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GLOMERULAR BASEMENT MEMBRANE THICKENING IN RENAL ALLOGRAFTS

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ABSTRACT

Diffuse thickening of glomerular basement membranes (GBM) was optically observed in the renal grafts of 15 patients (21 specimens) among 70 recipients (128 specimens). Most of these specimens with diffuse GBM thickening were examined ultrastructurally. All cases with the above change were only found in allografts. The correlation was observed between the degree of GBM thickening and the clinical course, which can be classified into 3 groups. Group I showed a marked thickening of subendothelial spaces of GBM due to the accumulation of electron-lucent material. The cases of Group I fell into renal failure within relatively short periods because of either continued or repeated acute rejections (4 cases at 10 weeks to 15 months after transplantation). Group II showed a relatively mild thickening of subendothelial spaces and mild thickening of laminae densae accompanied by chronic rejections (7 cases at 9 months to 10 years and 10 months after transplantation). Group III showed glomerulonephritis-like changes and chronic clinical courses (3 cases with membranoproliferative glomerulonephritis (MPGN)-like change and one case with membranous nephropathy (MN)-like change). The GBM thickening was milder in Group II than in Group I. Mesangiolysis was found in both groups, which was, however, milder in Group II. The one case with MN-like change in Group III was considered to be complicated with MN under chronic rejection. As to the 3 cases with MPGN-like changes in Group III, it can not be concluded at a preset, whether they are to be attributed to a special form of chronic rejection itself or to a chronic rejection on which glomerulonephritis or some other factor(s) may be superimposed.

Keywords: Transplanted kindney, glomerular basement membrane, Mesangiolysis, Transplant glomerulopathy, Rejection

INTRODUCTION

The most characteristic change in glomeruli of renal grafts is the thickening of subendothelial spaces due to the accumulation of electron-lucent material. This finding was first described by Hamburger[1] in 1964, which has been widely accepted. Mesangial interposition is also frequently observed in renal grafts.[2,3,4] These two changes which occur diffusely characterise so-called transplant glomerulopathy. This is histologically simillar to MPGN and is clinically characterized by heavy proteinuria and relatively well maintained renall function.

Although much attention has been focussed on the glomerulopathy, its pathogenesis has not yet been clarified. The author studied the changes in the GBM of renal grafts in relation to the clinical courses of patients. The correlation observed is presented in this paper. The author also emphasized frequent appearance of mesangiolysis,[5,6] which has not been pointed out by other investigators. Since the incidence of transplant glomerulopathy is relatively low, it may belong to the special type of glomerular changes in renal grafts. Since transplant glomerulopthy differed in detail from primary MPGN,[4,7,8,9,10] studies were also done on the

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difference between the cases reported here and the primary MPGN.

MATERIALS AND METHODS

One hundred and forty specimens of renal grafts from 78 patients have been histologically examined at The Institute of Medical Science The University of Tokyo in a period of about 10 years from July, 1970 to May, 1980. Except for 6 cadaveric ones, all other grafts were living. The latter ones were donated by either one of the parents or siblings. All the former ones were unrelated except for one from a patient's mother. Recipients with an allograft were continuously given prednisolone and azathioprine. At an acute rejection, either the administration of methyl prednisolone or the combined therapy with irradiation to grafts was performed. Among 140 specimens prepared, 97 specimens were obtained by percutaneous biopsy, 14 by resection, 17 by autopsy (a total of 128 specimens available from 70 recipients), and the remaining 12 specimens were unavailable because of marked necrosis or postmortem change. Where biopsied specimens contained glomeruli, even if only one, they were subjected to examination.

Changes in the GBM were focused in the present study, and optical observations were performed on the following points: 1) Whether the GBM thickening is local or diffuse. 2) Whether the thickening feature is of double membrane, spike or others. 3) Mesangiolysis.

Table 1. Glomerular Basementmembrane Change on Light Microscopy

	0 ≀ 1 Mo	1 ≀ 2 Mo	2 ≀ 3 Mo	3 ≀ 4 Mo	4 ≀ 5 Mo	5 ≀ 6 Mo	sum & rate within 6 Mo after transplant	6 Mo ≀ 1 Yr	1 ≀ 2 Yr	2 ≀ 3 Yr	3 ≀ 4 Yr	4 ≀ 5 Yr	over 5 Yr	sum & rate over 6 Mo after transplant
number of material	26	36	10	5	4	1	82	16	9	6	8	2	5	46
thickening of GBM	18	20	7	4	3	0	52 (63.4%)	12	7	4	8	1	5	37 (80.4%)
local	17	20	4	4	3	0	48 (58.5%)	9	4	2	3	0	2	20 (43.5%)
diffuse	1	0	3	0	0	0	4 (4.9%)	3	3	2	5	1	3	17 (37.0%)
double membrane	5	4	3	3	3	0	18 (22.0%)	6	3	4	5	1	2	21 (45.7%)
mesangiolysis	1	4	1	3	1	0	10 (12.3%)	5	4	4	3	1	2	19 (41.3%)
spike		1					1 (1.2%)	0	0	1	2	0	1	4 (8.7%)
MPGN-like change							0 (0%)	0	1	1	3	0	0	5 (10.9%)
MN-like change							0 (0%)						1	1 (2.2%)
cases showing diffuse thickening of GBM	4,500		4,556 1,055 77-11					5,510 5,517 74-31	5,766 5,522 7,373	5,487 73-9	3,940 7,065 7,808 76-15 80-4	73-11	5,640 77-8 79-29	

Abbreviations: GBM, glomerular basementmembrane ;
MPGN, membranoproliferative glomerulonephritis ; MN, membranous nephropathy.

Mesangiolysis is defined as disintegration of mesangium with the resultant balooned appearance of a glomerular tuft. The dilated lumen of a simplified tuft was filled with many red blood cells. The definition of diffuse thickening of the GBM was as follows: The GBM thickening of over 50% capillary loops of individual glomerulus was optically observed in more than a half of the total glomeruli in a specimen. When the occurrence of the GBM thickening was lower than the above, the lesion was defined as local thickening of the GBM. The majority of cases with diffuse thickening of the GBM were further examined under electron microscope. In order to observe the time course of the GBM change, the period of the first 6 months after transplantation was evenly divided into 6 periods, the period from the 7th month to the first year defined as one period, and the following 4 years and the period later than the 6th year divided into 5 periods (Table 1). The number of specimens obtained within 6 months after transplantation was 82, and that over 6 months 46.

Specimens for optical observations were processed in a conventional manner. Paraffin embedded sections cut at 2–3μ were stained with hematoxylin and eosin (H & E), periodic acid-Schiff (PAS), periodic acid-methenamine silver (PAM), Masson-Goldner technics, elastic Verhoff-van Gieson (VVG) technics, and phosphotungstic acid-hematoxylin (PTAH). Specimens for ultrastructural observations were first fixed in 5% glutaraldehyde buffered with 0.1 M sodium cacodylate, refixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812 resin. Ultrathin sections were cut on a Porter-Blum ultramicrotome and stained with uranyl acetate and lead acetate. These specimens were observed under a Nihon Denshi 100-B electron microscope.

RESULTS

Fifty two specimens (63.4%) showed the GBM thickening within 6 months after transplantation. Among them, 48 (58.5%) showed local thickening, 4 (4.9%) diffuse thickening, 18 (22.0%) double membrane formation clearly observable under light microscope, and 1 (1.2%) spike formation. Mesangiolysis was observed in 10 specimens (12.3%).

