Renal Allografts in HL-A Matched Recipients

Light, Immunofluorescence and Electron Microscopic Studies

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PREVIOUS EXAMINATIONS OF RENAL ALLOGRAFTS from patients undergoing therapy with immunosuppressants have demonstrated cellular interstitial infiltrates, vascular lesions, glomerular abnormalities, tubular injury, and hypertrophy of the juxtaglomerular apparatus. Among these changes, inflammatory cellular exudates, vascular abnormalities, and certain tubular injuries have been ascribed to graft rejection.[1–4] The glomerular alterations have been varied and range from frank recurrence of host disease to less specific abnormalities of capillary basement membranes.[5–8] The pathogenesis and significance of alterations of the juxtaglomerular apparatus in transplanted kidneys are less certain.[2]

In the usual setting of clinical transplantation, the large numbers of variables (*ie*, degree of histocompatibility, type and quantity of immunosuppression, original renal disease of the host, infections, and untoward mechanical factors) make it difficult to distinguish tissue changes related specifically to graft rejection and those that result from a recurrence of original host disease in the graft. The complexity of variables affecting renal grafts can be reduced or simplified, in part, by studying the tissue reactions that occur in well-matched renal allografts, since these patients receive little or no immunosuppressive therapy.[10, 11]

In the setting of closely matched donor and recipient, where less immunosuppressive therapy is required, it might be possible to evaluate grafted kidneys for (1) rejection reactions due to minor tissue histoincompatibilities and (2) the relationship, if any, of redevelopment, within the graft, of features of the original host disease. The HL-A

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Supported in part by Grants AM11797, MO1RRL30, AM13084, GM00726 and AM11092 from the US Public Health Service.

Accepted for publication July 6, 1970.

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matched patients with renal allografts, included in this study, were biopsied at intervals after transplantation. Biopsies of transplanted kidneys and host kidneys removed at the time of transplantation were compared by light, immunofluorescence, and electron microscopy.

Materials and Methods

The 12 patients included in this study received their kidneys from siblings who were typed as being HL-A identical with the respective recipient. The clinical information regarding these patients has been reported in detail,[11] but the features particularly pertinent to this study are included in Table 1. The patients are identified by their living donor (LD) number.

Eleven of the recipients were male and one was female. They ranged in age from 16 to 42 years at the time of transplantation. The host diseases were chronic glomerulonephritis (9 cases), chronic lobular glomerulonephritis (1 case), chronic pyelonephritis (1 case), and arteriolar nephrosclerosis (1 case). The latter patient and four of the patients with chronic glomerulonephritis had coexistant malignant arteriolar nephrosclerosis. Seven of the patients had episodes of threatened rejection. Six occurred during the first two post-transplant months and one was delayed for more than a year.

Biopsies of the grafts were carried out 2–47 months after transplantation. The grafts had been in place for 11–57 months after transplantation at the time of this report (June 1, 1970). One patient (LD 23) had biopsies of his graft 2 and 19 months after transplantation. Patient LD 6 was given a second renal allograft (cadaveric) in the twentieth month of his course because of renal failure caused by recurrence of the original host renal disease in the first graft.

The details of immunosuppressive therapy were included in a previous report.[11] Only azathioprine and prednisone were used. Four of the patients received only azathioprine (2 mg/kg/day). Prednisone (200 mg/day) was started when threatened rejection was diagnosed and was continued until there was no further improvement in creatinine clearance. One patient (LD 19) received no immunosuppression for a full year prior to biopsy.

Specimens obtained from the host's own kidney and portions of subsequent biopsies of the renal grafts were prepared for light, immunofluorescence, and electron microscopy as outlined below.

Light Microscopy. A portion of each specimen was fixed in buffered 10% formalin, processed, and embedded in the usual way. Sections cut at 2–3 μ were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), periodic acid-methenamine silver-Masson (PAMM), elastic Verhoff-Van Gieson (VVG) technics, or methyl green-pyronin (MGP reaction).

Electron Microscopy. Another portion of each kidney biopsy was cut into 1-mm fragments, immersed in cold 4% glutaraldehyde buffered with 0.1 M sodium cacodylate, fixed in 1% osmium tetroxide, and embedded in Epon. Ultrathin sections were cut on a Porter-Blum ultramicrotome and stained with either lead citrate or uranyl magnesium acetate and lead citrate. These specimens were examined and photographed using a Hitachi HS-7 or HS-8 electron microscope.

Immunofluorescence Microscopy. A third portion of each tissue was rapidly frozen in a matrix of gelatin and stored at −70 C until immunohistologic analysis. Sections from these blocks were cut in a cryostat microtome at 4 μ, picked up on glass slides, immersed in acetone for 5 minutes, washed twice in phosphate-buffered (0.02 M, pH 7.3) saline, and incubated in a moist container at room temperature for 30 minutes with the appropriate fluoresceinated immunoglobulin reagent. The

stained slides were then coverslipped using 50% glycerin diluted with an equal volume of buffered saline. Observations and photographs were made with a Leitz Ortholux microscope using an HBO-200 W mercury-vapor light source.

Antibody reagents used in this study included antiserums reactive with human 7S immunoglobulins, IgG, IgM, β_1C globulin, albumin or fibrinogen as well as anti-guinea pig complement. The first and last three of these antiserums were prepared and analyzed for specificity of reactivity in this laboratory as described elsewhere.[12] The remaining antiserums were purchased from Hyland Laboratories or Immunology, Inc as the whole serum and analyzed for monospecificity of reactivity prior to preparation of their immunoglobulin fractions. The sodium sulfate-precipitated immunoglobulin fraction of each respective antiserum was labeled with fluorescein isothiocyanate by methods described previously.[12-14] Appropriate controls for the respective immunohistochemical reactions were those outlined in detail in earlier studies on a variety of human and experimental renal diseases.[12, 15]

Results

Presentation of the results of this study will be divided into three major categories: (1) morphologic descriptions of the original host renal diseases; (2) presentation of the histologic alterations in biopsies of the renal allografts; and (3) in the discussion, a comparative analysis of changes in the grafts and respective diseased host kidneys.

Host Disease

The pathologic findings in the host kidneys are summarized in Table 2 and 3.

Chronic Glomerulonephritis. The 9 patients with chronic glomerulonephritis had typically far advanced marked glomerulosclerosis at the time of transplantation (Table 2 and Fig 1 and 11). Glomeruli frequently had local, hypereosinophilic, hyaline thickening of capillary walls (Fig 1, 11 and 21). The remaining parenchyma in these kidneys was severely altered by focal or widespread tubular atrophy, interstitial fibrosis, and focal interstitial aggregates of lymphocytes. Arterial and arteriolar nephrosclerosis of variable, but usually severe, degree was present in 8 of the 9 cases and accelerated malignant hypertensive vascular disease with fibrinoid necrosis and hemorrhage in arteriolar walls was present in 4 of these 8 cases.

When examined by electron microscopy, the glomerular basement membranes were wrinkled, often greatly thickened, and collapsed around mesangial areas composed of greatly increased amount of basement membrane matrix; they frequently contained massive electron-dense deposits (EDD) (Fig 3, 4, 13 and 22). Epithelial and endothelial cells in the most sclerotic glomeruli were often crowded out by these altered basement membranes. Foot processes and their

Table 1. Pertinent Clinical Information

Case	Age/Sex		Host renal disease*	Interval to rejection episode (mo)	Duration of graft at time of biopsy (mo)	Total duration† of graft at time of report (mo)	Histologic estimate of degree of rejection‡	Post-transplantation therapy	Comments
	Host	Donor							
LD 4	40/M	42/M	CGN, ANS (severe)	—	41	51	Mild	Imuran	
LD 13	25/M	23/F	CGN, ANS, MNS	14	14	30	Moderate	Imuran prednisone	
LD 18	42/M	47/M	CGN, ANS (severe)	—	8	22	Moderate	Imuran	
LD 16	22/M	21/M	CGN, ANS, MNS	1.5	14	25	Mild	Imuran prednisone	
LD 2	32/M	28/F	CGN, CNS, ANS	—	47	57	Mild	Imuran	
LD 32	37/M	42/F	CGN, ANS	0.1	2	14	Slight (subcapsular reaction)	Imuran prednisone	
LD 23	38/M	40/F	CGN, ANS, MNS	1	2, 19	19	Slight	Imuran prednisone	
LD 3	39/M	42/M	CGN§	1	43	53	Slight	Imuran prednisone	
LD 36	23/M	27/M	CGN, ANS, CPN	—	10	11	Moderate	Imuran	

LD 21	16/M	22/M	CPN	1	8	20	Mild	Imuran prednisone	
LD 19	25/F	35/F	ANS	0.3	14	22	Mild	Imuran prednisone	No immunosuppression therapy for 1 yr before biopsy of graft
LD 6									
1st graft	25/M	28/M	CLGN	—	18	20	Slight/marked	Imuran prednisone	Graft developed host disease,
2nd graft	27/M	Cadaver		—	2 (necropsy)	2 (necropsy)	Mild		new cadaveric graft implanted 2 mo antemortem

* CGN = Chronic glomerulonephritis; ANS = Arteriolar nephrosclerosis; MNS = Malignant, necrotizing arteriolar nephrosclerosis; CPN = Chronic pyelonephritis; CLGN = Chronic lobular glomerulonephritis.

† Time as of June 1, 1970.

‡ See Table 2 and 3 for summary of histologic alterations in the grafts and host kidneys.

§ Mixed membranous and proliferative variety.

filtration slit pores were locally or extensively obliterated. Mesangial areas of glomeruli were thickened in all 9 cases and contained moderate to marked amounts of EDD intermixed with or within mesangial basement membrane material.

The most general findings in these kidneys, by immunofluorescence microscopy, were predominantly IgM and, less extensively, IgG immunoglobulins, β_1C globulin, and fixation of guinea pig complement in local or diffuse globular or granular deposits in glomerular capillary walls (Fig 2 and 12). In 5 of 6 cases tested, IgM globulins were the predominant immunoglobulins localized in glomeruli. In 9 of 9 cases tested, IgG globulins were identified in glomerular capillary walls but generally in less extensive distribution than localized IgM. The patterns of globulin localization in glomeruli correlated closely with the distribution of EDD on electron microscopy. Three patients (LD 23, LD 16 and LD 2) all of whom had arteriolar sclerosis and two of whom had malignant arteriolar sclerosis, also had deposits of IgG and IgM immunoglobulins in blood vessel walls. (See Refs 16 and 17 for immunohistochemical analysis of malignant arteriolar nephrosclerosis).

