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LIGHT AND ELECTRON MICROSCOPIC STUDY OF BIOPSIES FROM THIRTY-THREE HUMAN RENAL ALLOGRAFTS AND AN ISOGRAFT $1\frac{3}{4}$-$2\frac{1}{2}$ YEARS AFTER TRANSPLANTATION*

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In the period between November 1962 and March 1964 at the University of Colorado Medical Center, 64 patients received renal allografts and two received renal isografts from living donors. The identical twins and 34 of the other patients are still alive (Starzl *et al.*, 1966).

Of those that died, 27 did so in the first year and three in the second year after renal transplantation. The pathological changes found in the renal allografts from 29 of these dead patients have already been published (Porter *et al.*, 1965; Starzl *et al.*, 1966).

The present report is a preliminary account of the light and electron microscopic changes found in biopsies obtained about two years after transplantation from 33 of the 34 surviving renal allografts and from one of the two isografts. The histopathological findings in each transplant are correlated with the relationship of donor to recipient, with the degree of compatibility of donor and recipient as shown by lymphocyte typing, with the number of clinical rejection episodes experienced by the patient, and with renal function.

MATERIALS AND METHODS

Patients. Each case is denoted by a number prefixed by the letters LD, for the allograft recipients and IDT, for the identical twin. This same code has been used previously (Starzl, 1964; Starzl *et al.*, 1965; Starzl *et al.*, 1966) and further details of any of the patients can be obtained by referring to these publications.

In all but two of the patients, splenectomy and bilateral nephrectomy were performed at or before the time of transplantation. The exceptions were LD2 who retained his right kidney and IDT2 in whom splenectomy was unnecessary. The thymus was removed from 12 of the patients, four before, and eight between 250–520 days after transplantation.

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At the time of open biopsy, the identical twin was receiving no immunosuppressive or antihypertensive drugs. Of the 33 patients with renal allografts, all were being given daily azathioprine, 24 were receiving 2.5–25 mg per day of prednisone, and 15 were being treated with one or more of the antihypertensive drugs, chlorothiazide, hydralazine, reserpine and methylDOPA.

Control Kidney from a Donor. A 32-year-old man was killed in an accident two years and four months after donating a kidney to his brother (LD15). His remaining hypertrophied left kidney (weight 300g) was carefully examined and used as a control in this study.

Lymphocyte Typing. Blood lymphocytes obtained from the patients and from their donors were tested against a panel of 65–121 different cytotoxic antisera in the presence of rabbit complement (Terasaki *et al.*, 1965). Those antisera tending to act alike had been previously classed together by a computer factor analysis program into groups. Each individual's lymphocytes were then "typed" with respect to the presence or absence of seven major factors by regression analysis (Terasaki *et al.*, 1966a & b). Incompatibilities between donors and recipients were then noted.

Tissue Processing. Renal tissue for examination by light microscopy was fixed in ten per cent neutral formalin or formol-saline, embedded in paraffin

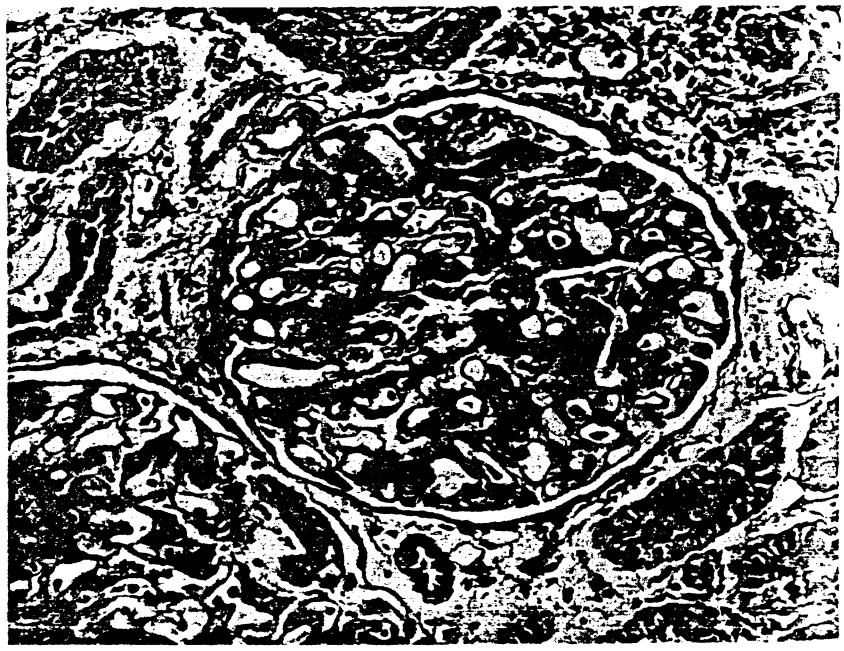

FIGURE 1. Biopsy of renal allograft one year and ten months after transplantation (LD54). PAS-positive material is deposited in the glomerular capillary walls and in the mesangium (PAS, × 340).