The GBM thickening in the period later than 6 months after transplantation was observed in 37 specimens (80.4%). Among them, local thickening was observed in 20 specimens (43.5%), diffuse thickening in 17 (37.0%), double membrane formation in 21 (45.7%), and spike formation in 4 (8.7%). Mesangiolysis was observed in 19 specimens (41.3%).

The number of specimens with diffuse thickening of the GBM was thus much greater in the group over 6 months after transplantation. Although the formation of double membrane was observed in more than 1/3 of all specimens later than the 3rd month after transplantation, the diffuse formation of double membrane was noted only in 3 cases. Spike formation was more frequent in the group later than 6 months, although the incidences were low. Mesangiolysis was also more frequently observed later than 6 months. The GBM of glomeruli subjected to mesangiolysis showed thinning or wrinkling.

Cases developing diffuse GBM thickening within 6 months after transplantation (Table 2).

Four specimens, *i.e.*, No. 4,500, 77–11, 1,055 and 4,556, from 3 cases were included here. Specimens 4,500 and 77–11 were from the same patient, the former being biopsied on the 16th day after transplantation, and the latter being autopsied on the 74th day for the complication with mycotic infection. Specimen 1,055 was resected on the 77th day after transplantation. The severe acute rejection soon after transplantation led these two patients to death or nephrectomy. These 2 cases showed marked thickening of the GBM on H & E and PAS

Table 2. Clinical Data of Group I and II

Group I

	sex	age at trans-plant	donor/age	length of transplant	serum creatinine mg/dl	protei-nuria	sort of material	cause of death or length of hemodialysis prior to nephrectomy
4,500	M	26	father/49	16 days	13.2	++	biopsy	
77-11				74 days	3.0	−	autopsy	candidiasis
1,055	F	27	mother/59	11 wk	11.8	+++	nephrectomy	7 wk
5,522	F	31	mother/52	15 mo	9.7	+++	nephrectomy	9 wk
5,510	M	32	sister/37	9 mo	6.0	+	biospy	
5,717				12 mo	13.4	unclear	nephrectomy	3 mo

Group II

74-31	M	24	mother/47	9 mo	2.9	+	autopsy	liver cirrhosis pneumocystis pneumonia
7,373	M	26	mother/57	18 mo	13.4	unclear	nephrectomy	6 mo
73-9	M	17	father/47	32 mo	12.4	+++	autopsy	chrnic rejection meningitis
80-4	M	36	father/67	48 mo	3.6	unclear	autopsy	pneumocystis pneumonia
73-11	F	25	mother/50	51 mo	10.2	+++	autopsy	chronic rejection meningitis
77-8	M	20	mother/44	98 mo	8.6	+	autopsy	chronic rejection esophagoenteritis
79-20	F	22	father/52	130 mo	9.3	+	autopsy	chronic rejection sepsis

staining. The degree and form of the thickening varied from glomerulus to glomerulus and also in each glomerular tuft. On PAS staining, the GBM thickening presented various appearances, which consisted of double membranes and/or thick inner portions of GBM faintly homogeneously or fibrillary stained with PAS. Glomerular capillary loops were markedly fused to each other. By PAM staining, the GBM thickening was not clearly observed except for some glomeruli. In specimen 1,055, many glomeruli showed mesangiolysis, whose basement membranes were thin and whose lumens wer dilated. Specimens 1,055 and 77−11 on ultrastructural observations showed the electron-lucent thickening of the subendothelial spaces with fibrin deposition, scattered tiny vacuoles just beneath the laminae densae, local mesangial interposition, wrinkling of basement membranes, and local fusion of foot processes.

Specimen 4,556 was the biopsied material on the 66th day when the hyperacute rejection on the first day after transplantation subsided to same extent. The GBM thickening in this case was milder than that in the above 2 cases. The wrinkling and adhesion of the GBM were remarkable on optical and ultrastructural observations. The thickening of subendothelial spaces was milder than that in the above 2 cases, with no fibrin deposition observed. Local mesangial interposition was noted. In these three cases, no deposit weas detected under electron microscope.

Cases developing diffuse GBM thickening later than 6 months after transplantation. In this group, 17 specimens from 13 cases at 9 months to 10 years and 10 months after transplantation were included. Based on the thickening form of the GBM and clinical course, these cases were divided into 3 groups.

Group I: Cases with a marked thickeing of subendothelial spaces. These cases resulted in relatively early renal failure after transplantation because of repeated acute rejections (Table 2).

Three specimens, 5,510, 5,717 and 5,522, from 2 patients were included in this group. The first two specimens were from the same patient. Specimen 5,510 was biopsied at 9 months, and specimen 5,717 resected at 1 year after transpantation. Specimen 5,522 was a nephrectomy at 1 year and 3 months after transplantation, from the same patient who supplied specimen 4,556. These two patients each suffered from 3 drastic acute rejections during the period from the transplantation to the nephrectomy. In this group, the thickening of subendothelial spaces was more remarkable than that in the cases with diffuse GBM thickening within 6 months. The capillary lumens were extremely narrowed in some portions, where endothelial cells were occasionally necrotized and subendothelial spaces were in direct contact with erythrocytes (Figs. 1 and 2). Although in the above lesion the glomerular capillary lumen appeared optically to be dilated like an aneurysm, it is, in fact, ultrastructurally narrowed as already mentioned. Therefore, Zollinger[11] has proposed calling the lesion pseudoaneurysm. As seen in specimen 1,055, many glomeruli showed mesangiolysis (Fig. 3). No deposit was observed in this group under electron microscope. This