Chronic Lobular Glomerulonephritis. One patient (LD 6) had end-stage chronic lobular glomerulonephritis at the time of transplantation (Table 3 and Fig 25). The rapid progression of the lesion to end stage in this patient was made evident by comparing the histologic changes over one year and then over an interval of one month prior to transplantation. An electron micrograph prepared from the first biopsy (Fig 27) shows mesangial nodules of basement membrane matrix making up the characteristic glomerular nodular alteration in this disease. When examined immunohistochemically, the nodules were free of positive-staining material but both IgM and IgG immunoglobulins appeared as granular and short linear deposits in some peripheral capillary walls around the nodules (Fig 26). Other than for some electron-dense mottling of basement membranes, no apparent correlates of localized globulin could be seen by electron microscopy. An occasional arteriole contained IgG deposits that were slightly positive for fixation *in vitro* of guinea pig complement.

Arteriolar Nephrosclerosis. As described above, arteriolar sclerosis was observed in 8 cases of chronic glomerulonephritis (Table 3). There was one other case with a major histopathologic diagnosis of arteriolonephrosclerosis (LD 19) who had malignant arteriolar sclerosis. There were IgG and IgM immunoglobulins and fibrin in blood vessel walls in this case. The electron micrographs from the kidneys

of this patient showed marked wrinkling or wavy alterations of glomerular basement membranes. She was of particular interest in that a diagnosis of scleroderma was suspected initially but serologic and pathologic studies were negative in this regard. Ultrastructural examination of her glomeruli revealed rare EDD intermixed with fragments of cellular material in the mesangium. Immunofluorescent examination showed no significant glomerular localization of plasma proteins in this case.

Chronic Pyelonephritis. One patient (LD 21) had chronic pyelonephritis (Table 3). The diagnosis was made histologically on the basis of marked glomerular sclerosis in the absence of any significant alterations in nonsclerosed glomeruli, frequent periglomerular capsular fibrosis, "thyroidization" among atrophic tubules, and interstitial fibrosis and chronic inflammatory infiltrate in areas of sclerosed glomeruli. Immunohistochemical examinations were negative. Ultrastructurally, a few paramesangial and subepithelial intramembranous EDD were observed, but these were far more local and never as extensive in distribution as those seen in chronic glomerulonephritis.

Graft Disease

Pathologic observations in the renal allografts are also summarized in Table 2 and 3. A single transplant was done in 11 of the cases. In one other case (LD 6) there were two renal allografts; the first was from an HL-A identical sibling and the second was from a cadaveric source.

The pathologic changes to be described, in particular the amount of interstitial inflammatory infiltrates, are graded according to their severity from 0 to 3+. A grade of ± (rare) indicates minor involvement; 1+ (slight) indicates more than one or two sites involved; 2+ (moderate) indicates changes in multiple areas; and 3+ (marked) indicates more extensive involvement of the biopsy specimen. The terms to describe the histologic distribution of renal lesions, local or diffuse, and focal or generalized, are those defined and used elsewhere.[15]

Interstitium. Inflammatory cell infiltration of the interstitium in the renal grafts varied from slight, widely interspersed (±) in 1 case; mild, more frequent (+) in 7 cases; moderate (++), more extensive in 3 cases; to severe (+++) in the first renal allograft of LD 6 when examined at necropsy and in the second biopsy of LD 23. These cellular infiltrates were characteristically heterogeneous, being made up of small and medium-sized mononuclear and lymphoid cells, plasma cells, and occasional macrophages and eosinophils (Fig 17–20

Table 2. Histopathology of the Host and Allograft Kidneys of Patients with Chronic Glomerulonephritis

Case and kidney	Light microscopy			Electron microscopy (glomeruli)		Immunofluorescence (glomeruli) immunoglobulins
	Glomeruli	Arterioles	Interstitium	Basement membrane	Deposits	
LD 4						
Host	Sclerosis G+++	Sclerosis +++	Sclerosis +++, chronic inflammation +++	Wrinkled, thickened + → +++	Mesangial ±	7S
Graft	↑ Mesangium +	Smudging ±	Inflammatory infiltration F+	Wrinkled L ±, thickened L±	Intramembranous small ±	0
LD 13						
Host	Sclerosis G+++	Sclerosis +++ MNS*	Sclerosis +++ chronic inflammation F++	Thickened +++, collapsed	Intramembranous small +	7S, IgG, IgM, C3-4, GpC-fix.
Graft	↑ Mesangium +, "glomerulitis"	Smudging ±, vacuolation	Inflammatory infiltration F++, (1 periglomerular)	Not done	Not done	7S, C3-4, GpC-fix.
LD 18						
Host	Sclerosis G+++, cellularity F++	Sclerosis +++	Sclerosis +++, chronic inflammation +++	Thickened ++, wrinkled collapsed	Subendothelial, intramembranous +++	IgM, C3-4, GpC-fix.
Graft	↑ Mesangium + ↑ JGA +	—	Inflammatory infiltration F + → ++, perivascular, periglomerular	—	Subendothelial, loose granular (rare)	0
LD 16						
Host	Sclerosis +++	Sclerosis +++ MNS	Sclerosis ++ foam cells +++	Thickened +++, tiny "crystals"	Intramembranous tiny "crystals"	IgM, C3-4

Graft	↑ Mesangium, FL+ ↑ JGA + → ++	Smudging F	Inflammatory infiltration F ± → + (perivenous, tubular)	Thickened L+	Paramesangial ++	7S, IgG, IgM, IgA, C3-4, GpC-fix.
LD 2						
Host	Sclerosis +++, cellularity +	Sclerosis ++	Sclerosis +++, chronic inflammation ++	Thickened D+++, wrinkling, collapse	Subendothelial, intramembranous	7S, GpC-fix.
Graft	↑ Mesangium + old crescents	—	Inflammatory infiltrate F+ (periglomerular X2)	Thickened L±	Paramesangial L + → ++, "viral" particles	7S, IgG, IgA
LD 32						
Host	Sclerosis ++, cellularity +	Sclerosis ++ MNS	Chronic inflammation + → ++	Not done	Not done	0
Graft	—	—	Inflammatory infiltration F±	Not done	Not done	0
LD 23						
Host	Sclerosis +++, cellularity +	Sclerosis +++ MNS	Chronic inflammation F+++ (nodular!)	Not done	Not done	IgG, IgM, IgA, C3-4 GpC-fix.
Graft Bx. No. 1	↑ Mesangium ±	Vacuolation F	Inflammatory infiltration F± (between arteries and vein)	Wrinkling	Paramesangial L, very small	0
Bx. No. 2	↑ Mesangium FL L+	—	Chronic inflammation ++	Not done	Not done	7S, IgM, IgG, C3-4, GpC-fix.
LD 3						
Host	Sclerosis ++, Membranous thickening	Sclerosis +++	Sclerosis +, chronic inflammation +	Thickened +++	Intramembranous +++	7S, GpC-fix.

Table 2. (Continued)

Case and kidney	Light microscopy			Electron microscopy (glomeruli)		Immunofluorescence (glomeruli) immunoglobulins
	Glomeruli	Arterioles	Interstitium	Basement membrane	Deposits	
Graft	↑ Mesangium + → ++ ↑ JGA±	↑ Intima FL	Subcapsular infiltrate; inflammatory infiltration ±	Wrinkling (paramesangial)	Paramesangial + (rare subepithelial)	7S, IgG, IgM, C3-4
LD 36						
Host	Sclerosis ++, pericapsular fibrosis	Sclerosis +++, MNS	Sclerosis ++, chronic inflammation F++	Not done	Not done	7S, IgM, C3-4, GpC-fix.
Graft	↑ Mesangium ± (1 sclerosed)	Smudging vacuolation	Inflammatory infiltration F++	Thickened F±	0	7S, C3-4

Nomenclature for histologic distribution of an alteration: F = focal, some of the structures involved; G = generalized, all of the structures involved; L = local, a portion of the structure involved; D = diffuse, nearly the entirety of the structure involved.

Nomenclature for severity of a finding: 0 = not present; ± = slight, infrequent; + = mild; ++ = moderate; +++ = severe.

* MNS = Malignant nephrosclerosis.

and 31). They were most often clustered in close proximity to or surrounding renal tubules and blood vessels (venules and arterioles) (Fig 18–20) and less often adjacent to glomeruli (Fig 17). Characteristically, those cells associated with tubules were often contiguous with disrupted peritubular capillaries and sometimes appeared to be injuring tubules by invading their basement membranes and epithelial cells (Fig 17–19 and 31). Many of the cells making up these infiltrates of the interstitium had a moderate amount of cytoplasm which stained intensely with methyl green-pyronin. In spite of this, only a few cells in some grafts could be detected to contain antibody globulins by immunofluorescent technics.

It was common to find masses of small lymphocytes immediately subjacent to the thickened sclerosed renal capsule. These lesions are generally regarded as nonspecific responses to mechanical capsular injury incurred during surgical procedures rather than to graft rejection. The most severe changes of this sort were seen in patient LD 19 who was known to have a long-standing perinephric abscess. The homogeneity of the subcapsular cellular exudates contrasts sharply with the heterogeneity of the cortical interstitial infiltrates resulting from transplant rejection reactions.

Blood Vessels. Vascular alterations were observed in transplanted kidneys in 5 cases, involved almost exclusively only selected arterioles, and consisted mainly of local thickening, smudging, or vacuolization of the muscular media. In no case was the elastica or intima significantly altered. These minor alterations in arterioles within the grafted kidneys showed no correlation with the presence of necrotizing arteriolar lesions in the previously removed host kidneys. In only 3 cases (LD 18, LD 21 and LD 6) were immunoglobulins identified in vessel walls and then only rare arterioles were minimally affected by tiny, local streaks of positive immunofluorescence along the intima or in the media. Either IgM or IgG immunoglobulins were found but complement could not be identified in these vessels. These minimal sites of localized immunoglobulins showed no apparent correlations with the presence of the minor alterations in arterioles described above.