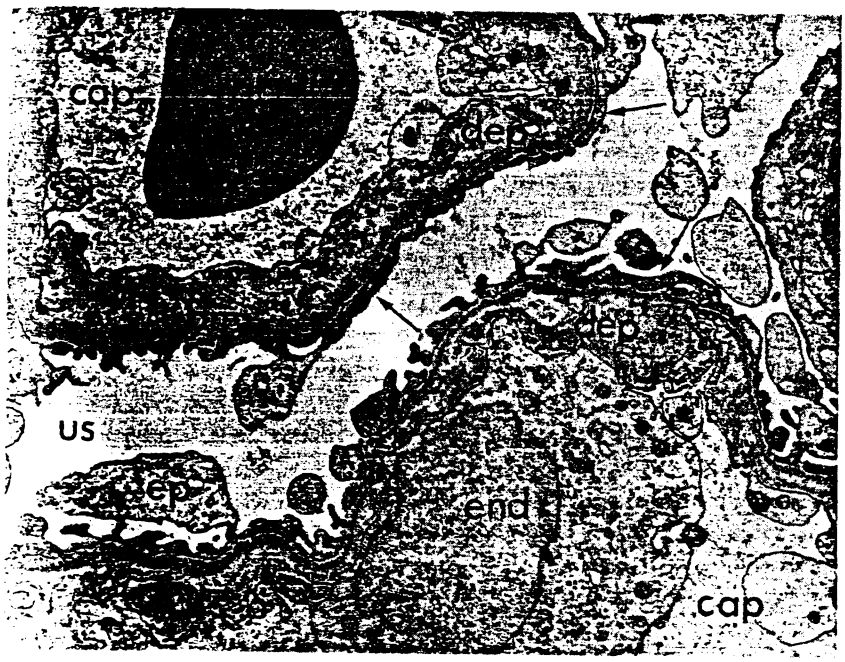

FIGURE 2. Biopsy of renal allograft one year and ten months after transplantation (LD41). There is a diffuse subendothelial accumulation of amorphous material (dep) on the basement membranes (bm) of two capillary loops. A few epithelial foot processes are fused (arrows). cap = capillary lumen. end = endothelial cell. ep = epithelial cell with foot processes. us = urinary space. rbc = erythrocyte. Electron micrograph. (Lead stain, × 6,320). (Compare with Fig. 5).

wax and serially sectioned. For electron microscopy, tissue was fixed in Palade's buffered osmium tetroxide solution and embedded in an epoxy resin, usually Epon 812, but sometimes Araldite. Thin sections, stained with phosphotungstic acid or lead, were examined in a Siemen's Elmiskop 1 electron microscope.

RESULTS

Histopathological Findings

Glomerular Changes

Lesions Resembling Glomerulonephritis. In 17 (51.5%) of the renal allografts the capillary walls of many or all of the glomeruli were thickened by material which stained positively with periodic-acid Schiff reagent (PAS). In some instances this was a diffuse change resembling Ellis type 2 (membranous) glomerulonephritis (FIGURE 1); more frequently, it was focal within a glomerular tuft and associated with large mesangial deposits of similar material producing a picture closely resembling lobular glomerulonephritis. These

changes were sometimes accompanied by localized areas of cellular proliferation; capsular crescents were rare.

Ultrastructurally, in all 17 cases, there were subendothelial accumulations of amorphous, finely granular material on the glomerular capillary basement membranes (FIGURE 2). There was often obliteration of the lamina interna rara by the deposits, but the lamina densa was generally normal (FIGURE 3). The density and compactness of the deposits varied: in several allografts, large areas were relatively electron translucent. The deposits pushed the endothelium lining the capillary loops inward and occasionally extended between adjacent endothelial cells. Fragments of endothelial cytoplasm were commonly incorporated in the subendothelial accumulations. In the majority of the renal allografts, the basement membrane thickening produced by these deposits was focal, while in others, it was diffuse. In many of the kidneys the mesangial matrix was greatly increased (FIGURE 4). In the most severely affected transplants, the endothelial cells were hypertrophic and hyperplastic; their cytoplasm was voluminous and frequently caused great narrowing or even obliteration of the capillary lumina. In all cases, there were areas in