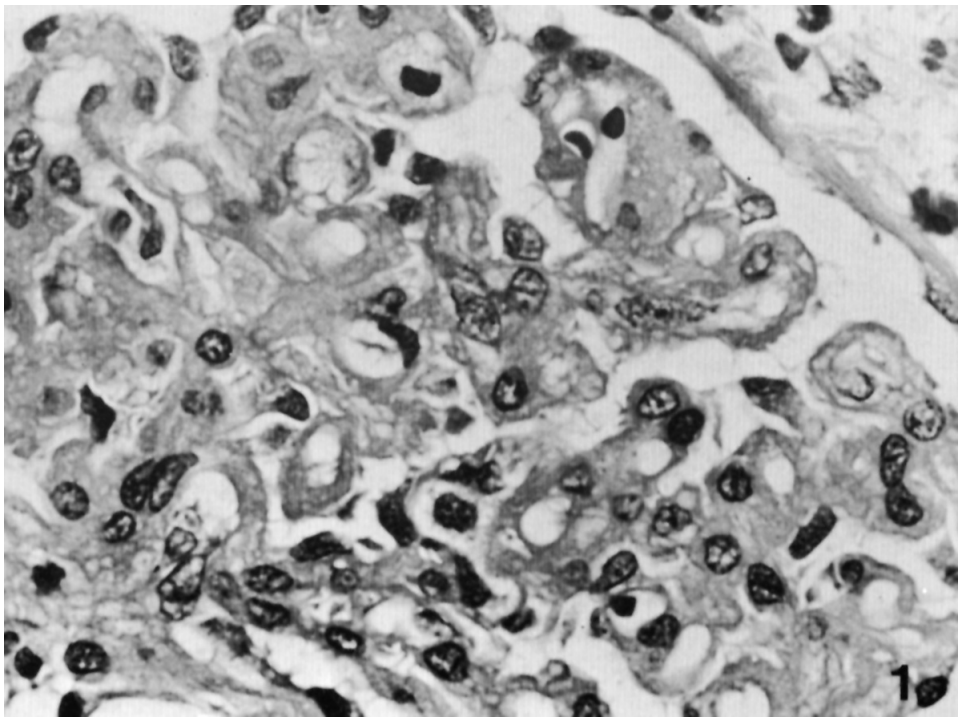

Fig. 1

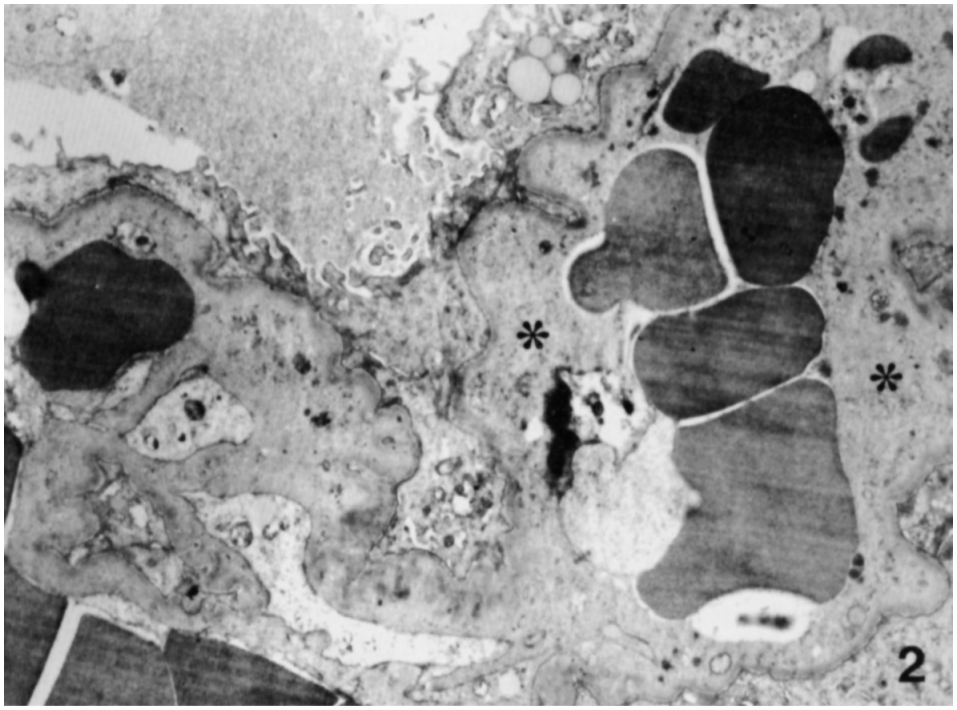

Fig. 2

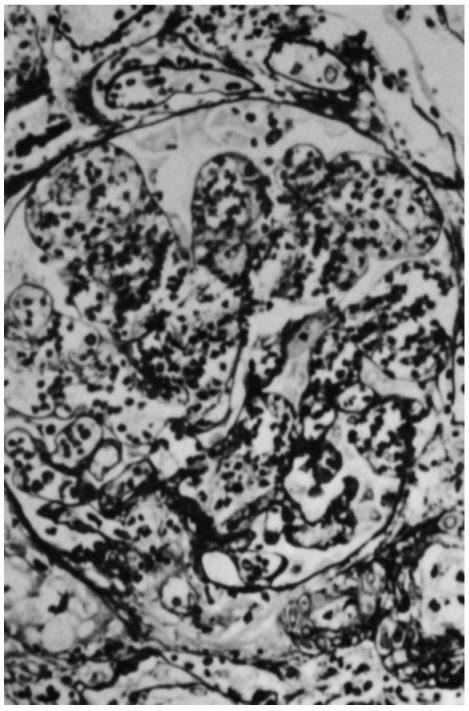

Fig. 3

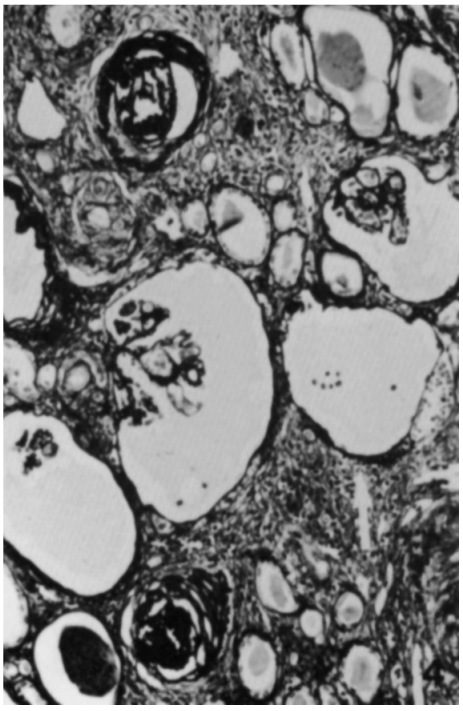

Fig. 4

group resembled the group with diffuse GBM thickening within 6 months in both morphological and clinical aspects. The degree of thickening, however, was more marked in this group.

Group II: The cases which had relatively mild thickening of subendothelial spaces and mild thickening of laminae densae. These cases took the course of chronic rejection (Table 2).

Seven specimens 7,373, 74–31, 73–9, 80–4, 73–11, 77–8 and 79–20, from 7 patients were included in this group. Except for specimen 7,373 resected at 1-1/2 years after transplantation, all others were autopsied ones. All these renal grafts had almost no function, except for cases 74–31 and 80–4, the former being at 9 months and the latter at 4 years after transplantation. All these 6 cases died of infectious complications. Many glomeruli showed sclerosis and fibrosis, and the rest had very mild to relatively mild thickening of the GBM when the specimens were stained with PAS or PAM. The basement membranes of glomeruli which had adhesion of capillary loops and mesangiolysis were finely wrinkled, but the capillary lumens were not dilated so markedly as in Group I. These capillary loops were noted to gradually disappear leaving only the filtrate in Bowman spaces (Fig. 4). This change was observed remarkably in case 79–20 which functioned for 10 years and 10 months, and mildly in the case 77–8 functioning for 8 years and 2 months. Ultrastructural findings were, in addition to the above changes, rare deposition of fibrin, local mesangial interposition, the GBM wrinkling with thickened subendothelial space or mesangial interposition, and sporadical tiny subendothelial and paramesangial deposits (present in cases 7,373, 74–31 and 80–4). The thickening of the GBM was milder than that in Group I, and mesangiolysis was also milder (Figs. 5 and 6). The renal grafts of 74–31 and 80–4 were still functioning well, without mesangiolysis being observed.