Glomeruli. Eleven of the renal allografts showed some degree of glomerular changes, which consisted mainly of local or diffuse mild or moderate thickening of mesangial regions (Fig 5, 7 and 14). One exception to these minor glomerular changes was LD 6 who showed recurrent chronic lobular glomerulonephritis in an allograft from his brother (Fig 28–30). The second renal allograft in this patient was from a cadaver and was in place for only one month prior to the

Table 3. Histopathology of the Host and Allograft Kidneys of Patients with Chronic Pyelonephritis, Arteriolar Nephrosclerosis and Chronic Lobular Glomerulonephritis

Case and kidney	Light microscopy			Electron microscopy (glomeruli)		Immunofluorescence (glomeruli) immunoglobulins
	Glomeruli	Arterioles	Interstitium	Basement membrane	Deposits	
CHRONIC PYELONEPHRITIS						
LD 21						
Host	Sclerosis +, pericapsular fibrosis +++	—	Sclerosis ++, chronic inflammation ++	Thickening L+, wrinkling	Subepithelial (rare), paramesangial (rare)	0
Graft	↑ Mesangium ±	—	Inflammatory infiltration +	Thickening, wrinkling (para mesangial) ±	0	0
ARTERIOLAR NEPHROSCLEROSIS						
LD 19						
Host	Sclerosis F+, thick walls D+	Sclerosis +++	Sclerosis +, chronic inflammation F+	Wrinkling	Cell remnants in intramembranous deposits (rare)	0
Graft	↑ Mesangium FL±	—	Subcapsular chronic inflammatory +++, inflammatory infiltration F+.	Thickening L±	0	0

CHRONIC LOBULAR GLOMERULONEPHRITIS						
LD 6						
Host (Bx No. 1)	Cellular ++ lobular +++	—	Sclerosis ±	Not done	Not done	Not done
Host (Bx No. 2)	Lobulated +++	—	Sclerosis +, chronic inflammation F+	Wavy D, thickening D+	0	Not done
Host (Bx No. 3)	Lobulated +++, sclerosis +++	—	Sclerosis +	—	—	IgM
Graft 1 (Bx)	Cellular ++ lobulated ++	—	Sclerosis F++, inflammatory infiltrate ±	Wavy D, thickening D+	0	IgM
Graft 1 (necropsy)	Lobulated +++, cellular +	—	Inflammatory infiltrate +++ (nodular)	Not done	Not done	IgM
Graft 2 (necropsy)	—	—	Inflammatory infiltrate F+, edema	Wrinkling L+	0	0

Nomenclature for histologic distribution of an alteration: F = focal, some of the structures involved; G = generalized, all of the structures involved; L = local, a portion of the structure involved; D = diffuse, nearly the entirety of the structure involved.

Nomenclature for severity of a finding: 0 = not present; ± = slight, infrequent; + = mild; ++ = moderate; +++ = severe.

recipient's death. No significant glomerular changes were observed by light microscopy in this second graft, but very small, local deposits of IgM immunoglobulins were observed in occasional peripheral capillary loops of a few glomeruli.

Another case in which the graft displayed more than the usual minor glomerular alterations was LD 2. By light microscopy, local thickening of glomerular capillary walls and mesangial regions, and formation of fibrous capsular crescents in two glomeruli were noted in this case (Fig 5 and 7). Ultrastructurally, there was widening of the glomerular mesangium by basement membrane matrix and prominent paramesangial EDD (Fig 8). IgG immunoglobulins and minor sites of complement were found locally distributed in the glomerular stalk, capillary walls, and rare segments of membranes roughly in the same distribution as the electron-dense deposits (Fig 6). Of particular interest was the identification of small loci of IgA immunoglobulins in glomerular mesangium. Similar patterns of limited distribution of IgA immunoglobulins were found in some glomeruli of the renal graft of LD 16 (Fig 23) and of IgM immunoglobulins in glomeruli of the renal graft of LD 3 (Fig 15.). Although glomerular abnormalities were not notable by light microscopy in these two patients, electron microscopy showed a thickened mesangium with small paramesangial and intramesangial EDD in LD 16 (Fig 24) and thickened mesangium with rare EDD in LD 3 (Fig 16).

Ultrastructural glomerular changes in other renal grafts in this study were minimal in degree, consisting of local variable thickening of basement membrane and occasional very small paramesangial subendothelial or intramembranous EDD. In one case (LD 18) peculiar small "pools" of loosely granular subendothelial deposits or alteration in the lamina rara interna of glomerular capillaries were noted. In 5 cases, slight to moderate local loss of foot processes of epithelial cells was noted. In one case (LD 2) "virus-like" particles were identified at several subepithelial sites along the glomerular capillary basement membrane (Fig 9 and 10). All of the above ultrastructural changes could not be appreciated by light microscopy. Observations by immunofluorescence microscopy in these cases were generally negative except for rare, isolated small mesangial or capillary mural streaks of either IgG or IgM immunoglobulins.

Tubules and Juxtaglomerular Apparatus. With the exception of those instances of tubular damage in association with peritubular inflammatory cell infiltrates, there were few tubular alterations. Hydropic swelling of proximal tubular cells in LD 19, dilated tubules in LD 23

and LD 2, and occasional hyaline casts in several cases were observed. Alterations of juxtaglomerular apparatus were slight (Fig 14); some minor degree of focal hypertrophy of these structures was seen in 4 cases (LD 18, 16, 23, 3).

Discussion

Comparative Analysis of Renal Lesions of the Host and Grafted Kidneys

A number of general considerations result from comparing the alterations observed in transplanted kidneys with those observed in the respective diseased host kidneys removed at the time of transplantation. First of all, the distribution and severity of rejection reactions, including interstitial chronic inflammatory cell infiltrates, tubular damage, or minor alterations in arterioles, could not be correlated with the type of original host renal disease, the severity of arteriolar disease in resected host kidneys, or the presence of malignant arteriolar nephrosclerosis in the host. Also, the presence of ultrastructural and immunohistologic alterations suggestive of "recurrence" in the graft of something resembling glomerular lesions in the host kidneys in patients with chronic glomerulonephritis did not correlate with the severity or extent of concomitant rejection reactions in the respective recipients' grafts. It is difficult to determine whether the ultrastructural electron-dense deposits in or adjacent to glomerular mesangial regions (most strikingly noted in the grafts of 3 of 9 patients whose original disease was chronic glomerulonephritis) bore direct relationship to the similar, but obviously more marked and advanced changes in the respective host kidneys. However, in 2 of these 3 cases, the resemblance of the electron-dense glomerular deposits in the grafts to those changes in the host kidney and the association of the deposits with the most extensive mesangial distribution of localized antibody globulins was sufficient to suggest early stages in the development of host-type "chronic" glomerular lesions.

The patients with chronic pyelonephritis, arteriolar nephrosclerosis or chronic lobular glomerulonephritis failed to develop ultrastructurally electron-dense deposits or immunohistologic glomerular alterations described in patients with chronic glomerulonephritis. This suggests some specificity of the relationship of mesangial electron-dense deposits in grafts with chronic glomerulonephritis in host kidneys. In this regard, it is also of particular interest and possible significance that the graft in the patient with chronic pyelonephritis did not develop anything resembling the few isolated electron-dense glomerular

deposits identified in the diseased host kidney. In the patient with chronic lobular glomerulonephritis, there was obvious correlation of light, immunofluorescence, and ultrastructural microscopic glomerular alterations in the first graft with those in the host kidney; here the glomerular lesions in the graft evolved over about 18 months and progressed rapidly in the last two months before death, being nearly identical to the progression of host glomerular lesions over the month immediately preceeding transplantation.

The nature of the loosely granular, irregular subendothelial glomerular capillary deposits in the graft from LD 18, who had chronic glomerulonephritis and severe arteriolar nephrosclerosis, is not clear. This particular alteration, otherwise infrequently observed in this study, may represent either irregular formation in the graft of abnormal basement membrane material in the lamina rara interna region of glomerular capillary walls or deposition of plasma proteins underneath the endothelial cells, irregularly elevating portions of overlying endothelial cells. In at least one case, not included in this study since the host and donor were not HL-A identical, similar but more extensive subendothelial deposits were seen in both the host and donor kidneys.

It should be noted that arterial and arteriolar nephrosclerotic lesions and malignant necrotizing arteriolar lesions noted in certain host kidneys were not seen in the respective renal grafts, in spite of the fact that the grafts had been in place for from 10 to 47 months (arterial nephrosclerosis) and 14 months (malignant arterial nephrosclerosis). This is in contrast to previous unpublished experience (DTR and PMB) showing that renal grafts placed in patients with malignant necrotizing arteriolitis in their own kidneys may develop similar necrotizing arteriolar changes in the graft when the diseased host kidneys are not resected at the time of transplantation. Bilateral nephrectomies had been performed in each of the HL-A identical recipients at the time of transplantation.

Comparative Analysis of Results of the Present Study with Those of Others

The classic pathologic picture of immunologic rejection in first-set allografts in unsensitized individuals is an inflammatory cellular response with lymphocytes and plasma cells surrounding tubules, blood vessels, and glomeruli.[1–4] More recently, early changes of endothelial vascular injury have been recognized and it has been shown that rejection can occur in the face of extensive immunosuppression.[1–4,18,19] It has also been emphasized that severe arteriolar vascular changes, consisting of thickening, splitting and reduplication of the internal elastica, are part of the

long-term effects of graft rejection.[1-4,20] It has been suggested that the chronic vascular changes reflect the later results of earlier more acute lesions such as fibrinoid necrosis.[1-4,6,20] Although this course of events is reasonable, direct proof is not entirely satisfactory. There have been few immunofluorescent studies of renal grafts and only occasionally have deposits of immunoglobulins been observed within the walls of acutely damaged vessels of human allografts.[19,21] Porter *et al*[20] have, however, recognized acute necrotizing lesions in as many as 35% of canine allografts biopsied during graft rejection.

Our studies suggest that minor histocompatibility differences between HL-A identical donor and recipient may be sufficient to lead to some structural changes similar to certain of those seen in frank graft rejection as described above. However, it should be emphasized that the alterations observed in renal grafts in the present study were never as great as has been noted in allografts[2] or xenografts in other studies[18] and that our patients exhibited minimal or no clinical evidence of graft rejection or loss of function.

The cellular exudates observed in our patients are qualitatively like those seen in more classic instances of graft rejection.[1-4,9,18] They are made up of lymphocytes and plasma cells with occasional eosinophils and macrophages. These cellular aggregates are frequently associated with disrupted peritubular capillaries and are often immediately adjacent to damaged cells and/or basement membranes of proximal renal tubules. As in instances of more obvious graft rejection, these cellular aggregates lie adjacent to vessels and glomeruli as well as tubules but invasion of arteriolar walls or Bowman's capsule is uncommon.

The most striking difference between these grafts and those reported in studies by others is the rarity of vascular lesions in our patients. It has been reported[2, 22] that many of the kidneys examined during rejection show some degree of fibrinoid necrosis within vessel walls and that as many as 70% of grafts have evidence of more chronic changes, such as splitting or fragmentation of the internal elastica. A similar association between rejection and vascular damage was reported in canine renal allografts.[20, 21] The patients studied here showed neither frank fibrinoid necrosis nor significant alterations of vascular elastica, but some had smudged or vacuolated media in occasional renal arterioles. As might be expected, we also found infrequent localization of antibody within vessel walls. These findings of relatively mild cellular infiltrates and vascular injury in renal allografts with HL-A matching support our contention that even in the face of minimal immunosuppression the minor histocompatibility differences have only minor

significance in inducing histologic alterations compatible with graft rejection. Final assessment of the degree of renal damage that may result from minor differences in histocompatibility can, of course, not be made until successive biopsies are carried out in these patients. In the one instance where we had a second renal biopsy 17 months after the first (LD 23), there was increased evidence of a cellular inflammatory response to the graft. However, even in this case morphologic changes were less severe than those seen in frank graft rejection, and there were none of the vascular stigmata of chronic graft rejection. In addition, the patient has continued to maintain excellent renal function.