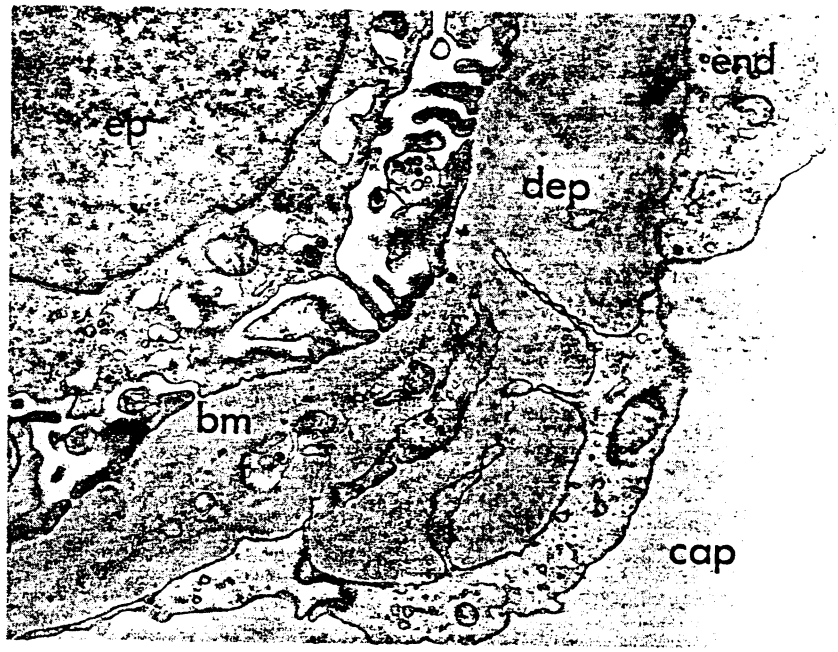

FIGURE 3. Biopsy of renal allograft two years and one and one-half months after transplantation (LD18). Segment of glomerular capillary wall in which the basement membrane (bm) is thickened by a subendothelial accumulation of fine granular material (dep). Endothelial cell fragments (f) are embedded in this amorphous deposit and the lamina interna rara is obliterated. cap = capillary lumen. end = endothelial cell. ep = epithelial cell with foot processes. Electron micrograph. (Lead stain, × 12,350).

the ischaemic areas of those kidneys with severe vascular narrowing. Glomerular hypertrophy was a feature of all the transplants.

Hyperplasia of the Juxta-Glomerular Apparatus

The juxta-glomerular bodies were enlarged in the renal isograft, in the control donor kidney and in 31 of the 33 renal allografts (FIGURE 6). In the majority this hyperplasia was equal in all zones of the kidney. Only in those cases with widespread narrowing of interlobular arteries and many ischaemic foci was the hyperplasia uneven, tending to be least in the atrophic areas. There was a great increase in the number of lacis cells.

Vascular Changes

Peritubular Capillaries. Even in areas of scarring, these fine vessels were intact though usually with thickened basement membranes. In many of the allografts, lymphocytes and monocytes were common in the lumina of the peritubular capillaries.

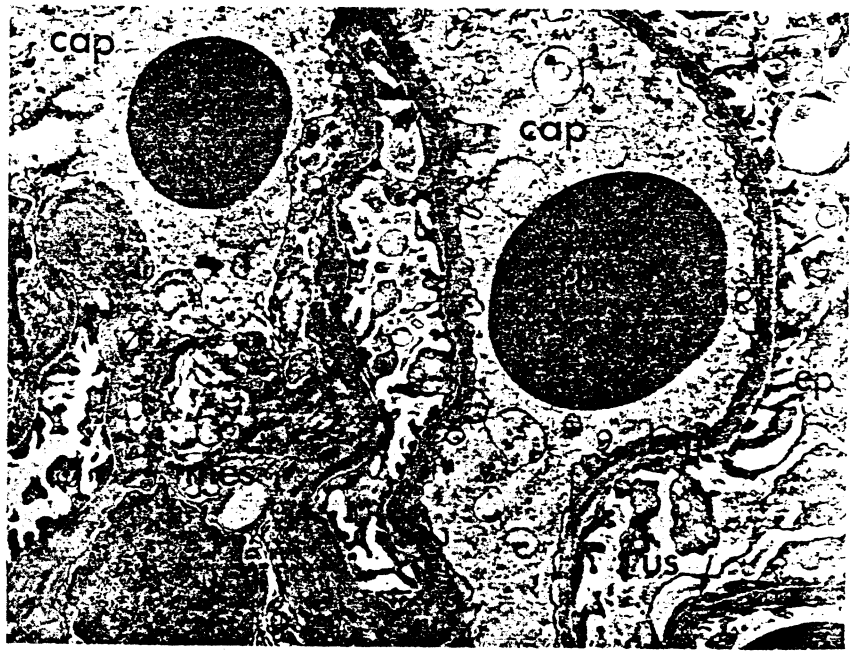

FIGURE 5. Biopsy of renal isograft two years and two and one-half months after transplantation (IDT2). Part of three glomerular capillary loops. A few epithelial foot processes are fused (arrows). The capillary basement membrane (bm) is normal in this area. cap = capillary lumen. ep = epithelial cell with foot processes. us = urinary space. rbc = erythrocyte. mes = mesangium. Electron micrograph. (Lead stain, × 6,525).

