular electron-lucent vesicular structures are visible along the periphery of the densities. A thin basal lamina follows the contour of the tubular epithelium. ×14,800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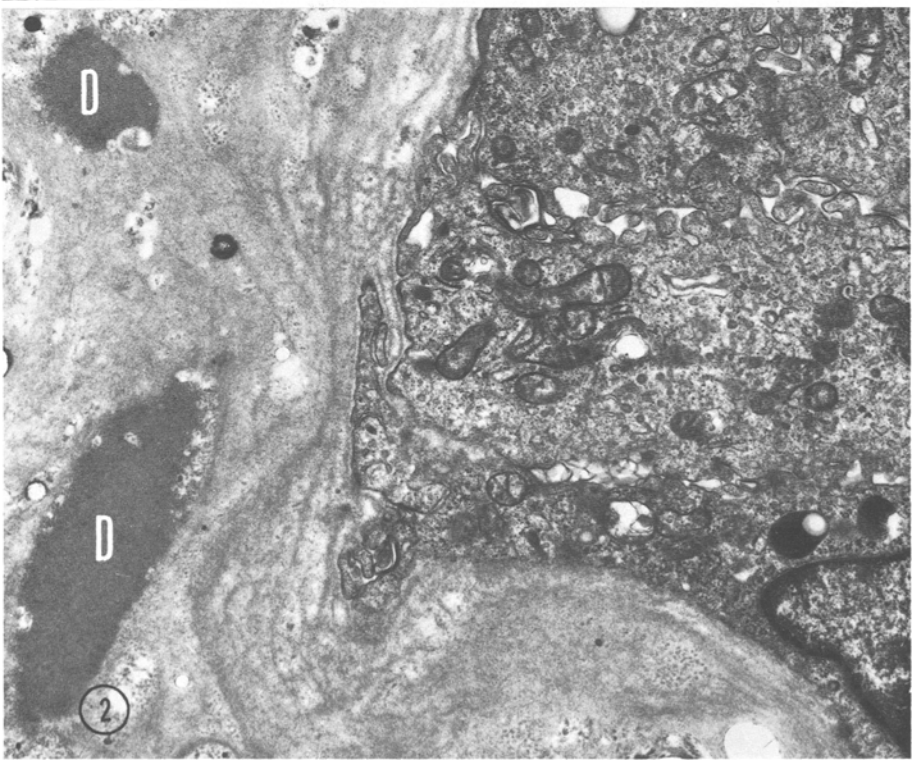
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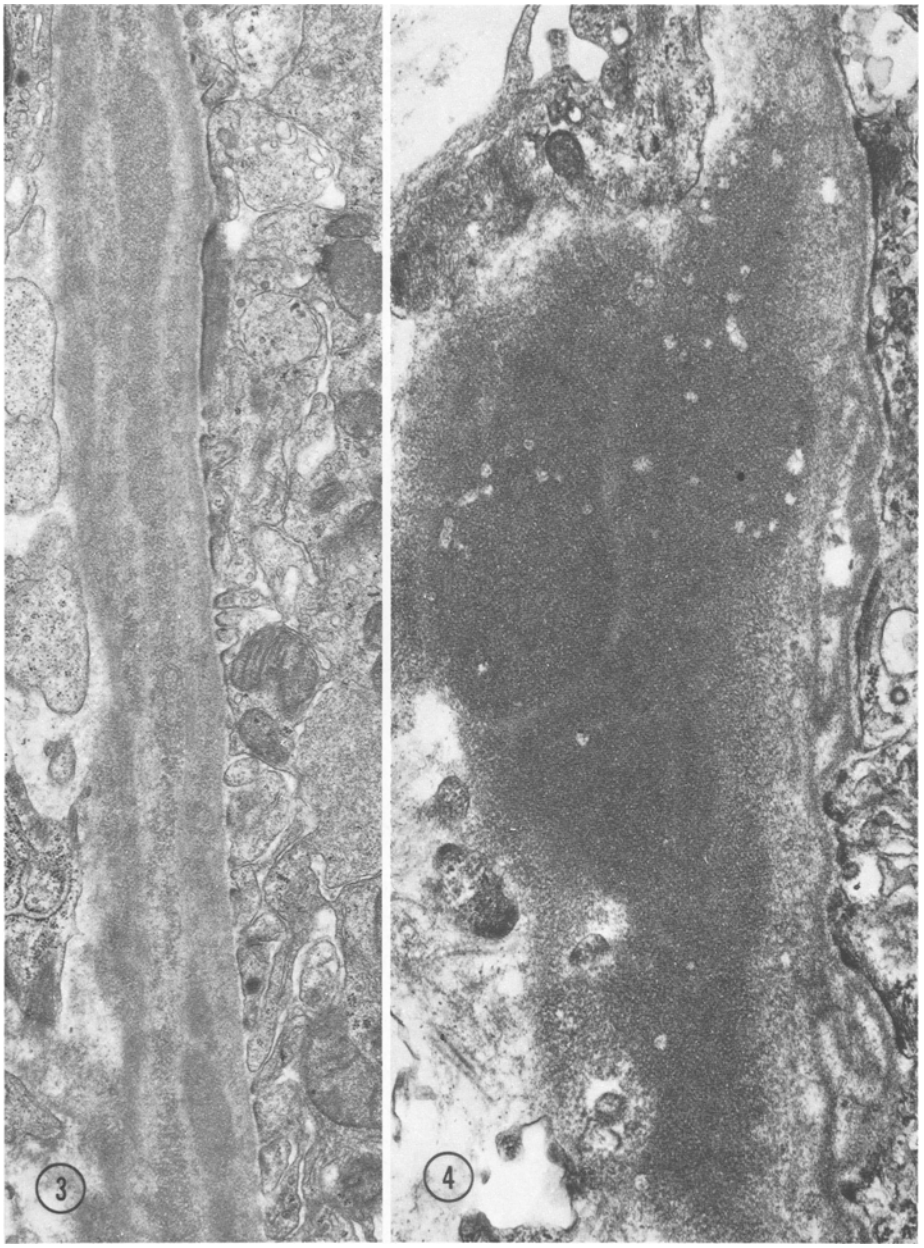