There is considerable evidence to indicate that glomeruli are frequently altered in either renal isografts [7, 23] or renal allografts.[5–8] Recurrence of host disease has, in fact, now become one of the more frequent complications of isografts.[8, 23] The type, incidence and significance of glomerular lesions in renal allografts are less well established. Hamburger *et al* [5] first emphasized the frequent occurrence of glomerular changes in allografts and Porter *et al* [6, 7] have more recently examined a number of these changes in detail. The latter authors reported that 76% of renal allografts had altered glomerular capillary basement membranes associated with subendothelial electron-dense deposits. Ninety percent of the glomeruli showing these structural changes had IgM immunoglobulins in a linear or finely granular distribution. This deposition was often associated with fixed complement and less often with deposits of IgG immunoglobulins and fibrinogen. It was thought that these changes did not represent recurrence of host disease but rather that they resulted from host antibodies reacting with glomerular basement membranes of the graft. In 3 other cases,[6, 7] it was felt that subepithelial deposits in glomeruli were more suggestive of actual recurrence of host disease. Dixon *et al* [8] have also investigated the disease processes in renal allografts with particular attention being given to the relationships between pathogenetic mechanisms in host and graft kidneys. Of 13 patients in whom the original disease was an antiglomerular basement membrane nephritis, seven had apparent recurrence of the original host disease in the renal graft. Of 26 patients in whom the host disease was caused by antigen-antibody complexes, only six developed apparent recurrent disease in their renal grafts.

It has been suggested that in clinical situations where isografts or allografts are matched to the recipients with respect to HL-A tissue antigens, the low level of immunosuppression used may, in fact, increase the chance of developing recurrence of host renal disease in the

renal graft.[8] In this regard, it is of interest that Porter *et al*[6] found the lesser degrees of glomerular change in grafts of patients judged retrospectively to be most nearly matched in histocompatibility. However, there is no indication as to whether or not this affected the dosage of immunosuppressive agents. These drugs are frequently used in the treatment of glomerulonephritis and might, in themselves, suppress the recurrence of host disease.

The patients included in our study showed less change than might have been anticipated from the observations of others. It may be important in this regard that none of the recipients in the present study showed the morphologic features characteristic of glomerulonephritis due to antiglomerular basement membrane antibody.[24] Similarly, none of the renal allografts examined by us showed changes consistent with this form of nephritis. Six of the grafts had glomerular EDD of variable extent. In two of these (LD 2 and 16) the deposits were rather striking, and in one of these two cases (LD 2), there was ultrastructural histologic suggestion of a chronic-type glomerulonephritis beginning to recur in the graft.

One other patient (LD 6) was of particular interest in that his first graft showed clear evidence of recurrent chronic lobular glomerulonephritis. Porter *et al*[7] had reported a similar case. However, it is of interest that one of the published electron micrographs from their case shows subepithelial electron-dense deposits more compatible with nephritis induced by antigen-antibody complex. This further suggests that the glomerular lesions observed in renal allografts may, in fact, be more complex pathogenetically than simply a reinstitution of the previously existing host renal disease. Histopathologc alterations in renal homografts may be the singular or combined result of recurrence of original host disease, transplantation rejection, mechanical and infectious processes related to surgical procedures and therapy, or institution of renal disease due to new immunopathogenetic "hypersensitivity" mechanisms independent of original host disease and transplant rejection reactions.

Summary and Conclusions

Twelve recipients of renal allografts from HL-A identical donors were studied to determine the degree of rejection caused by minor histocompatibility antigens in humans and the relationships between changes in the renal allografts and their host disease. Biopsies of these renal allografts were made 2–42 months after transplantation.

In none of the cases was graft rejection sufficient to cause loss of

the graft nor were the histologic alterations as extensive as those noted in other series. Eleven of the cases showed some degree of lymphoid cellular infiltrate but eight of these biopsies were only mildly involved. Even in the 2 cases in which the most extensive changes could be seen, there was no apparent loss of renal function. That minor histocompatibility differences might lead to increasing damage is suggested by the single graft recipient in whom a second biopsy was made 17 months after the first biopsy. Minor vascular alterations were evident in 5 cases but there was no evidence of acute fibrinoid necrosis and none of the vascular changes ordinarily associated with long-term graft rejection.

The host diseases were chronic glomerulonephritis (9 cases), chronic lobular glomerulonephritis (1 case), chronic pyelonephritis (1 case) and arteriolarnephrosclerosis (1 case). Five of the patients had fibrinoid arteriolar necrosis in the host kidneys. Eleven of the 12 HL-A identical grafts displayed changes in glomeruli. For the most part these were of minor degree, consisting of fusion of epithelial foot processes, electron-dense membrane deposits, and increase in mesangial cells and matrix. The significance of these changes in terms of recurrence of host disease is not known. There were 2 patients with changes in the grafts similar to those of the host. One was a patient whose original disease was chronic lobular glomerulonephritis and who had frank recurrence of his host disease within 18 months of transplantation. The host disease in the second patient was chronic glomerulonephritis. A biopsy of his graft 47 months after transplantation showed focal lesions that were highly suggestive of recurrent host disease.

This study suggests that genotyping for major histocompatibility differences is important in that it permits successful maintenance of HL-A identical human allografts for long periods of time with low levels of immunosuppression.

References

1. Porter KA: Morphological aspects of renal homograft rejection. Brit Med Bull 21:171–175, 1965
2. *Idem:* Renal transplantation, Pathology of the Kidney, Edited by RH Heptinstall. Boston, Little, Brown, and Co, 1966, pp 601–643
3. Dammin GJ: The pathology of human renal transplantation, Human Transplantation. Edited by FT Rapaport, J Dausset. New York, Grune and Stratton, Inc, 1968, pp 170–199
4. Rowlands DT Jr, Bossen EH: Immunological mechanisms of allograft rejection. Arch Intern Med 123:491–500, 1969

5. Hamburger J, Crosnier J, Dormont J: Observations in patients with a well tolerated homotransplanted kidney: possibility of a new secondary disease. Ann NY Acad Sci 120:558–577, 1964

6. Porter KA, Dossetor JB, Marchioro TL, Peart WS, Rendall JM, Starzl TE, Terasaki PI: Human renal transplants. I. Glomerular changes. Lab Invest 16:153–181, 1967

7. Porter KA, Andres GA, Calder MW, Dossetor JB, Hsu KC, Rendell JM, Seegal BC, Starzl TE: Human renal transplantation. II. Immunofluorescent and immunoferritin studies. Lab Invest 18:159–171, 1968

8. Dixon FJ, McPhaul JJ Jr, Lerner R: Recurrence of glomerulonephritis in the transplanted kidneys. Arch Intern Med 123:554–557, 1969

9. Porter KA: Pathological changes in transplanted kidneys, Experience in Renal Transplantation. Edited by TE Starzl. WB Saunders Co, Philadelphia, 1964, pp 299–359

10. Amos B: Immunologic factors in organ transplantation. Amer J Med 44: 767–775, 1968

11. Stickel DL, Seigler HF, Amos DB, Ward FE, Price AR, Anderson EE: Immunogenetics of consanguinous allografts in man: II. Correlation of renal allografting with HL-A genotyping. Ann Surg (in press)

12. Burkholder PM: Immunohistopathologic study of localized plasma proteins and fixation of guinea pig complement in renal lesions of diabetic glomerulosclerosis. Diabetes 14:755–770, 1965

13. Marshall JD, Eveland WC, Smith CW: Superiority of fluorescein isothiocyanate (Riggs) for fluorescent-antibody technique with a modification of its application. Proc Soc Exp Biol Med 98:898–900, 1958

14. Goldman M: Fluorescent Antibody Methods. New York, Academic Press, Inc, 1968

15. Burkholder PM, Bradford WD: Proliferative glomerulonephritis in children. A correlation of varied clinical and pathologic patterns utilizing light, immunofluorescence, and electron microscopy. Amer J Path 56:423–467, 1969

16. Fennell RH Jr, Reddy CRRM, Vazquez JJ: Progressive systemic sclerosis and malignant hypertension. Immunohistochemical study of renal lesions. Arch Path 72:209–215, 1961

17. Burkholder PM: Malignant nephrosclerosis. An immunohistopathologic study on localized gamma-globulin and fixation of guinea pig complement in human kidneys. Arch Path 80:583–589, 1965

18. Rowlands DT Jr, Kirkpatrick CH, Vatter AE, Wilson WEC: Immunologic studies in human organ transplantation. IV. Serologic and pathologic studies following heterotransplantation of the kidney. Amer J Path 50:605–622, 1967

19. Najarian JS, Foker JE: Interpretations of transplanted kidney morphology. Transplantation Proc 1:1022–1031, 1969

20. Porter KA, Calne RY, Zukoski CF: Vascular and other changes in 200 canine renal homotransplants treated with immunosuppressive drugs. Lab Invest 13:810–824, 1964

21. Horowitz RE, Burrows L, Paronetto F, Dreiling D, Kark AE, Popper H: Immunologic observations on homografts. II. The canine kidney. Transplantation 3:318–325, 1965

22. Porter KA, Rendall JM, Stolinski C, Terasaki PI, Marchioro TL, Starzl TE: Light and microscopic study of biopsies from 33 human renal allografts and an isograft 1¾–2½ years after transplantation. Ann NY Acad Sci 129:615–636, 1966

23. Glassock RJ et al: Human renal isografts: a clinical and pathologic analysis. Medicine 47:411–454, 1968

24. Dixon FJ: The pathogenesis of glomerulonephritis. Amer J Med 44:493–498, 1968

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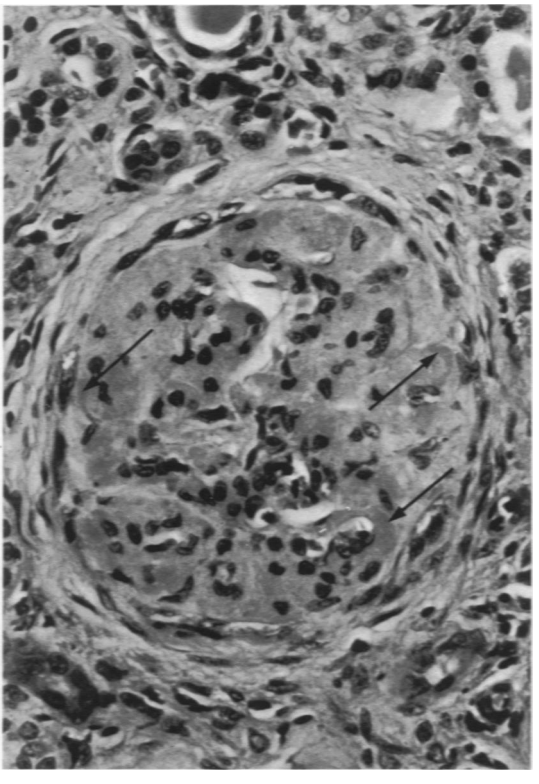
Legends for Figures

Fig 1–4 are all of LD 2 host kidney.