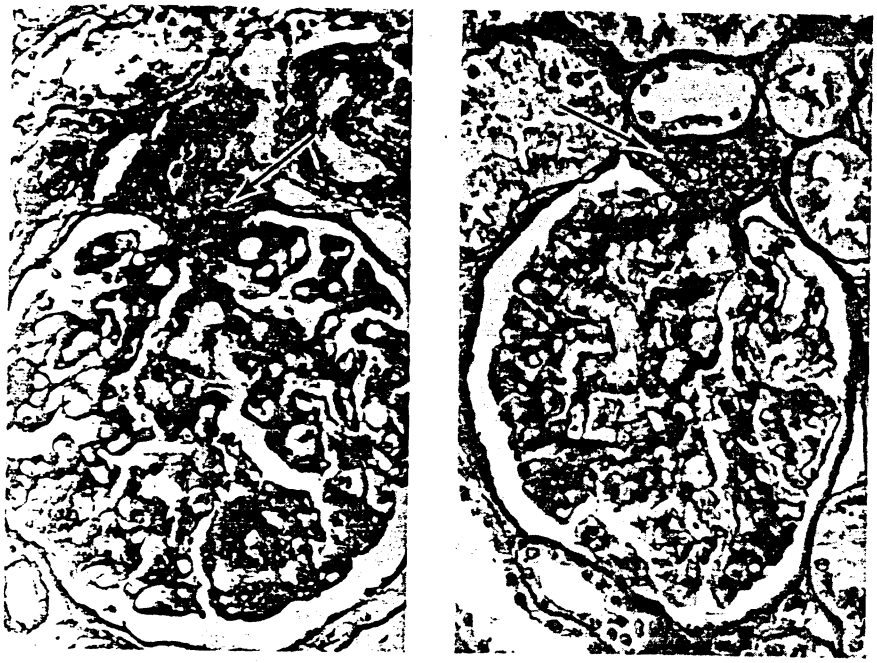

FIGURE 6a. Biopsy of renal allograft one year and nine months after transplantation (LD15). The juxta-glomerular apparatus (arrow) is enlarged and there is thickening of the glomerular capillary walls.

FIGURE 6b. Other kidney from donor to LD15. This man was killed in an accident two years and four months after giving the kidney illustrated in 6a. to his brother. Enlargement of the juxta-glomerular apparatus is as pronounced in this hypertrophied kidney as it is in the transplant. (PAS, × 300).

Arterioles. Collections of homogeneous "hyaline" material, which stained pink with eosin and positively with PAS, were present in the arteriolar walls of 16 (48.5%) of the renal allografts (FIGURE 7). Both afferent and efferent arterioles were affected. Twelve of these were kidneys with glomerular basement membrane deposits. Ultrastructurally, the arteriolar deposits consisted of finely granular, moderately electron dense material, deposited in the intima beneath the endothelial cells (FIGURE 8). In some arterioles, the deeper part of the deposit adjacent to the muscle cells had a finely fibrillar structure, but no periodicity could be detected in these fibrils. Occasionally, fragments of muscular cytoplasm were found embedded in the intimal deposits. The smooth muscle cells were compressed and some contained dense bodies, probably lipofuscin pigment. Endothelial cells over the deposits were sometimes hyperplastic or swollen.

Interlobular Arteries. These vessels were normal in the renal isograft and in the control donor kidney. In 23 (69.7%) of the renal allografts, the intima was thickened (FIGURE 9). This change affected a variable number of the

arteries in each kidney and was usually focal within an individual vessel. Frequently, there was reduplication of the internal elastic lamina; occasionally, the elastic layer was ruptured. Ultrastructurally, the finer vessels showed the same amorphous subendothelial deposits encountered in the arteriolar walls. The material was found infiltrating the internal elastic lamina and between the medial muscle cells. Areas showing a fibrillar structure were also frequent. In addition, other, usually larger vessels, showed variable numbers of smooth muscle cells between the endothelium and the internal elastic lamina (FIGURE 10). So far, only a few vessels have shown collagen in the thickened intima, even though, under light microscopy, this layer was frequently positive for fibrous tissue by van Gieson's stain. The endothelial lining was usually intact but the cells were often swollen and contained dense bodies. In some interlobular arteries the lumen was almost occluded.

Veins and Lymphatics. The walls of small veins were sometimes invaded by mononuclear cells. The lymphatics were normal in all the transplants.

Cellular Infiltration

Eight transplants were free from invading cells. The remainder (75.8%)

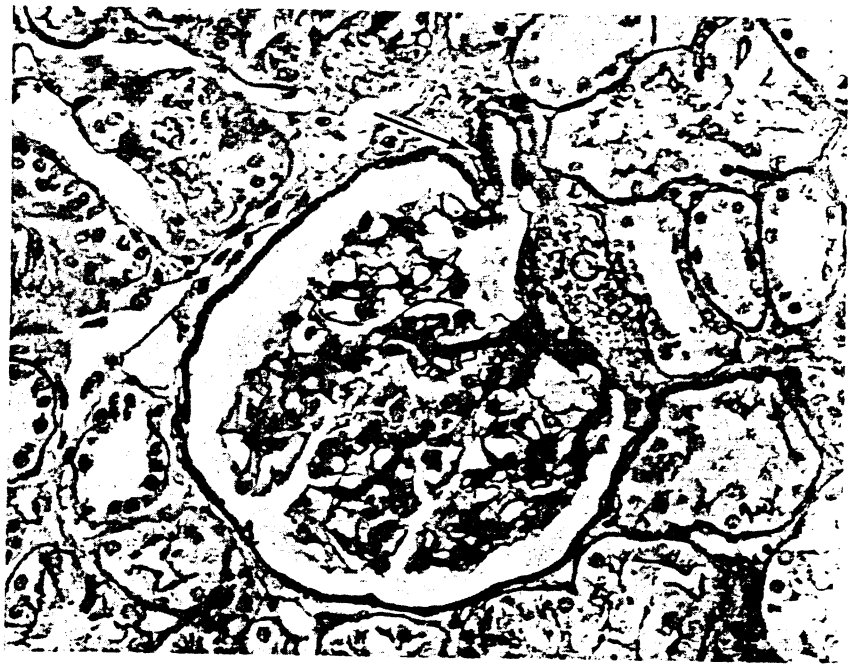

FIGURE 7. Biopsy of renal allograft one year and ten months after transplantation (LD63). Homogeneous hyaline material is present in the wall of an afferent arteriole (arrow). There is also hypertrophy of the juxta-glomerular apparatus (JGA) (PAS, × 350).