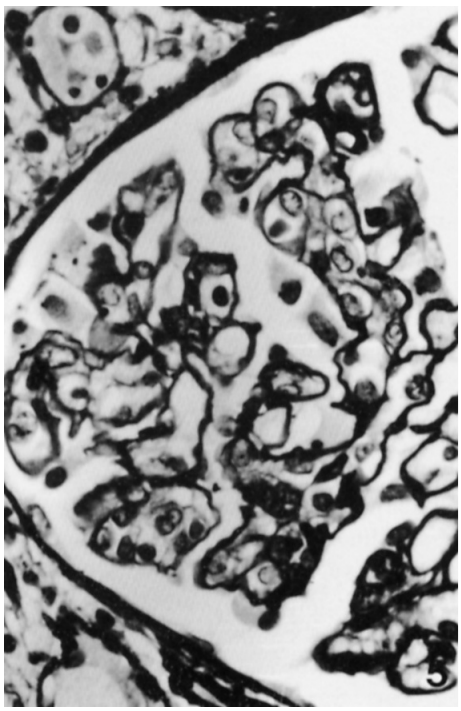

Fig. 5

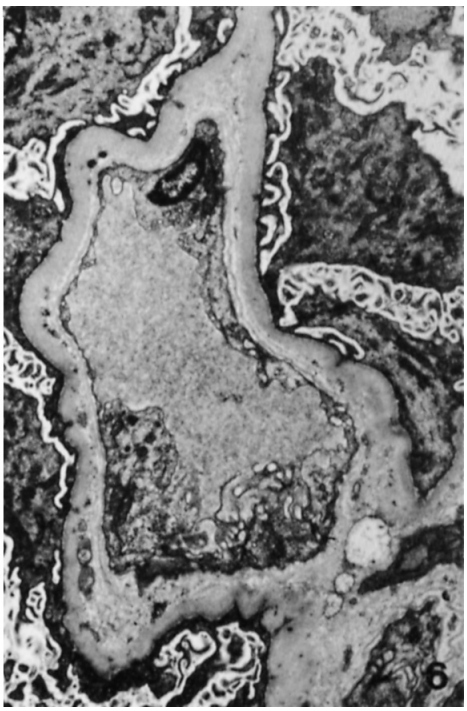

Fig. 6

Table 3. Clinical Data of Group III

	sex	age at transplant	donor/age	interval from transplant to biopsy	at biopsy: serum creatinine	at biopsy: proteinuria	histology	original disease	on May '80 (2 yr. after biopsy): serum creatinine	on May '80 (2 yr. after biopsy): proteinuria
3,940	M	32	father/65	3-1/2 yr	2.5 mg/dl	+++	MPGN-like	CGN	death due to meningoencephalitis one month after biopsy	
5,487	M	27	mother/50	3 yr	2.1	unclear	MPGN-like	CGN	2.1 mg/dl	+++
5,766	M	25	unrelated cadaver/26	2 yr	2.7	++	MPGN-like	CGN	4.4	+++
7,065		25	unrelated cadaver/26	3 yr and 2 mo	3.5	+++	MPGN-like	CGN		
7,808		25	unrelated cadaver/26	3 yr and 11 mo	4.4	+++	MPGN-like	CGN		
5,640	M	23	mother/47	5-1/2 yr	2.1	±	MN-like	RPGN	2.2	±

5,766, 7,605 and 7,808 are from the same patient. MPGN, membranoproliferative glomerulonephritis : MN, membranous nephropathy ; CGN, chronic glomerulonephritis ; RPGN, rapidly progressive glomerulonephritis.

Table 4. Optical Change of Glomerulus in Group III

	double membrane	spike	mesangial region: widening	mesangial region: cell increase	aneurysm-like change	endothelium: swelling	endothelium: increase	epithelium: swelling	epithelium: increae
3,940	+++	−	+++	++	+	−	−	−	+
5,487	++	±	±	−	−	−	−	±	±
5,766	++	−	±	±	−	−	−	+	+
7,065	+++	±	+	+	±	−	−	−	−
7,808	+++	++	++	+	−	−	−	±	−
5,640	±	+++	−	−	−	−	−	−	±

± very slight ; + mild ; ++ moderate ; +++ marked

Group III: The cases which showed glomerulonephritis-like changes. These cases had chronic clinical courses (Tables 3, 4, and 5). Seven specimens from 4 patients belonged to this group. Among them, MPGN-like changes were seen in 6 specimens from 3 patients, namely, No. 3,940, 76−15, 5,487, 5,766, 7,065 and 7,808, and a MN-like change seen in one specimen 5,640 from one case. Specimens 3,940 and 76−15 were from the same patient, the former was biopsied at posttransplant 3-1/2 years and the latter autopsied at 1 month thereafter. Specimen 5,487 was biopsied at posttransplant 3 years. Specimens 5,766, 7,065, and 7,808 were all from the same patient, biopsied at posttransplant 2 years, 3 years and 2 months, and 3

Table 5. Ultrastructural Change of Glomerulus in Group III

	electron-lucent material accumulation in subendothelial space	mesangial interposition	dissection of GBM	deposits					thickening of subepithelial space	foot process fusion
				subendothelial	paramesangial	subepithelial	intramembranous	fibrin		
3,940	+++	+++	+	+	++	−	−	+++	−	+++
5,487	±	++	−	+	++	−	±	−	−	+
5,766	++	++	±	++	++	−	±	±	+	+
7,808	+++	+++	−	+	++	++	−	+	++	+++
5,640	±	±	−	±	−	++	−	−	−	+

± very slight ; + mild ; ++ moderate ; +++ marked

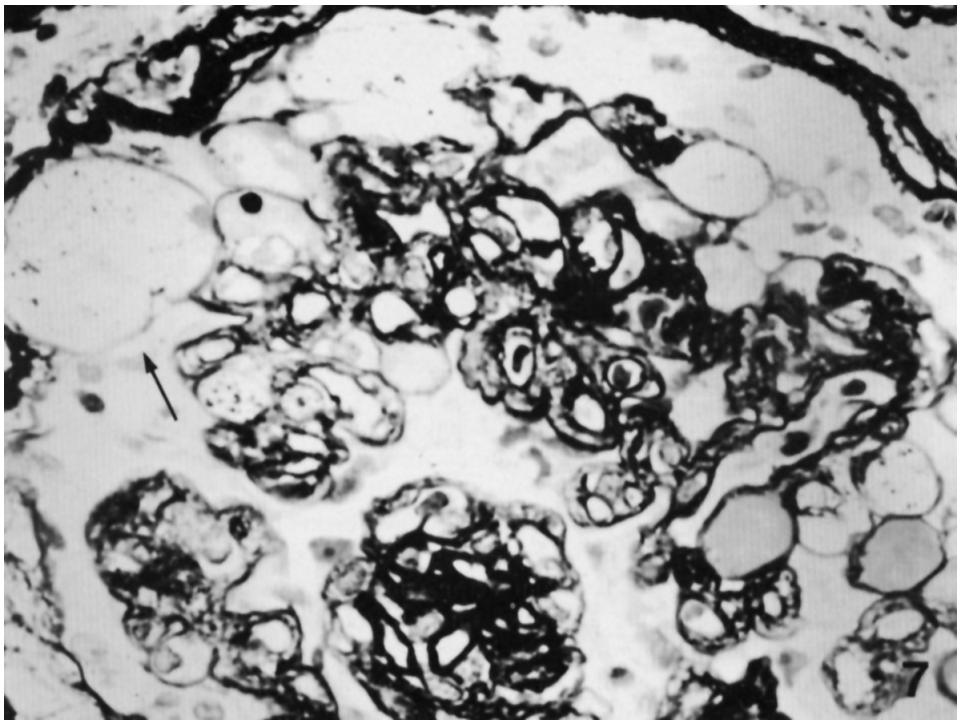

Fig. 7

years and 11 months respectively. Specimen 5,640 was biopsied at posttransplant 5-1/2 years.