Fig 1.—Host kidney from LD 2. Glomerulus with residual cellularity, collapse of capillary loops, thickening of mesangial regions, local hypereosinophilic deposits *(arrows)* in thickened capillary walls, and fibrous thickening of Bowman's capsule. H&E. × 270.

Fig 2.—Glomerulus with local globular deposits of host antibody globulin in mesangial regions and in some thickened capillary walls probably corresponding with hyaline capillary sites in Fig 1 and the electron-dense deposits in Fig 3. Fluorescent anti-human 7S immunoglobulins. × 390.

Fig 3–4.—Glomerular capillary walls are very thick by virtue of thickened basement membrane *(bm)*, electron-dense subendothelial and intramembranous dense deposits *(d)* and enlarged endothelial cells, which have reduced capillary lumens to size of red blood cells *(rbc)*. Mesangial cells *(me)*, epithelial cell *(ep)*. Electron micrographs. **Fig 3,** × 3800. **Fig 4,** 3900.



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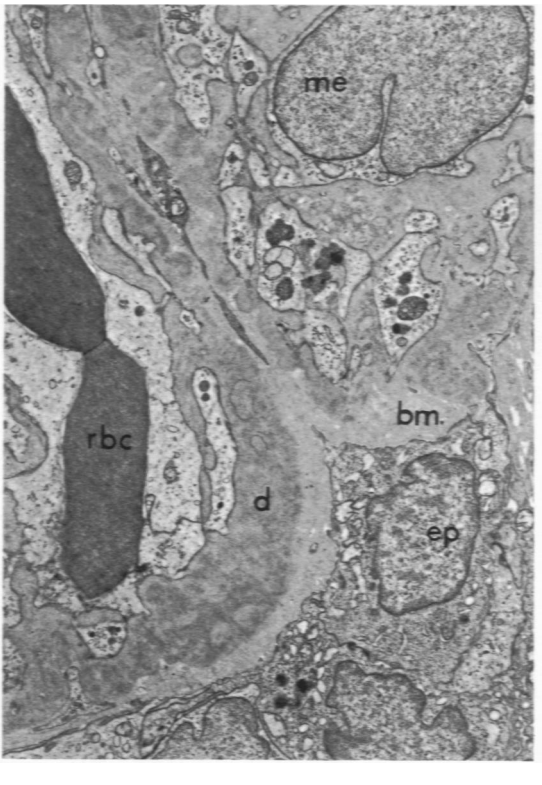

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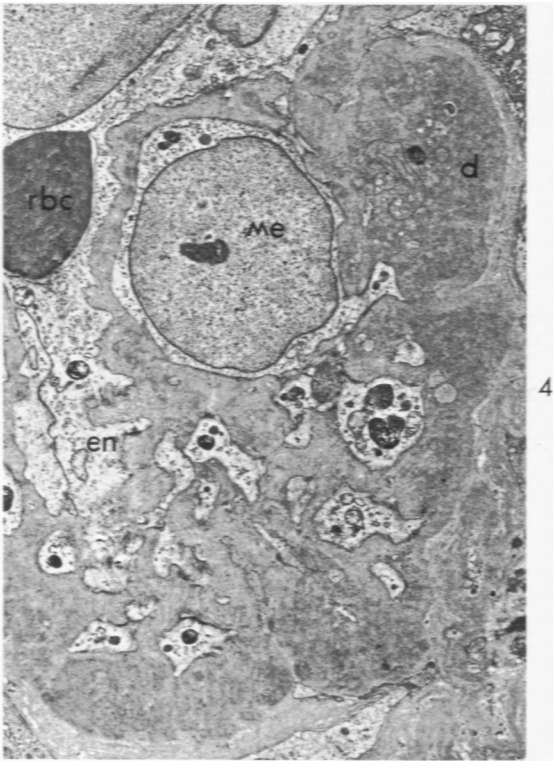

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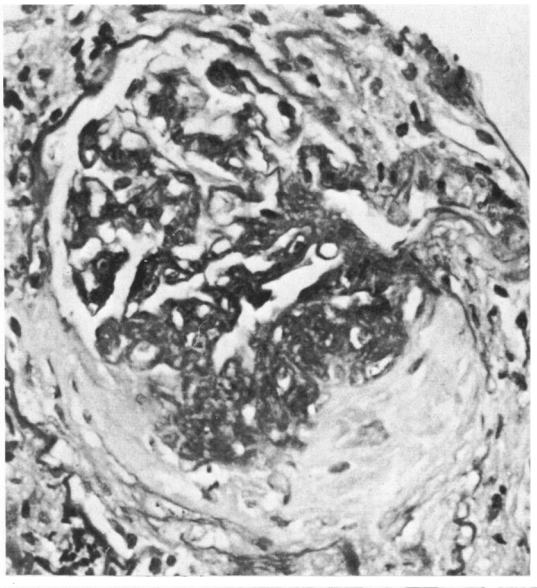
Fig 5–8 are all of LD 2 allograft kidney.

Fig. 5.—Allograft kidney from LD 2. This is one of two glomeruli in this graft showing local sclerotic collapse of portion of tuft adherent to adjacent greatly thickened Bowman's capsule; otherwise slight mesangial thickening is present. H&E. × 330.

Fig 6.—Very local globular or elongate deposits of antibody globulin (possibly corresponding with electron-dense deposits in Fig 8) in capillary walls or mesangium of glomerulus. Fluorescent anti-human 7S immunoglobulins. × 185.

Fig 7.—Local slight-to-moderate thickening of mesangial areas in a more representative glomerulus. H&E., × 190.

Fig 8.—Electron-dense deposits in paramesangial and mesangial basement membrane *(bm)* of glomerulus. Mesangial cell *(me)*, endothelial cell *(en)*, epithelial cell *(ep)*. Electron micrograph. × 8300.



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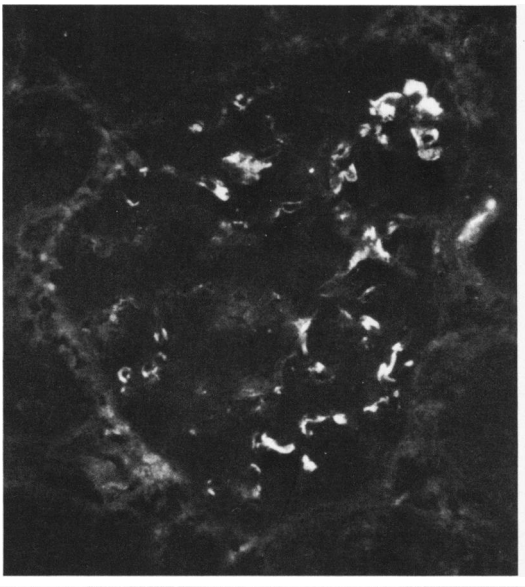

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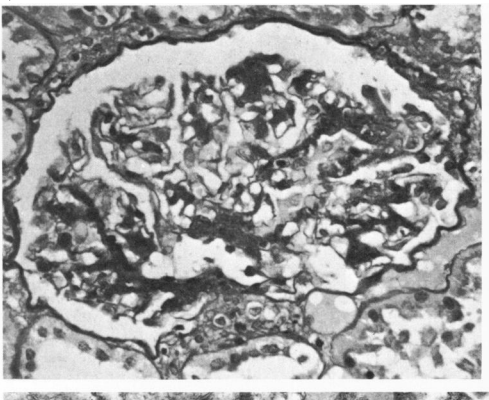

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Fig 9 and 10 are of LD2 allograft kidney.

Fig 9.—Several collections of "virus-like" particles in space between epithelial cell *(ep)* and glomerular capillary basement membrane *(bm)*; endothelial cell *(en)*. Electron micrograph. × 10,600.

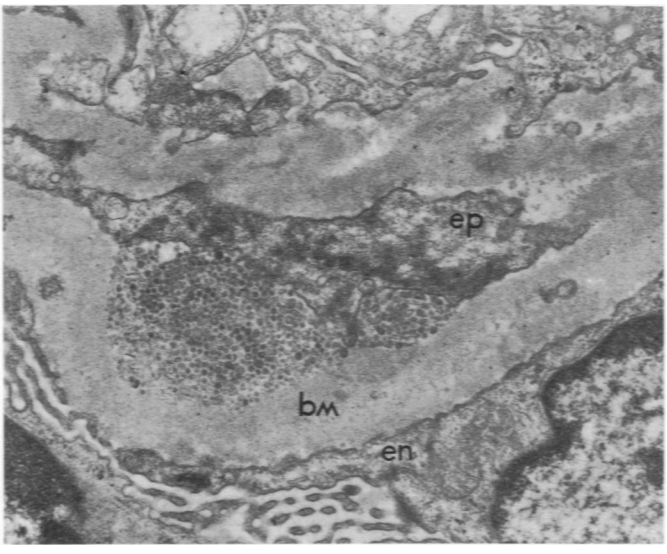
Fig 10.—Higher magnification of "virus-like" particles in another area similar to that in Fig 9. Double membrane envelopes can be discerned around most of particles; a few free membrane fragments are also seen *(double arrows)*. Electron micrograph. × 71,000.

Fig 11–13 are of LD 3 host kidney.

Fig 11.—Host kidney from LD 3; Glomerulus shows residual cellularity, thickening of capillary walls and mesangium, and some "lipid"-laden foamy capsular epithelial cells. H&E. × 260.

Fig 12.—Glomerulus shows fairly diffuse, lumpy, coarsely granular distribution of antibody globulin along capillary walls and in mesangium. Fluorescent anti-human 7S immunoglobulins. × 135.

Fig 13.—Many irregular and coalescent dense deposits *(d)*, possibly corresponding with deposits of antibody globulin in Fig 12, are seen in greatly thickened glomerular capillary basement membrane *(me)* and mesangial membrane matrix. Mesangial cell *(me)*, epithelial cell *(ep)*. Electron micrograph. × 4600.