Fig. 3. Proximal tubule from the allograft kidney from case # 1. Laminal electron densities of variable darkness are present in the TBM space. The TBM adjacent to the tubular epithelium at the right does not generally appear as a homogeneous structure. × 14,800

Fig. 4. TBM space of proximal tubule from first biopsy of case # 2. The TBM space contains an aggregated electron density which is seen to consist of packed dark masses, some of which have vesicular structures at their periphery. The TBM space also contains a partially doublelayered basal lamina adjacent to the tubular epithelium at the right. × 24,700

sent. TBM densities were visible in toluidine blue-stained sections. In the repeat biopsies, tubules were generally intact, but some were collapsed and had markedly thickened TBMs. The interstitium was focally edematous and fibrotic, and inflammatory cells were distributed randomly throughout.

Electron microscopy confirmed the presence of numerous electron densities in the first biopsy. Many of the electron densities were laminal, but some were irregular in outline and had the appearance of aggregated electron densities (Fig. 4). The latter interpretation is strengthened by the finding of small vesicles along what is presumed to be the periphery of the discrete electron densities that formed the aggregated electron densities.

Examples were found in this first biopsy where the TBM was focally obscured by electron densities. In such instances, the TBM was usually located at some distance from the base of the epithelial cells and a very thin basal lamina was found immediately adjacent to the basal border of the tubule cells. Only occasional laminal electron densities in TBM spaces were observed in the second biopsy from case # 2. The third biopsy, however, had all three forms of electron densities.

In these biopsies, collections of small vesicles were frequently arrayed in aggregates along, or seemingly within, the TBMs. These vesicles, when located in the middle of a TBM, produced an appearance of focal splitting of the TBM (Fig. 5). It was uncertain if the vesicles found at the edge of discrete electron densities, as in Figs. 2 and 4, were identical to those illustrated in Fig. 5. Some vesicles were surrounded with a membrane-like material, but many vesicles appeared as holes.

Case # 3 (Two Biopsies)

Except for TBM densities, the tubules in both biopsies were essentially normal by light and electron microscopy.

Electron microscopy revealed rare, small discrete electron densities in some TBMs in the first biopsy.