Optical and ultrastructural findings on specimen 3,940: On light microscope, there were remarkable double membrane formation, the marked widening of mesangial region associated with proliferation of mesangial cells, and the aneurysm-like dilatation of thinned glomerular capillary loops (this sort of change was already described (Fig. 7). On ultrastructural observations, the accumulation of electron-lucent material in the central

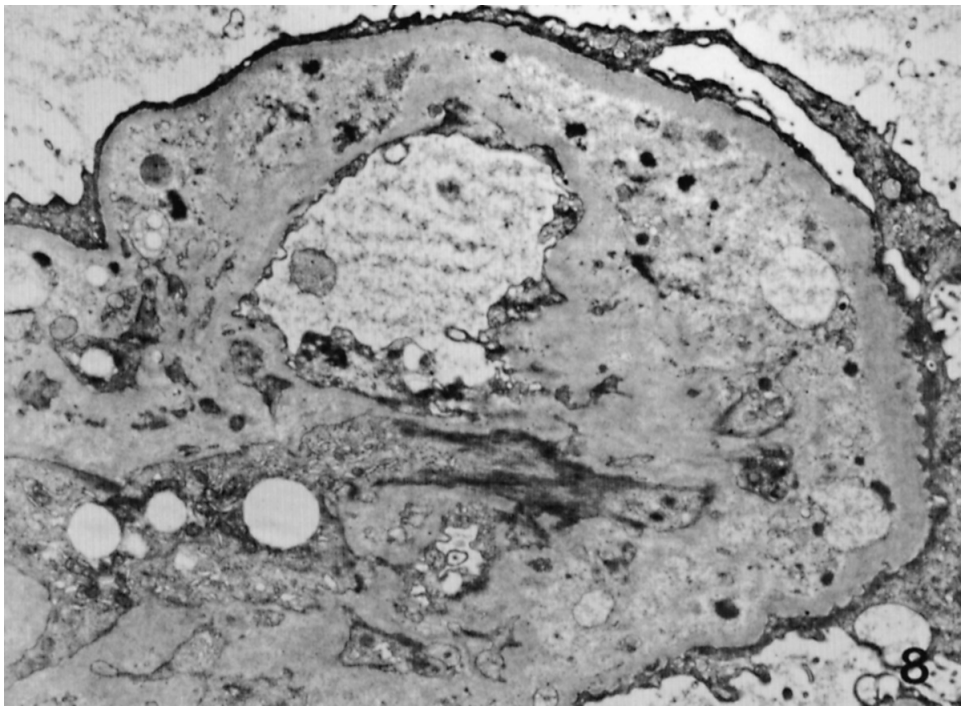

Fig. 8

portion of the GBM appeared as if the lamina densa were split. In such lesions, there were marked fibrin deposition and vacuoles of various sizes (Fig. 8). In cases without that obvious dissociation of the GBM, the central area of the GBM carrying vacuoles was occasionally electron-lucent. A certain cytoplasm of mesangial interposition so markedly swelled into a large vacuole which seemed as a capillary lumen with an original lumen closed. Mesangial interposition was also remarkable, and in some areas, the interposition and GBM-like material layered alternately to form the marked GBM thickening. Mesangial regions were widened by increase in both mesangial cytoplasm and matrix. Mesangial cytoplasms were often swollen edematously. In mesangial cytoplasms, moreover, there were many lipid droplets and myelin-like structure. Deposits, small in size, were observed in subendothelial and paramesangial areas, and those in the latter areas were occasionally large and dense. Deposition of fibrin was observed not only in subendothelial and paramesangial regions but also in capillary lumens and endothelial cells. Endothelial cells were swollen by vacuolar change, and epithelial cells showed the extensive flattening and fusion of foot processes, lost in some portions. Epithelial cytoplasms were markedly degenerative. Specimen 76–15 showed similar findings to those of 3,940. Mesangiolysis was observed in both specimens.

Optical and ultrastructural findings on specimen 5,487: Diffuse double membrane formation was observed on light microscope. The degree of this change, however, was not so pronounced as that in specimen 3,940. Mesangial lesions were also minimal. Ultrastructurally, in some parts, glomerular basement membranes were markedly thickened by multilayer formation with mesangial interposition and GBM-like materials. Mesangial regions and cytoplasms showed similar changes to those in specimen 3,940, but much milder.

The location, size, and density of deposits were also similar to those in specimen 3,940, and in addition tiny intramembranous deposits were rarely observed. Endothelial cells were vacuolar and swollen. Epithelial cells were also swollen and showed foot process fusion in some portions. The thickening of subendothelial spaces, caused by the accumulation of electron-lucent material, was very mild.

Optical and ultrastructural findings on specimens 5,766, 7,065, and 7,808: On light microscope, the formation of double membranes, the widening of mesangial regions and the proliferation of mesangial cells were found to progress with time. Spikes, which were not recognized in specimen 5,766, were observed in one of serial sections of specimen 7,065 obtained 1 year and 3 months later than specimen 5,766, and specimen 7,808, which was taken 9 months later than specimen 7,065, showed more frequent spike formation. In specimen 7,065, small numbers of tufts subjected to mesangiolysis consisted of wrinkled capillary loops. Ultrastructural observations on specimen 5,766 showed the thickening of subendothelial spaces, caused by the accumulation of electron-lucent material, where GBM-like material was formed in either a linear or a granular fashion, and there were irregularly shaped deposit-like substances with slightly higher density than that of lamina densa, and also were clearly identifiable deposits. Moreover, there were frequent mesangial interposition, the multilayer formation with this interposition and GBM-like material, and many small vacuoles right under laminae densae (Figs. 9 and 10). Mesangial regions were widened but only to minimal extent. The finding on the deposits was nearly the same as that in the above 2 cases, but the paramesangial deposits were not so dense as those in the previous 2 cases, and the sizes were smaller. A significant difference from the previous 2 cases was fine subepithelial projections of basement membranes and the linear formation of GBM-like material immediately beneath the epithelium. The epithelial foot processes were fused at those portions. No spike formation was detected on optical observations. Specimen 7,808 showed the similar changes to those in specimen 5,766, but much more remarkable. In addition, subepithelial deposits were clearly observable (Figs. 11 and 12). The thickening of subendothelial spaces became more pronounced where fine GBM-like materials were found to be formed in a longitudinal arrangement or randomly with a number of small vacuoles. Unlike the previous 2 cases, the cytoplasm of mesangial interposition showed little vacuolar swelling and atrophy, to give high electron density. Relatively high density deposits were observed just beneath the lamina densa. A characteristic finding of specimen 7,808, under electron microscope, was the marked thickening of subepithelial spaces associated with the deposits (Figs. 15 and 16). In the area without deposits observed, there was the linear and reticular formation of GBM-like material or collection of tiny vaculoes. Subepithelial deposits were mostly spherical, but some of them were irregularly shaped, and generally larger than subendothelial depositis. Similar deposits were also observed in paramesangial areas. Laminae densae showed thinning in places. Mesangial regions were widened by the increase in mesangial cells and matrix. Mesangial cytoplasms were swollen in some parts and markedly atrophied in other parts.

Epithelial foot processes were diffusely fused and lost in places. The cytoplasm of epithelial cells showed marked degeneration and atrophy. The adhesion and distortion of capillary loops were also remarkable. As regards the immunofluorescence in specimen 7,808, all of the immunoglobulins (IgG, A, M), complement C3, and fibrinogen were observed along the outer side of the capillary loops. IgG was stained in a thick linear fashion (Fig. 13), while all others were stained granularly, among them IgA being most strongly stained (Fig. 14).