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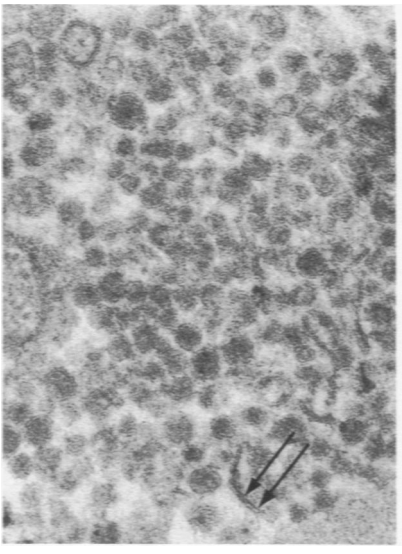

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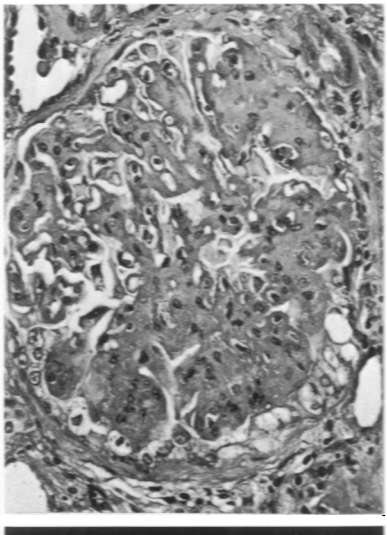

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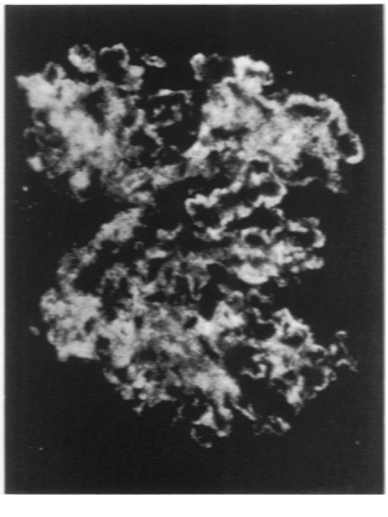

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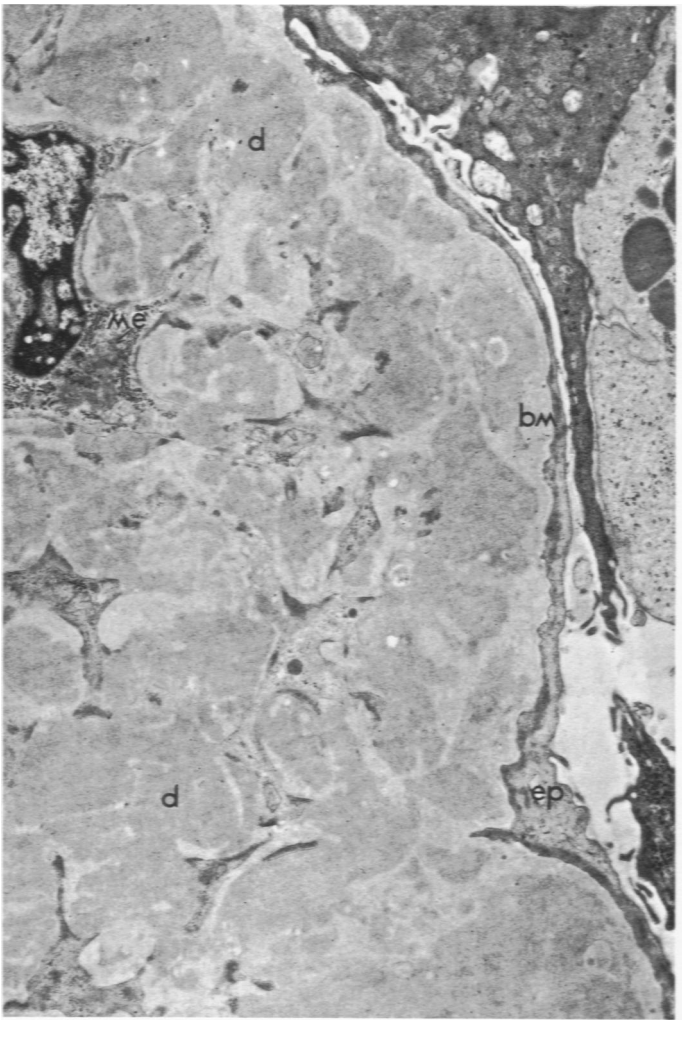

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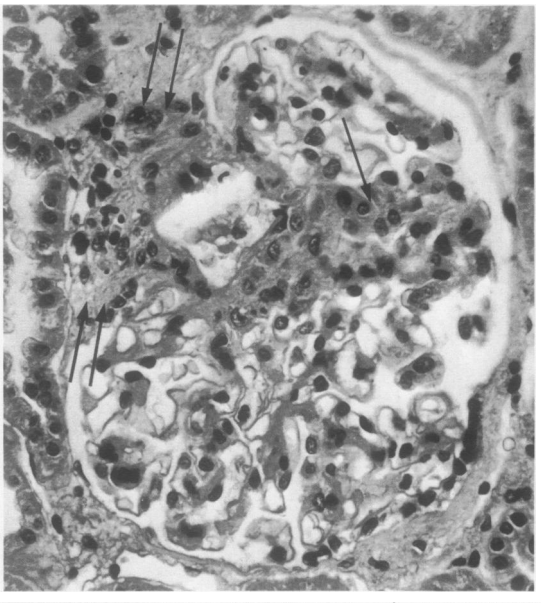

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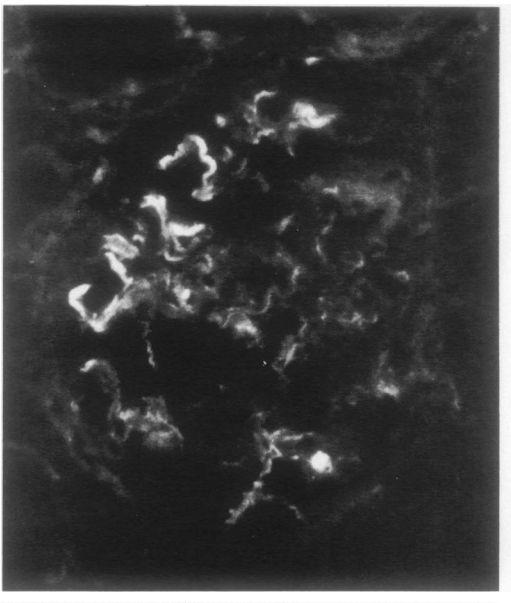

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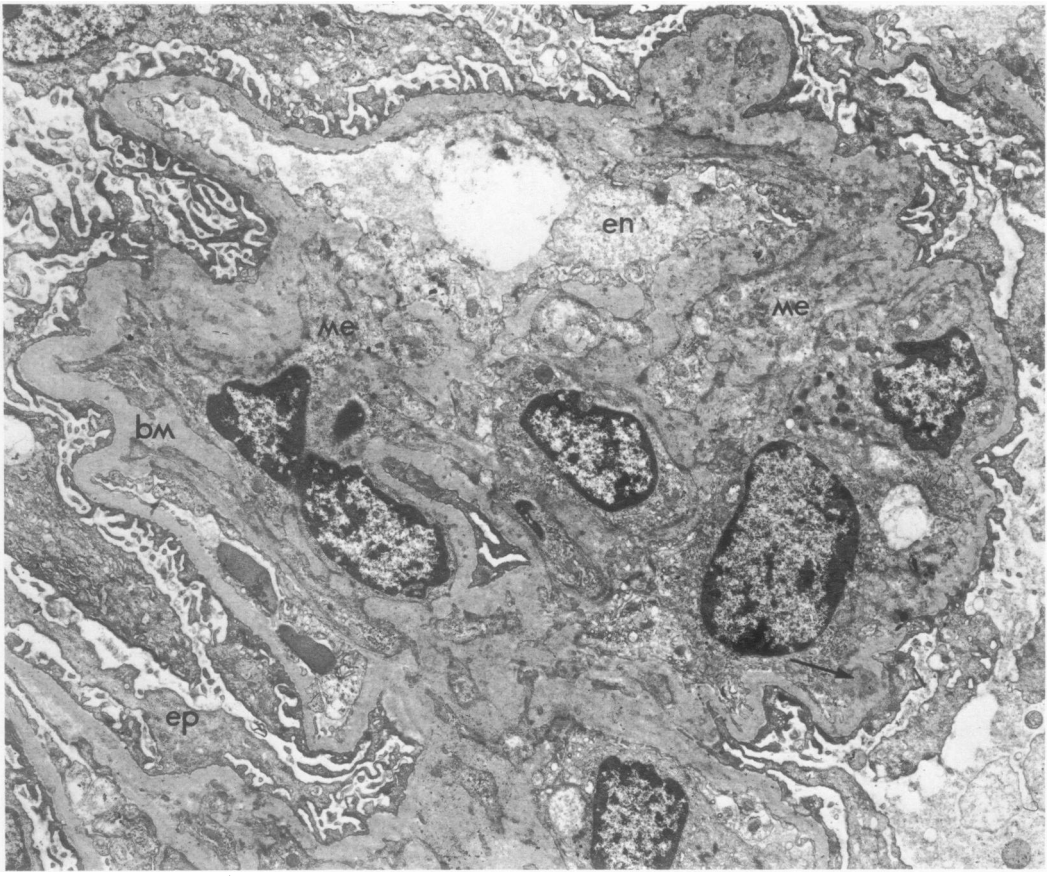
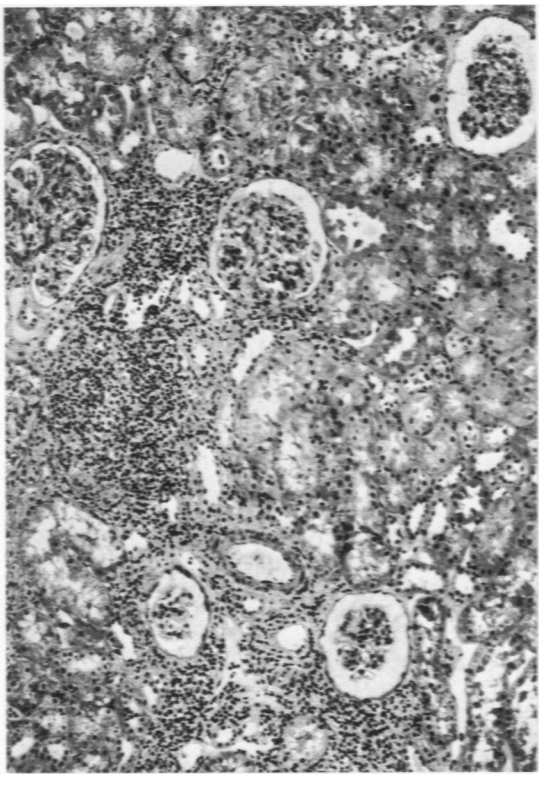


Fig 14–16 are all of LD 3 allograft kidney. **Fig 14.**—Juxtaglomerular apparatus *(outlined by two pairs of arrows)* of glomerulus is slightly enlarged and mesangium is locally thickened *(single arrow)* particularly in region of glomerular axis. H&E stain. × 320. **Fig 15.**—Very local globular deposits of IgM in segment of glomerular capillary walls and mesangium. Fluorescent anti-human IgM. × 240. **Fig 16.**—Mesangium of glomerular capillary loop is greatly expanded by increased number of mesangial cells *(me)* intermixed with basement membrane *(bm)* matrix, tiny intramembranous electron-dense deposits *(arrow)*. Epithelial cell *(ep)*. Electron micrograph. × 4000.