In the second biopsy the TBMs contained scattered large discrete and aggregated electron densities. Some electron densities were noted to consist of very fine granules.

Case # 4 (Two Biopsies)

Light microscopic examination of the first biopsy showed a mild, focal infiltrate of lymphocytes and occasional neutrophils. In the repeat biopsy, performed three months later, the interstitium was slightly edematous with resultant tubular disortion.

Electron microscopy of the first biopsy revealed a relatively normal proximal tubular epithelium. The TBMs contained numerous irregular, discrete electron densities and less numerous aggregated electron densities (Fig. 6). The combination of electron densities, focally thickened TBM and scattered vesicles gave

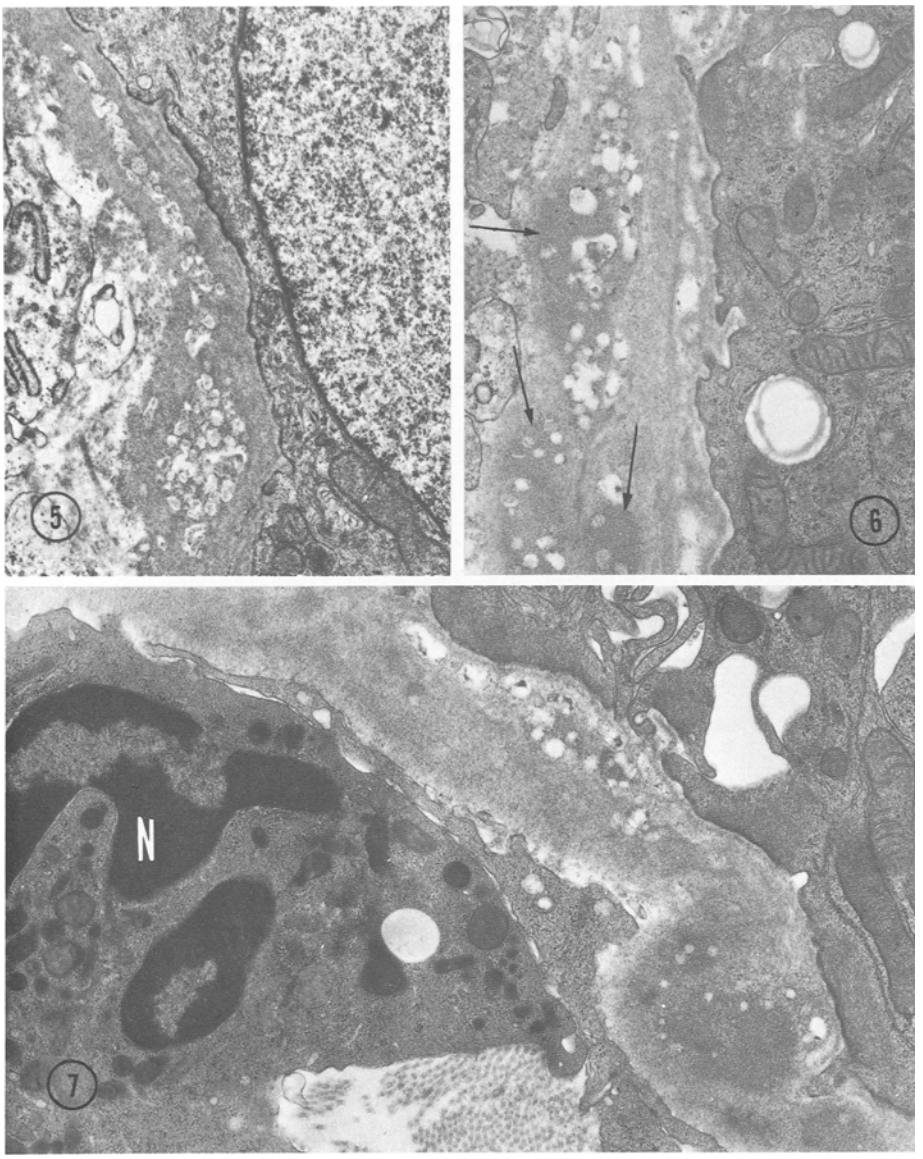

Fig. 5. TBM space of proximal tubule from repeat biopsy from case # 2. Aggregates of small vesicular structures, similar to those previously described, are present within the TBM and produce a focal thickening in the TBM. The tubular epithelium is at the right. × 14,800