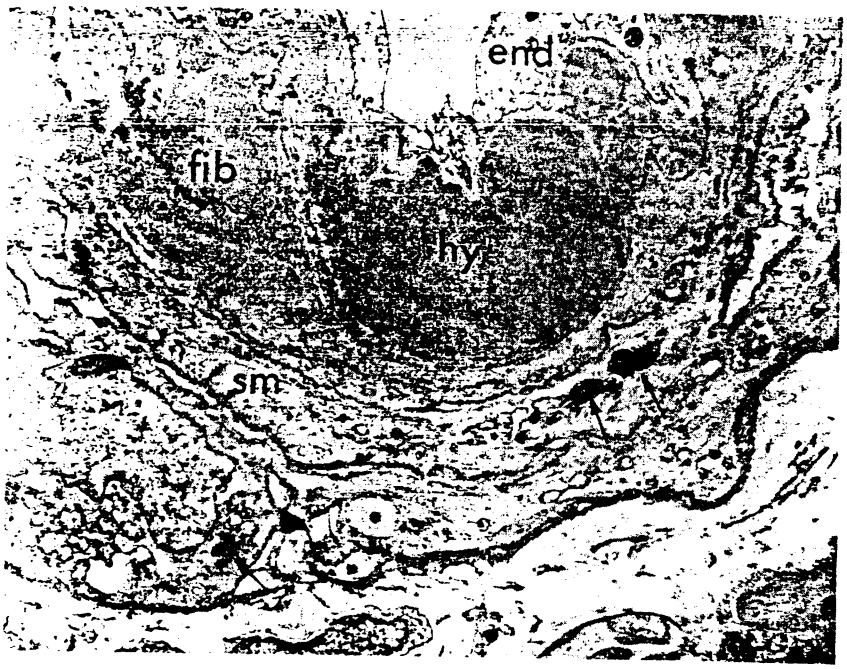

FIGURE 8. Arteriole from renal allograft (LD63) shown in Fig. 7. Hyaline material [illegible] is present between the endothelium (end) and the medial smooth muscle cells (sm). [illegible]jacent to the granular hyaline deposit there is an accumulation of material having a [illegible]cate fibrillar structure (fib). The muscle cells contain dense pigment bodies (arrows). [illegible]ectron micrograph. (Lead stain, × 3,200).

[illegible]ntained varying numbers of focal, dense collections of mononuclear cells. In [illegible]ve of those with cellular infiltration less than ten percent of the cells posses[illegible]ed cytoplasm which stained red with pyronin; in the other 10, between 10 and [illegible] percent of the cells had pyroninophilic cytoplasm. In a few grafts, occasion[illegible]infiltrating cells were found in stages of mitosis. Ultrastructurally, some of [illegible]e pyronin-positive infiltrating cells proved to be lymphoid cells with abun[illegible]ant cytoplasm lacking rough endoplasmic reticulum but full of free ribosomes [illegible]ranged in rosettes (FIGURE 11). The Golgi apparatus was well developed in [illegible]ese cells and the nucleus was often indented. Other pyroninophilic infiltrat[illegible]g cells proved to be plasma cells with abundant rough endoplasmic reticulum [illegible]udded with ribosomes (FIGURE 12). The number of large lymphoid cells [illegible]aried from case to case but they were, generally, surprisingly frequent. In a [illegible]w cases, practically all the pyronin-positive cells were plasma cells. Macro[illegible]ages and small lymphocytes were also found in the interstitial infiltrate. [illegible]osinophils comprised up to ten percent of the invading cells in a few renal [illegible] grafts. A few small lymphocytes and rare mast cells were found in the [illegible]erstitium of the renal isograft.

Interstitial Fibrosis

A striking feature of almost all the renal allografts was a band of dense fibrosis found in the superficial cortex immediately beneath the capsule. Fibrosis confined to this subcapsular zone was present in five allografts; fibrosis deeper in the renal cortex was found in the other 26 allografts (78.8%). Some focal fibrosis was even present in the renal isograft.

Tubular Atrophy

Foci of tubular atrophy were prominent in all those cases with obliterative vascular lesions. A few other allografts also showed occasional small areas of tubular damage. Altogether, 75.8 percent of the allografts were affected in some degree. The tubules in the control donor kidney and in the renal isograft were normal. In one allograft (LD30), several of the distal convoluted tubules were severely affected by cytomegalic inclusion disease.