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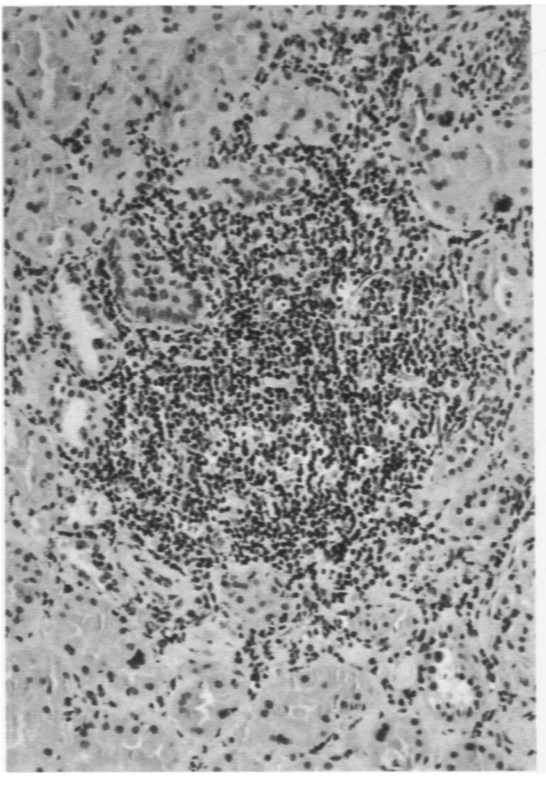

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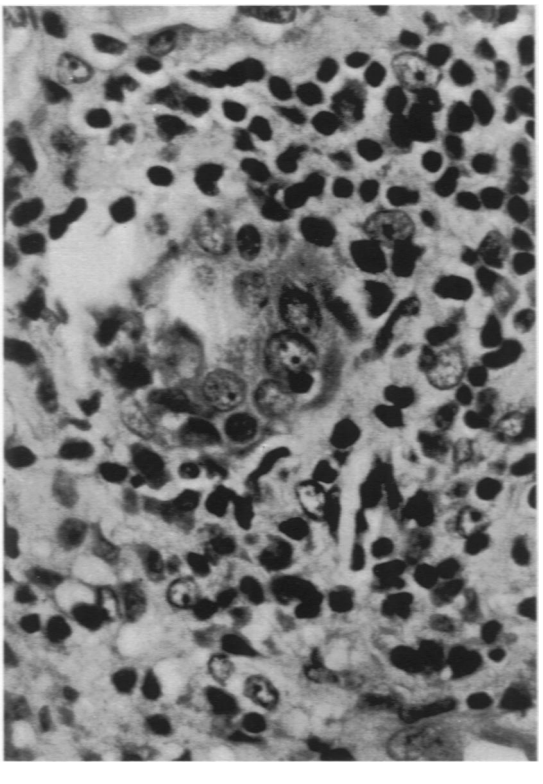

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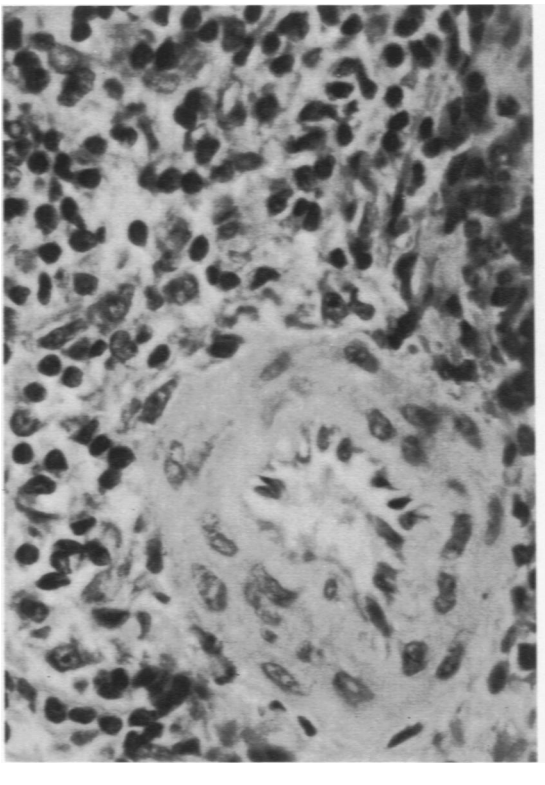

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Fig 17.—Allograft kidney from LD 13. Local infiltrates of chronic inflammatory cells (lymphoid, plasmacytic, and monocytic cells) in interstitium around tubules and adjacent to arterioles and glomeruli in moderate degree of rejection reaction. H&E. × 95. **Fig 18.**—Allograft kidney from LD 36. Atypical "nodular" chronic inflammatory infiltrate of interstitium with varying degrees of tubular destruction. H&E. × 165. **Fig 19.**—Allograft kidney from LD 23. Mononuclear cell infiltrate around, invading and damaging convoluted tubule. H&E. × 660. **Fig 20.**—Allograft from LD 13. Mononuclear chronic inflammatory infiltrate surrounds arteriole, which shows only slight "smudging" of its media. H&E. × 660.

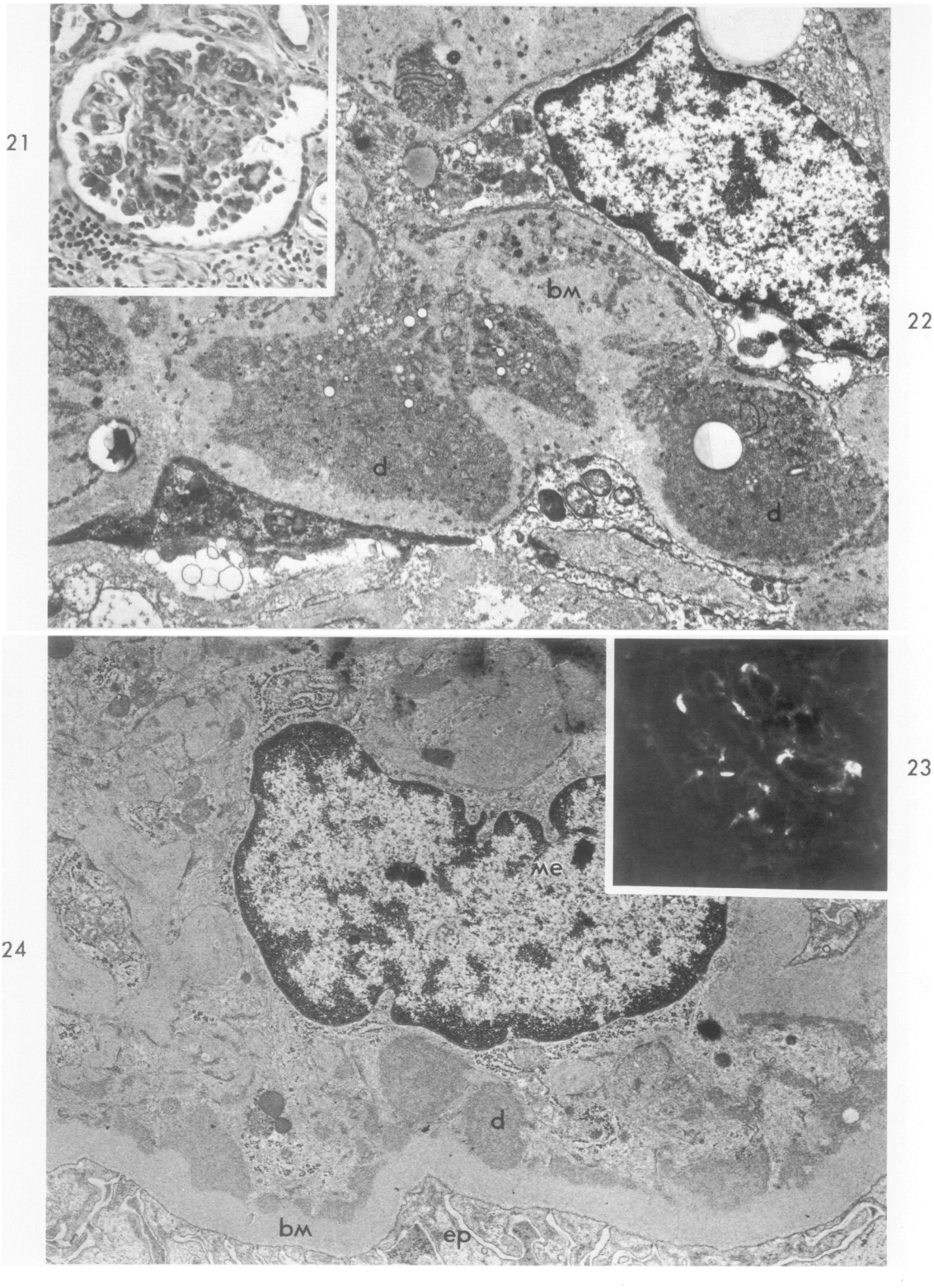
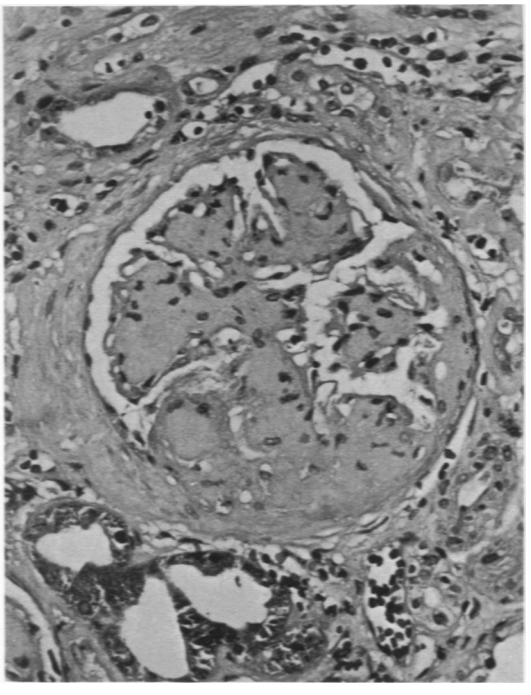


Fig 21.—Host kidney from LD 16. One of least diseased glomeruli in this biopsy shows extensive mesangial thickening, residual increased cellularity and local marked thickening of capillary walls. H&E. × 140. **Fig 22.**—Host kidney from LD 16. Small segment of capillary wall in glomerulus more "sclerosed" than that in Fig 21 shows rather large dense intramembranous *(bm)* deposits *(d)* containing fragments of cytoplasmic and cell membrane structures. Electron micrograph. × 7300. **Fig 23.**—Allograft kidney from LD 16. Very local globular deposits of IgA immunoglobulin in glomerular mesangial regions and capillary walls. Fluorescent anti-IgA. × 140. **Fig 24.**—Allograft kidney from LD 16. Several dense deposits (*d*), possibly corresponding with localized antibody globulins as shown in Fig 23, are located beneath glomerular capillary basement membrane (*bm*) in paramesangial location. Mesangial cell *(me)*, epithelial *(ep)* foot processes. Electron micrograph. × 8000.