Fig. 6. Basal portion of a proximal tubule from the first biopsy from case # 4. The TBM space consists of a thin basal lamina adjacent to the epithelium plus a thick multilayered lamina in which vesicular structures are embedded. Discrete and aggregated electron densities (*arrows*), with associated vesicular structures, are also present within the TBM space. × 14,800

Fig. 7. Proximal tubule from same biopsy as in Fig. 7. A neutrophil (N), within the interstitium, is adjacent to a TBM space which contains discrete electron densities. × 9,000

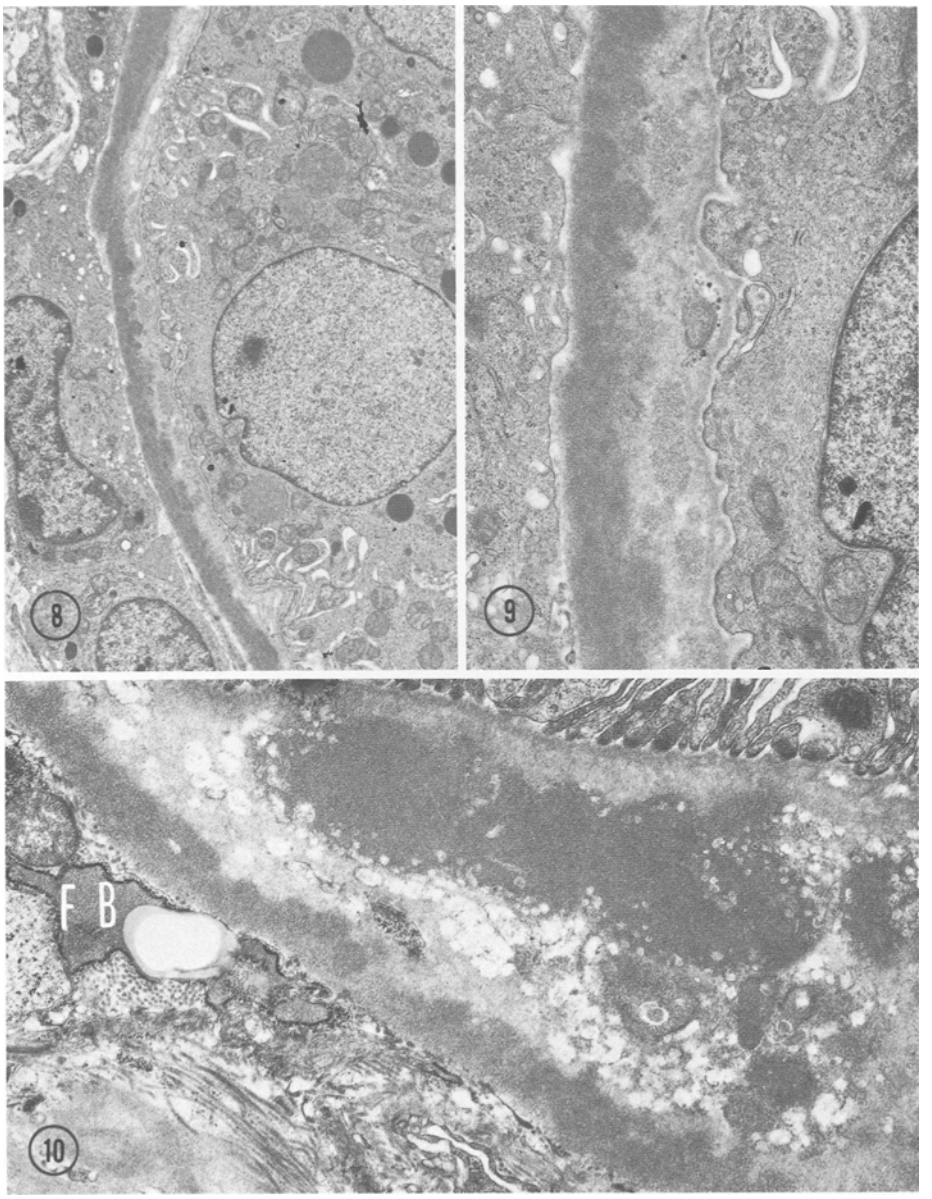

Fig. 8. A laminal density is present within a TBM space from the repeat biopsy from case # 4. Tubular epithelium is on the right. ×4,600