Correlation of Histopathological Findings with Relationship of Donor to Recipient

In TABLE 1, each of the 33 renal allografts is placed in one of seven histopathological categories. Only major morphological changes were considered in

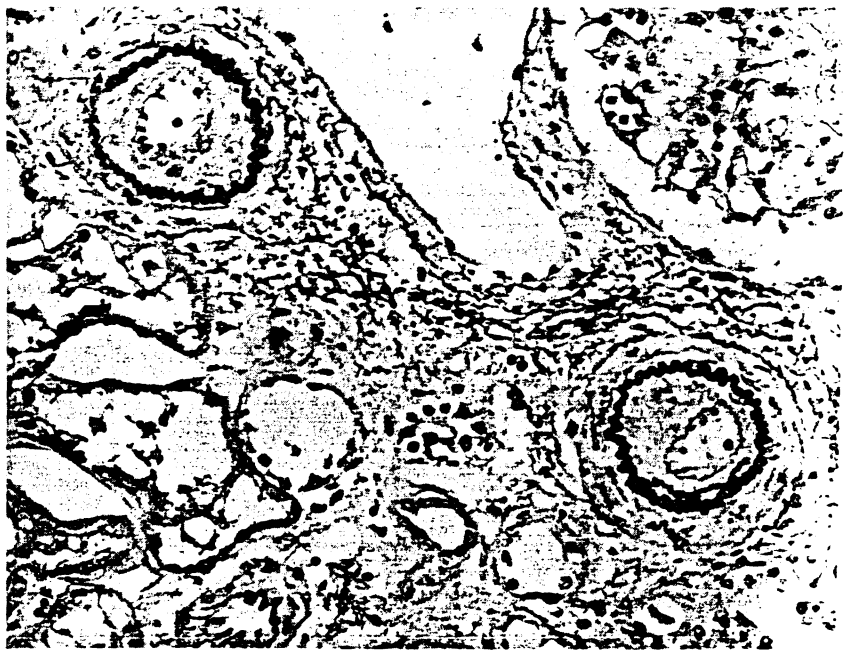

FIGURE 9. Biopsy of renal allograft one year and ten months after transplantation (LD12). The lumina of two small interlobular arteries are narrowed by intimal thickening. (Weigert's elastic counterstained with van Gieson, × 300).

FIGURE 10. Biopsy of renal allograft one year and eleven and one-half months after transplantation (LD30). Part of the wall of a small interlobular artery. There is an accumulation of partly granular and partly fibrillar material (dep) together with some smooth muscle cells (sm) between the endothelial cells (end) lining the artery and the internal elastic lamina (el). med = medial muscle. Electron micrograph (Lead stain, × 7,000).

making this classification. It can be seen that all the renal transplants from unrelated donors were severely damaged. Four of the kidneys from donors who were blood relatives of the recipient were undamaged; the other 19 showed lesions of varying severity.

Correlation of Histopathological Findings with Results of Lymphocyte Typing of Donor and Recipient

In making this correlation 35 renal allografts were considered. Details of the two additional cases (LD 36 and 47), which were from the same series, are given elsewhere (Starzl *et al.*, 1966). The histopathological categories in TABLE 2 are the same as those used in TABLE 1. The lymphocyte grouping depended upon whether donor and recipient were compatible or incompatible when their lymphocyte "types" were considered. These "types" were identified by the application of regression analysis to the results obtained when the cells were tested with a panel of grouped antisera (Terasaki *et al.*, 1966a & b).

When the results of the two methods of classifying the renal allografts, histological and serological, are compared, it will be seen that, in general,

those cases with only minor morphological changes were compatible by lymphocyte typing, whereas many of those with obliterative vascular lesions, glomerular capillary basement membrane thickening, or dense cellular infiltration, were incompatible with their donors in one or more major lymphocyte groups.

If TABLE 2 is divided at I to form a fourfold contingency table (TABLE 3), a comparison can be made between the number of allografts with and without important changes and the number of those allografts regarded as compatible or incompatible by lymphocyte typing. The results of this test show that the apparent association of lymphocyte compatibility with minor histological changes in the graft and of lymphocyte incompatibility with major changes in the graft is not just due to chance, but is statistically significant ($P = 0.008$).

Similarly, division of TABLE 1 at II (TABLE 4) demonstrates a significant association ($P = 0.008$) between lymphocyte incompatibility in two groups or in group six alone and severe histological damage to the graft.