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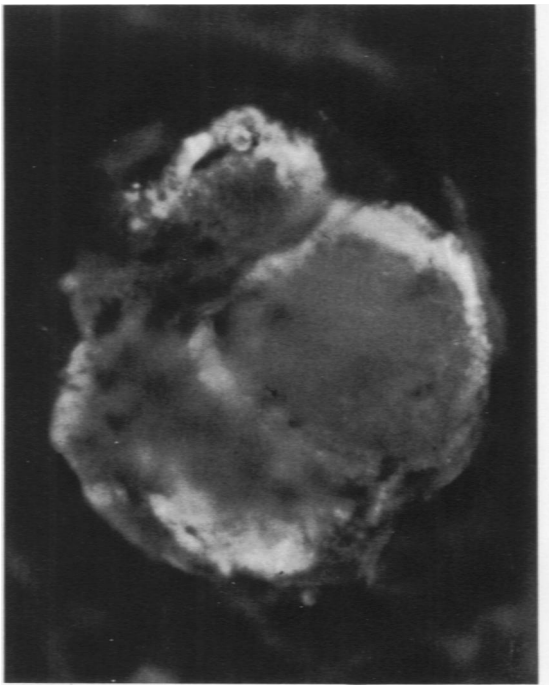

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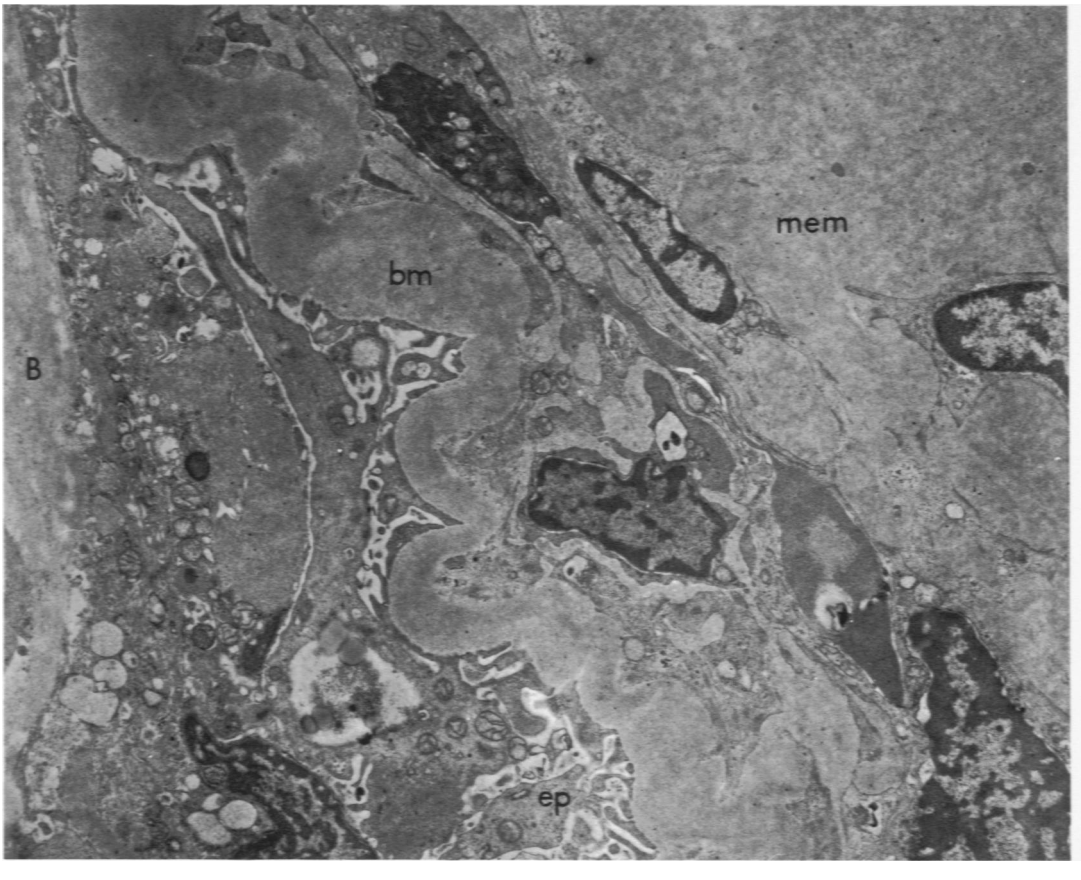

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Fig 25–27 are all of LD 6 host kidney. **Fig 25.**—Glomerulus with marked "nodular" expansion of mesangial region by noncellular membrane matrix resulting in lobular pattern, fibrous thickening of Bowman's capsule with local adhesion to several glomerular capillary loops. H&E. × 185. **Fig 26.**—Glomerulus with coalescent coarse granular deposits of antibody globulins in portions of peripheral capillary walls; the nodular mesangial regions are essentially free of such deposits. Fluorescent anti-human 7S immunoglobulins. × 300. **Fig 27.**—Intermittent thickening and waviness of mottled basement membrane *(bm)* of peripheral portion of glomerular capillary, marked thickening of mesangial region by fused membrane matrix *(mem)*. Epithelial cell *(ep)*, Bowman's glomerular capsule *(B)*. Electron micrograph. × 5900.

Fig 28–31 are all of first LD 6 allograft kidney.

Fig 28.—First allograft kidney from LD 6. Nodular thickening of glomerular mesangial regions by membrane matrix and mesangial cells, resulting in lobular pattern similar to, but more cellular than, that seen in original host disease (Fig 25). H&E. × 160.

Fig 29.—Glomerular capillary shows waviness and local electron-dense mottling of thickened basement membrane *(bm)*; mesangium again is thickened by fused membrane matrix *(mem)* with more mesangial cellular elements than in original host disease (Fig 27). Electron micrograph. × 6200.

Fig 30.—Local deposits of IgM immunoglobulin in limited segments of peripheral capillary walls. Fluorescent antihuman IgM. × 120.

Fig 31.—Mixed cellular inflammatory infiltrate consisting of neutrophilic leukocytes *(poly)* and mononuclear lymphoid cells *(ly)* is in intimate contact with thickened, mottled tubular basement membrane. Tubular epithelial cells *(ep)*, interstitial bundles of collagen *(c)*. Electron micrograph. × 3700.

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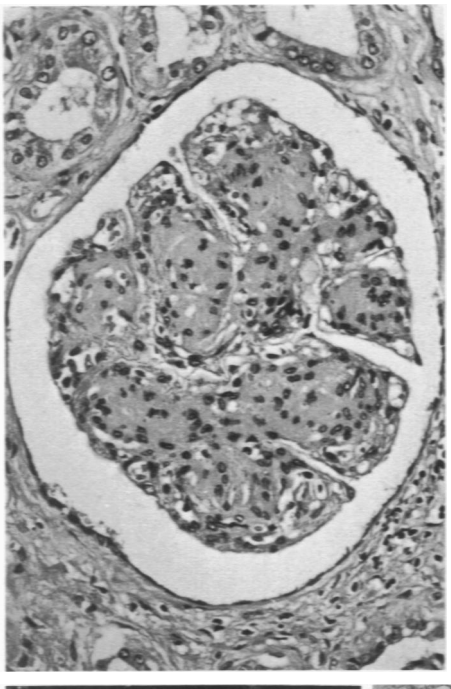

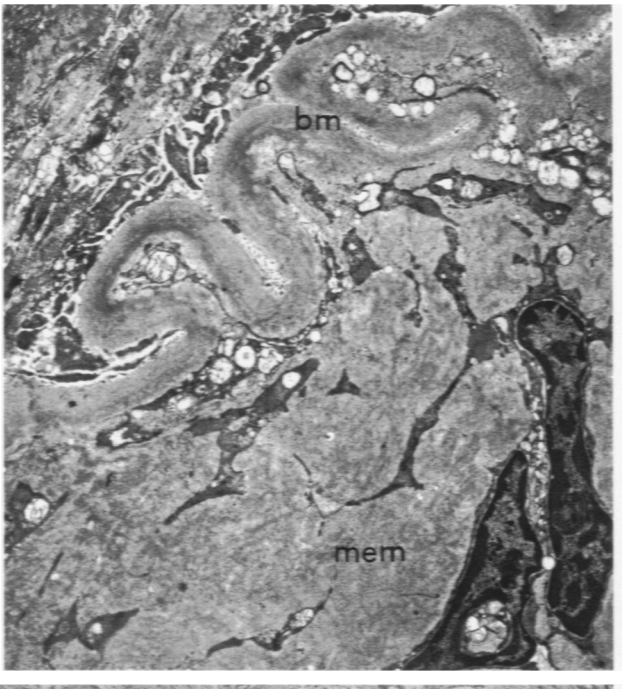

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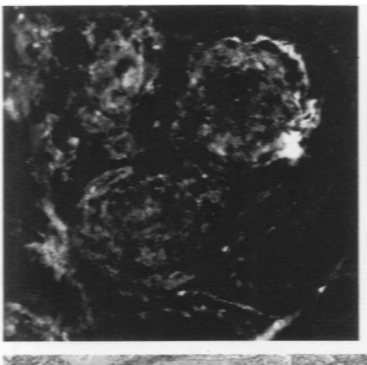

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[*End of Article*]