Fig. 9. Higher magnification of Fig. 8. The laminal density appears to consist of packed discrete densities. It is separated from a thin basal lamina by a less dense matrix containing accumulations of finely granular material. Similar accumulations were also observed in previous cases. ×14,800

Fig. 10. Proximal tubule from case # 6. The focally widened TBM space contains electron densities of different morphologies. The density adjacent to the fibroblast (*FB*) appears laminal, in part, and aggregated, in other parts. Larger aggregated electron densities, with associated vesicles, are also present. ×14,800

the TBM space a "moth-eaten" appearance. The TBM space illustrated in Fig. 6 contained a multilaminar TBM that was connected by numerous thin lamina to the narrow basal lamina adjacent to the tubule cells. Inflammatory cells present in the interstitium were occasionally observed in contact with the interstitial side of a TBM near electron densities (Fig. 7).

As in the earlier biopsy, the repeat biopsy showed normal proximal tubule cells. The TBM spaces contained electron densities of variable morphology. Some were small, discrete and contained vesicles. Others were laminal, dark and smooth (Fig. 8), and closely resembled the dense transformation seen in glomerular basement membranes in type II MPGN. At higher magnification, the luminal side of the laminal electron density appeared to be made up of aggregated electron densities (Fig. 9). The TBM space illustrated in Fig. 9 also contained focal accumulations of very fine granules surrounded by a lighter matrix.

Cases # 5–# 9 (Single Biopsies)

Proximal tubular epithelium was considered normal an cases # 5, # 6, # 8 and # 9. Case # 7 showed focal tubular atrophy as well as marked inflammatory infiltrates and interstitial fibrosis. Generally, the tubules appeared normal in this case. Case # 8 showed focal inflammatory infiltrates. Electron microscopic findings were variable from case to case. Electron densities were common in case # 5. The TBM spaces in case # 6 contained aggregated electron densities as well as laminal electron densities similar to those seen in glomeruli (Fig. 10). Numerous TBM densities were visible in the toluidine blue-stained Epon sections. Great variation in contents of the TBM spaces were noted in case # 7. In some instances, discrete electron densities were associated with a multilaminated TBM; other TBM spaces contained large quantities of vesicles and focal accumulations of fine granules. Case # 8 had isolated, large discrete electron densities and laminal electron densities in areas of inflammation and necrosis. Some TBM spaces around undamaged tubules contained focal accumulations of tightly packed fine granules which, en masse, resembled discrete electron densities. Case # 9 contained laminal electron densities and very dark, discrete electron densities. Close inspection of the laminal electron densities showed that they were probably composed of packed discrete electron densities organized into parallel layers.

Discussion

Type II MPGN is characterized by dark, thick, laminal electron densities associated with the basement membrane of glomerular capillaries and occasionally the basement membranes of Bowman's capsule and tubules (Berger and Galle, 1963; Galle et al., 1971; Jenis et al., 1974; Galle and Mahieu, 1975; Habib et al., 1975; Turner et al., 1976). Clinical findings usually include proteinuria, hematuria, often the nephrotic syndrome, and persistently low C3 concentrations

in the plasma (Antoine and Faye, 1972; Germuth and Rodriguez, 1973; Jenis et al., 1974; Galle and Mahieu, 1975; Habib et al., 1975; Turner et al., 1976). Studies on transplanted kidneys have shown that electron densities reoccur in graft kidneys within one year and precede glomerular proliferative changes and onset of clinical symptoms (Turner et al., 1976). Observations on our case # 1 are consistent with the findings of Turner et al. (1976). These authors concluded that the appearance of the dense "material within the basement membrane throughout the kidney clearly depends upon some humoral factor(s) which persist following transplantation."