Correlation of Histopathological Findings with Number of Clinical Rejection Episodes Experienced by the Patient

Only one of the ten patients with a normal or slightly damaged renal allo-

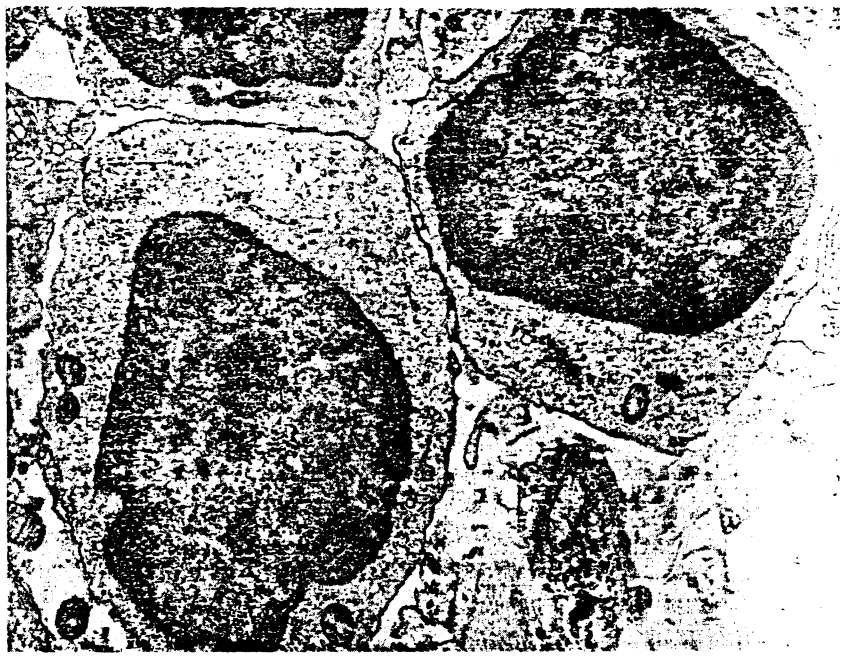

FIGURE 11. Biopsy of renal allograft two years after transplantation (LD27). There are three lymphoid cells in the interstitium. The cytoplasm of each contains many free ribosomes. Electron micrograph. (Lead stain, × 6,000).

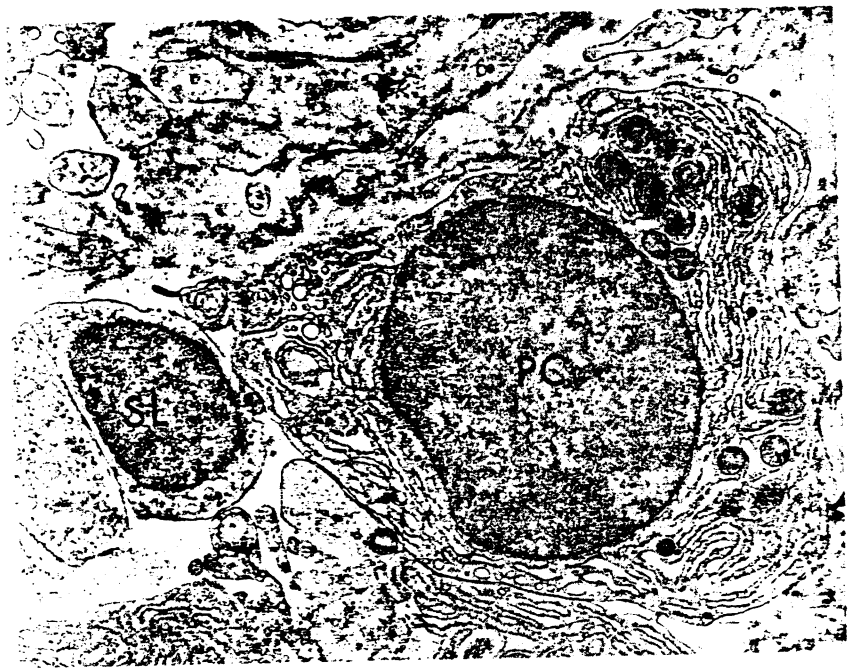

FIGURE 12. Biopsy of renal allograft one year 11½ months after transplantation (LD30). A plasma cell (PC) with abundant rough endoplasmic reticulum and a small lymphocyte (SL) are lying in the interstitium. Electron micrograph. (Lead stain, × 6,000).

graft had suffered a second rejection episode (TABLE 1). Of the ten with severely damaged kidneys, five had experienced two or more bouts of clinical rejection.

Correlation of Histopathological Findings with Renal Function

TABLE 5 shows that there is a close correlation between histological damage to the graft and decreased renal function as indicated by creatinine clearance and blood urea nitrogen levels. In making this correlation, two new histopathological categories were used. All the renal allografts which showed glomerular capillary basement membrane thickening, whether or not this was the major or only change in the kidney, were grouped together. Similarly, all those allografts with interlobular artery narrowing, however slight or focal, were placed in a separate class.

TABLE 1
CORRELATION OF HISTOPATHOLOGICAL FINDINGS IN RENAL ALLOGRAFT BIOPSIES WITH RELATIONSHIP OF DONOR TO RECIPIENT AND WITH NUMBER OF CLINICAL REJECTION EPISODES EXPERIENCED BY PATIENT*

Histopathology	Relationship of Donor to Recipient				No. of Rejection Episodes		
	Sibling	Parent	Other blood relatives	Unrelated	None	1	2 or more
No important changes	LD2 LD6 LD58	LD33			LD58	LD2 LD6 LD33	
Slight interstitial fibrosis and/or minimal cellular infiltration	LD14 LD49	LD39 LD42 LD50	LD53		LD14 LD49	LD39 LD42 LD53	LD50
Glomerular capillary basement membrane thickening	LD3 LD15		LD51 LD60		LD3 LD60	LD15 LD51	
Obliterative vascular changes	LD12	LD17 LD34 LD52			LD52	LD17 LD34	LD12
Obliterative vascular lesions + glomerular capillary basement membrane thickening	LD48	LD22 LD37				LD37	LD22 LD48
Cellular infiltration	LD25	LD13				LD25	LD13
Obliterative vascular lesions + glomerular capillary basement membrane thickening + cellular infiltration	LD18 LD45	LD1 LD40 LD41 LD55		LD27 LD30 LD54 LD63	LD63	LD1 LD18 LD40 LD45	LD27 LD30 LD41 LD54 LD55

*Patients are identified by the code numbers used in the text.