Optical and ultrastructural findings on specimen 5,640: Diffuse spike formation was observed on light microscope (Fig. 17). PAS staining showed local thickening of the inner

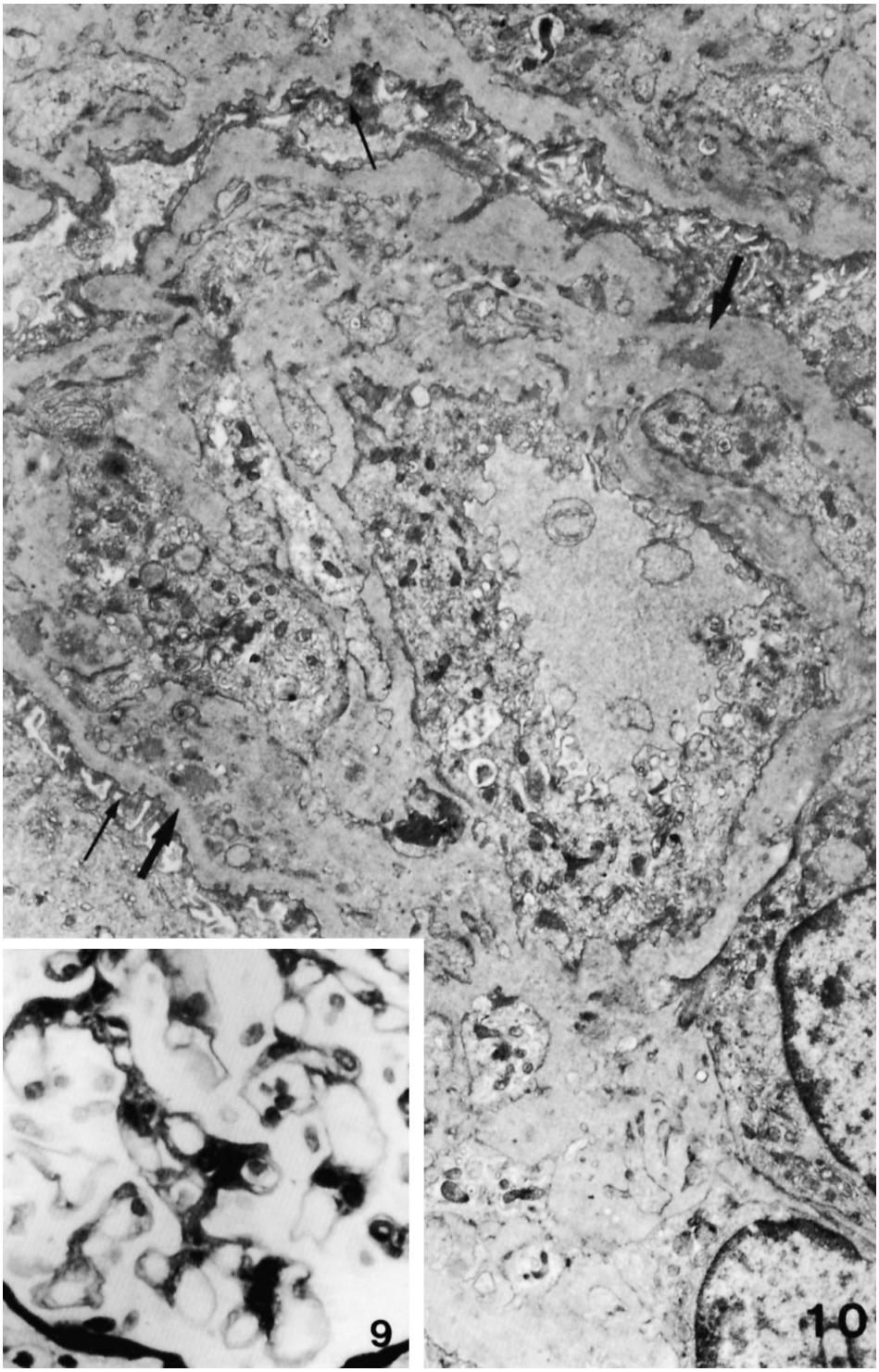

Fig. 9

Fig. 10

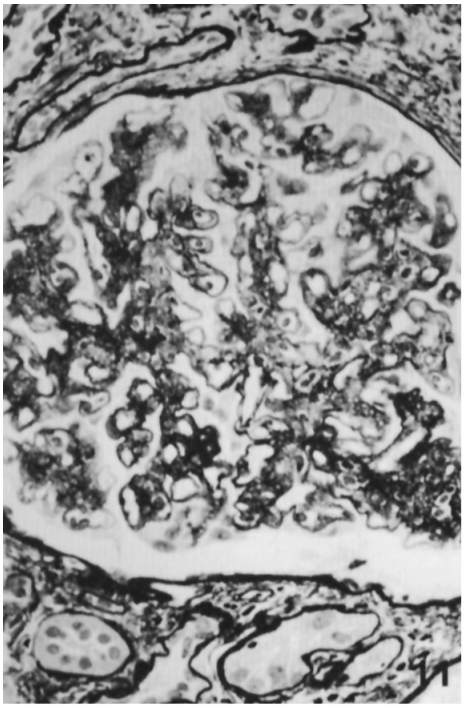

Fig. 11

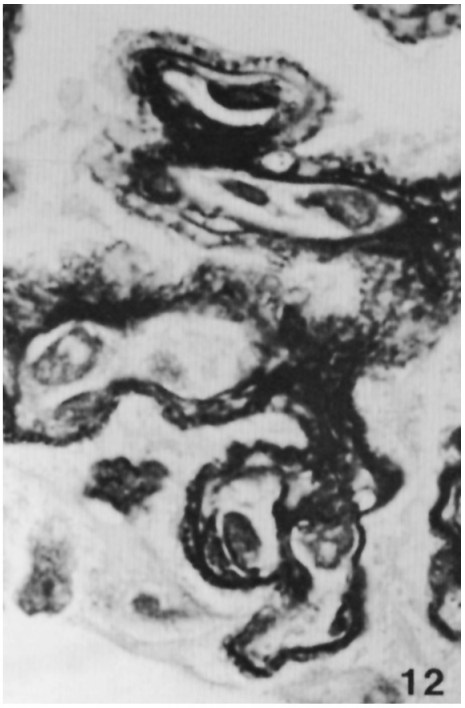

Fig. 12

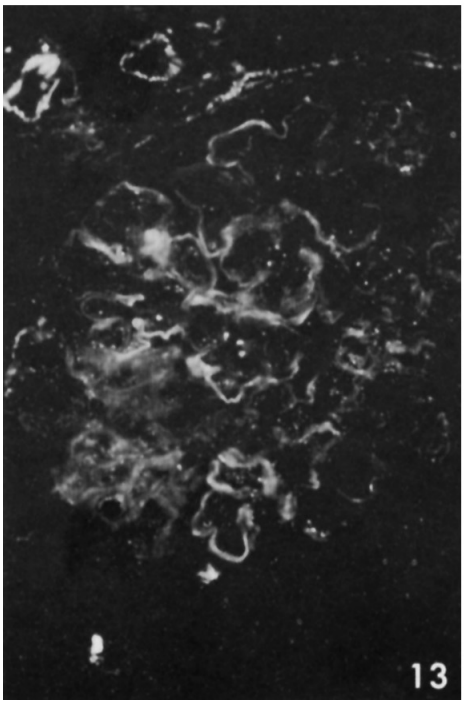

Fig. 13

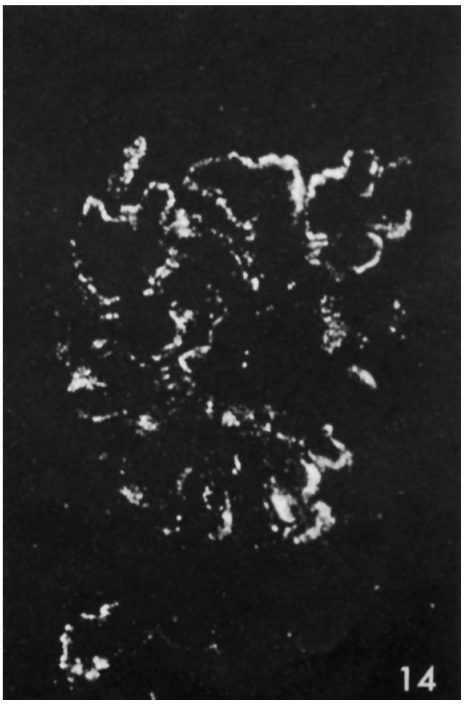

Fig. 14

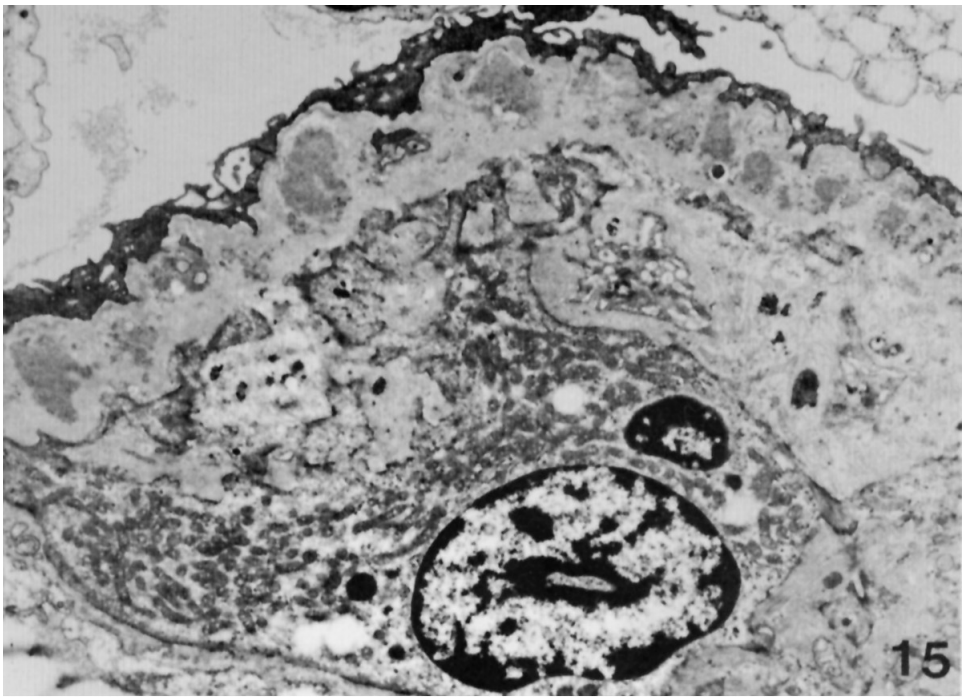

Fig. 15

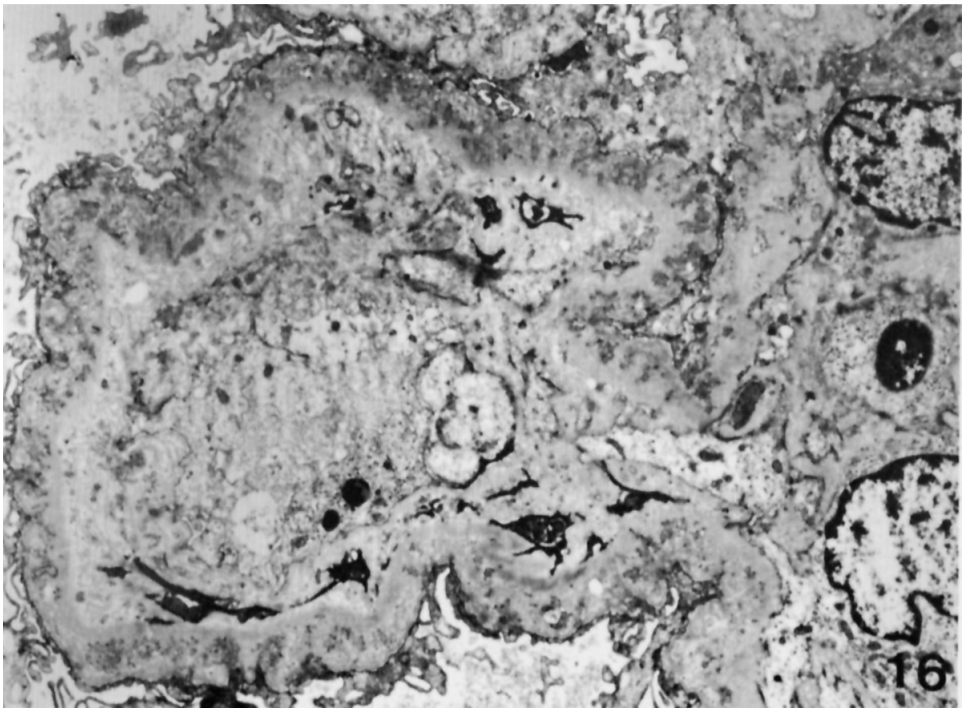

Fig. 16

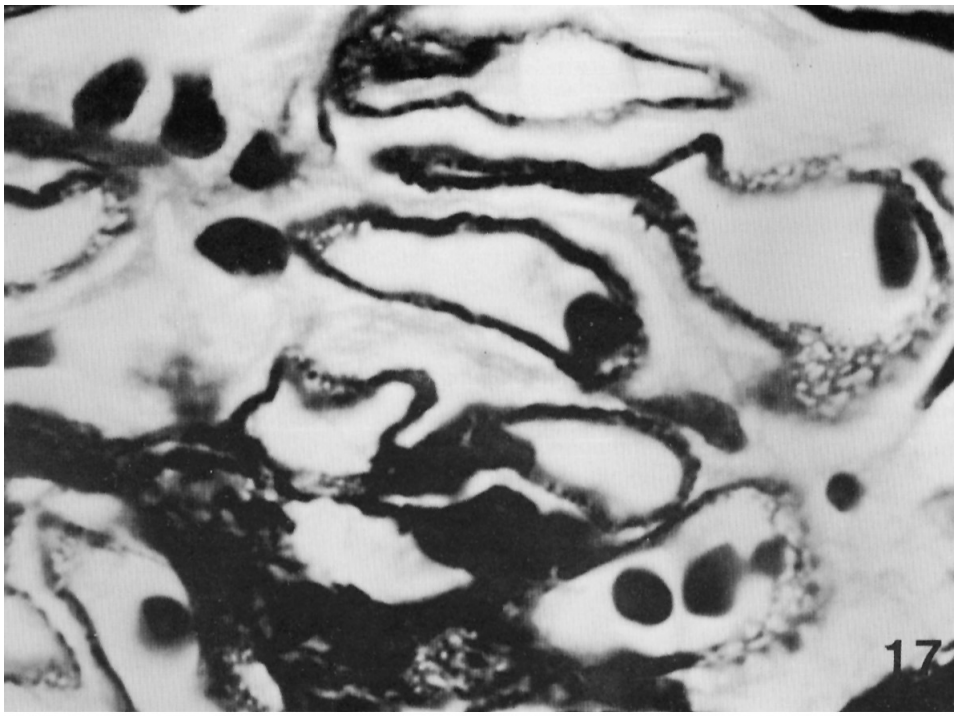

Fig. 17

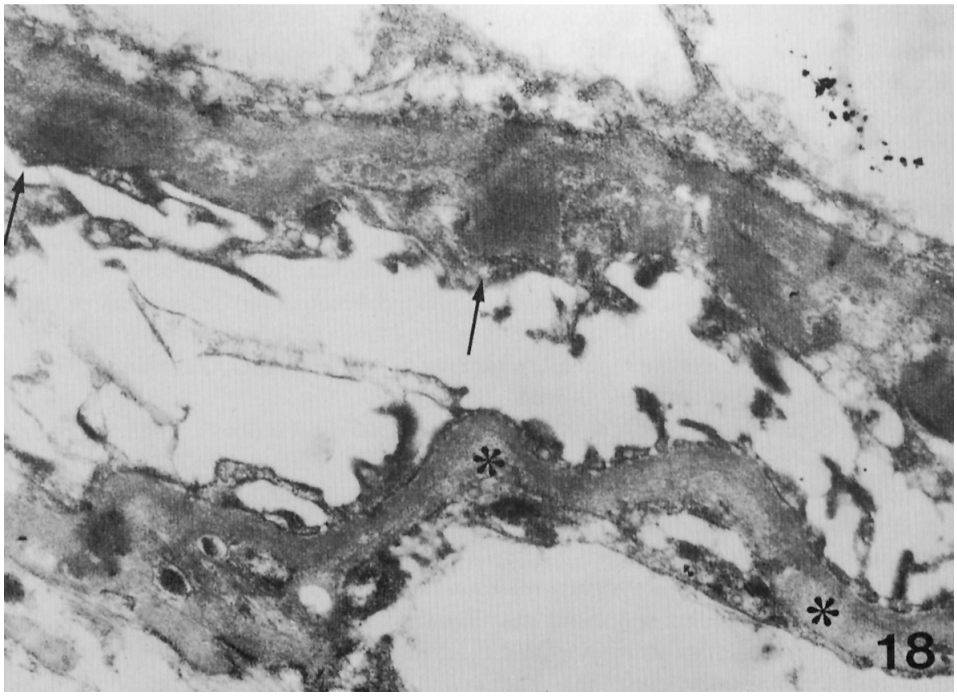

Fig. 18

side of the basement membranes. In addition, a small number of glomeruli showed ischemic changes. Epithelial cells showed mild swelling and proliferation. The specimens for the electron microscope, prepared from the paraffin embedded block, clearly showed subepithelial deposits, and also electron-lucent thickening of subendothelial spaces and presence of vacuoles just beneath laminae densae or mesangial interposition in wrinkled capillary loops (Fig. 18). Foot processes were fused with each other. Case 5,640 was considered to be in a state of chronic rejection with membranous nephropathy.

In all these four cases, the original renal diseases were clinically diagnosed without biopsies performed. None of the cases showed nephrotic syndrome before transplantation.

Changes other than those of the glomeruli in the cases with diffuse GBM thickening.

Excepting specimen 4,556, marked arterial stenosis due to fibrous intimal thickening was observed in all the specimens which contained blood vessels of appropriate sizes. The thickening of blood vessel walls in specimen 4,556 was mild, and attributable to the donor's. The degeneration and loss of tubules and interstitial fibrosis were mild to moderate in specimens 4,556, 5,640, 74–31 and 80–4, while considerably advanced in all other cases. The cellular infiltration of the stroma was marked in Group I, but mild in the other two groups.

DISCCUSION

It is well known that the thickening of the subendothelial space is a characteristic feature of chronic rejection. Attention has not been paid to the correlation between the degree of the thickening and the clinical course. Under continuation of a severe rejection for a certain period of time, the thickening develops markedly, while it is mild, if renal failure develops promptly or a chronic rejection is prolonged. Immunofluorescent studies showed in group I and II that IgG and IgM were focally and segmentally stained in glomerular basement membranes in a granular or sometimes in a linear fashion. Complement C3 was stained less frequently than the above two and IgA was stained only rarely. Fibrinogen was also stained frequently. Similar results have also been reported by other investigators.