Though considerable attention has been given to the glomerular densities in type II MPGN, relatively little consideration has been given to the extraglomerular densities. In the present study, 14 biopsy specimens from 9 patients previously found to have type II MPGN by routine examination of glomeruli were re-examined for proximal tubule basement membrane densities and associated tubular and interstitial changes.

Densities were found within the TBM spaces in all biopsies; however, only 6 of the 9 cases were observed to have laminal electron densities. In the remaining three cases, laminal TBM electron densities could not be found. Rather, discrete and/or aggregated electron densities were present. In comparison, dark, laminal densities occurred within glomerular basement membranes of all 14 biopsies and were much more numerous and extensive in size than the tubular densities.

A number of investigators have described densities (deposits) in the TBMs of patients afflicted with systemic lupus erythematosus (Andres and McCluskey, 1970; Klassen et al., 1972; Brentjens et al., 1975). During the course of our study, we also observed TBM densities in biopsies of three patients who had systemic lupus erythematosus. These densities were of the discrete type and were morphologically similar to those described by the above investigators. We did not observe laminal densities in the biopsies, but, in two of the three cases studied, aggregated densities were present. It would appear that laminal TBM densities are the only type that correlates exclusively with type II MPGN. We have also examined biopsies in two cases of type I MPGN and have failed to find TBM densities of any type.

The origin of the densities in type II MPGN is uncertain. The TBM electron densities in the allograft kidney (case # 1) examined in our study tended to be laminal, but were considerably less dark than the laminal electron densities seen in more advanced stages of the disease. This observation suggests that the laminal density may develop as an incremental dense alteration or addition of material to the TBM. On the other hand, the work of Galle and Mahieu (1975) suggest that the densities result from a biochemical modification of the basement membrane rather than from the deposition of material of extra-renal origin. In their work, solubilization and analysis of the protein content of abnormal glomerular basement membranes revealed no protein that was not present in similarly solubilized normal basement membranes. Furthermore, Galle and Mahieu found that no serum proteins could be detected by immunoelectrophoresis of abnormal basement membrane eluates or by analytic disc gel analysis of solubilized abnormal basement membranes. The implication of these findings is that gamma globulins were not present in the densities. This apparent lack

of gamma globulins is corroborated by immunofluorescent studies (Klassen et al., 1972; Jenis et al., 1974; Andres and McCluskey, 1975; Habib et al., 1975).

Jenis et al. (1974) have suggested that the densities may result from a local initiation of the alternate pathway for complement activation. These investigators considered that type II MPGN most likely represents an unusual response to injury and that it may be induced by a variety of agents.

With respect to the source of the laminal electron densities, the focal accumulations of fine granules, the discrete electron densities, and the aggregated electron densities may reflect stages in a sequence leading to the formation of the laminal electron densities. This interpretation is supported by the high magnification observation that some laminal electron densities appear as tightly packed discrete electron densities (i.e., aggregated electron densities). Though this sequence is not incompatible with the concept of a biochemical modification of the basement membrane (Galle and Mahieu, 1975), it seems more likely that the laminal electron densities result from a process of accretion rather than a biochemical alteration.

Though our study has not shown a consistent relationship between either tubular or interstitial alterations and the presence of TBM electron densities, it is our impression that laminal electron densities were generally larger and aggregated electron densities were more common in areas of tubule degeneration and interstitial inflammation.

The significance of the vesicular structures, which are found free in the TBM space as well as associated with discrete electron densities, is not known. We have observed similar vesicular structures in the TBM spaces of biopsies from patients with diseases other than type II MPGN. These structures may originate from debris released from living or necrotic cells that subsequently become adherent to electron densities or trapped between lamina of a multilaminated TBM.

Based on the consistent finding of electron densities in tubular basement membrane spaces in type II MPGN, we consider it feasible to use the presence of these densities as a diagnostic acid. In those cases where biopsies have afforded material only for light and electron microscopy, but not for immunofluorescence microscopy, and when glomeruli were not present in tissue blocks employed for electron microscopy, we consider that a search for electron densities in the TBM spaces would be warranted. Though, as already noted, the presence of electron densities within the TBM spaces is not pathognomonic for type II MPGN, the finding of electron densities, especially laminal densities, would help confirm considerations based on clinical and light microscopic data.

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