[3,12,13] In the present 3 cases which showed MPGN-like changes, all immunoglobulins, complement C3 and fibrionogen were fairly strongly stained. Fibrillar or granular GBM-like materials were frequently found in thickened subendothelial spaces. Then at the final stage they supposedly became so dense as to be indistinguishable from the lamina densa, in some cases like specimen 3,940. The boundary between the original lamina densa and the newly formed basement membrane, however, can usually be located because of the frequent presence of mesangial interposition or vacuoles at the boundary. It may be inferred that such a boundary might dissociate when a drastic rejection attack was superimposed. Accordingly, the lesion involved can be regarded as locus minoris resistantiae.

The mild thickening of the laminae densae observed in Group II does not seem to be evoked by rejection nor by some other immunological reactions. Protracted administration of prednisolone reportedly causes the thickening of basement membranes.[14]

The double membrane structure on the optical observation corresponds to the accumulation of electron-lucent material or mesangial interposition in the central area of basement membranes on the ultrastructural observation. The double membrane was found in the early stage after transplantation, and was observed in more than one third of all specimens in 2 months after transplantation, but its diffuse development was rarely observed. Other changes were gradually superimposed. These changes included the wrinkling of basement membranes, mesangiolysis, sclerosis, fibrosis etc.

Suzuki *et al.*[5] ultrastructurally demonstrated the mesangiolysis caused by snake poison. Shigematsu *et al.*[6] described it optically in Wegener's granulomatosis. Mesangiolysis was not rarely found in transplanted kidneys. In the tuft carrying meangiolysis in chronic rejections, the capillary loops were thickened by wrinkling, in contrast to the thin capillary loops at acute rejections. In the former, the capillary lumen was not so large, and the capillary loops gradually disappeared, leaving only the Bowman space. This characteristic change was observed in the grafts which fell into renal failure after a very long survival. In chronic end states both endothelial and epithelial cells frequently decreased. Also, mesangial regions and naked basement membranes formed a simple structure to be in slight contact with each other. In some case, only visceral epithelial cells proliferated. Mesangiolysis was mostly observed in end stages regardless of acute or chronic rejections.

Pathogenesis of so-called transplant glomerulopathy has been an attracting subject for many investigators, however, it has not been solved yet. Although its definition varies from investigator to investigator, it is almost unanimously accepted that a change involved primarily consists of mesangial interposition. The 3 cases which showed MPGN-like changes in the present study are included under this condition. These three cases differed from primary membranoproliferative glomerulonephritis in the following points: 1) The thickening of subendothelial spaces due to the accumulation of electron-lucent material. 2) The dissociation of basement membranes right under laminae densae or at closer sites to capillary lumens. 3) The deposition of fibrin. 4) The swelling and vacuolation of mesangial cytoplasms (occasionally including endothelial and epithelial cells), and the trend of forming multi-layers by mesangial interposition and GBM-like material. 5) Segmental tendency of mesangial interposition (rare to become circumferential). 6) Marked thickening of subepithelial spaces as observed in specimen 7,808 (Fig. 17). Besides the paper presented here, only Busch *et al.*[15] and Sonnabend *et al.*[16] have reported the change in item 6 above. It was rather difficult to distinguish specimen 5,487 from primary MPGN only with the change of glomeruli. But the chronic rejection undoubtedly existed judging from the more pronounced change in the arteries than in the glomeruli. McPhaul *et al.*[17] and Cameron *et al.*[10] have discussed whether the change is to be defined as chronic rejection or glomerulonephritis. As for glomerulonephritis, not only primary glomerulonephritis but also those caused by viral[16,18] or bacterial infection must be taken into consideration. Since allografts can not be saved from the attack of rejection, their complication with glomerulonephritis undoubtedly gives rise to extremly intricate morphological appearances. It can be inferred that protracted administration of immunosuppresive agents might modify the pattern of glomerulonephritis.[15] Based on these difficulties it is almost impossible to distinguish these two lesions.

A number of reports have been published on the complication with membranous nephropathy.[3,19,20] Since spikes, namely, subepithelial deposits can rarely be observed in transplanted kidneys, it seems reasonable to postulate the complication with membranous nephropathy, when many spikes are observed in transplanted kidneys.

Since the specimens in the present study were mostly obtained from transplanted kidneys at relatively early stages after transplantation, the possible thickening in later stages may have been excluded from the data presented here.

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