TABLE 2
CORRELATION OF HISTOPATHOLOGICAL FINDINGS IN RENAL ALLOGRAFTS WITH DEGREE OF COMPATIBILITY OF DONOR AND RECIPIENT AS SHOWN BY SEROTYPING OF LYMPHOCYTES*

*Patients are identified by the code numbers used in the text.

TABLE 2

CORRELATION OF HISTOPATHOLOGICAL FINDINGS IN RENAL ALLOGRAFTS WITH DEGREE OF COMPATIBILITY OF DONOR AND RECIPIENT AS SHOWN BY SEROTYPING OF LYMPHOCYTES*

Histopathology	Lymphocytes of donor and recipient							
	Compatible	Incompatible						
		In One Major Group of Antisera						In Two Major Groups of Antisera
		Group 1	2	3	4	5	6	
No important changes	LD2 LD6 LD33 LD58							
Slight interstitial fibrosis and/or minimal cellular infiltration	LD14 LD42 LD49 LD53	LD39		LD50				
Glomerular capillary basement membrane thickening	LD3 LD15	LD60						LD51
Obliterative vascular lesions	LD12 LD52	LD34						LD17
Obliterative vascular lesions + glomerular capillary basement membrane thickening	LD22		LD37		LD48			
Cellular infiltration					LD25			LD13
Obliterative vascular lesions + glomerular capillary basement membrane thickening + cellular infiltration	LD1 LD63		LD54	LD27		LD55	LD18 LD30 LD47	LD36 LD40 LD41 LD45

*Patients are identified by the code numbers used in the text.

TABLE 3
ASSOCIATION OF LYMPHOCYTE COMPATIBILITY BETWEEN DONOR AND RECIPIENT, AS SHOWN BY SEROTYPING, WITH ABSENCE OF APPRECIABLE HISTOLOGICAL DAMAGE TO THE RENAL ALLOGRAFT*

Histopathology	Number of Allografts that were by Lymphocyte Typing		Total
	Compatible	Incompatible in one or more groups	
No significant change or slight interstitial fibrosis and/or minimal cellular infiltration	8	2	10
One or more major lesions	7	18	25
Total	15	20	35

*$Chi^2 = 5.91$. $P = 0.008$.

TABLE 4
ASSOCIATION BETWEEN LYMPHOCYTE INCOMPATIBILITY OF DONOR AND RECIPIENT IN TWO MAJOR GROUPS OR IN GROUP SIX ALONE AND SEVERE HISTOPATHOLOGICAL CHANGES IN THE RENAL ALLOGRAFT*

Histopathology	Number of Allografts that were by Lymphocyte Typing		Total
	Compatible, or incompatible in one group other than group six	Incompatible in group six or in two major groups	
Minor to moderate changes	20	3	23
Obliterative vascular lesions + glomerular capillary basement membrane thickening + cellular infiltration	5	7	12
Total	25	10	35

*$Chi^2 = 5.86$. $P = 0.008$.

TABLE 5
CORRELATION OF HISTOPATHOLOGICAL FINDINGS IN RENAL ALLOGRAFT BIOPSIES WITH RENAL FUNCTION AS MEASURED BY CREATININE CLEARANCE (Ccr) AND BLOOD UREA NITROGEN (BUN)

Histopathology	No. of cases	Mean Ccr ml/min	Mean BUN mg/100 ml
No important changes	4	139.8	15
Slight interstitial fibrosis and/or minimal cellular infiltration	6	94.2	20.3
Glomerular capillary basement membrane thickening	17	85.4	26.6
Interlobular artery narrowing	23	83.5	23.7
Obliterative vascular lesions + glomerular capillary basement membrane thickening + cellular infiltration	10	71.6	30.4

DISCUSSION

The four morphological changes most frequently encountered in these biopsies from long-surviving renal allografts were hyperplasia of the juxta-glomerular apparatus, subendothelial deposits on the glomerular capillary basement membranes, narrowing of the interlobular arteries and cellular infiltration.

Hyperplasia of the juxta-glomerular apparatus in many of the allografts was almost certainly a compensatory phenomenon concerned with the maintenance of normal levels of renin and possibly erythropoietin. That similar changes were present in both a renal isograft and in the kidney remaining in the donor about two years after transplantation is evidence in favor of this view. Also, the plasma renin levels in over 50% of these patients with long-standing renal allografts were within normal limits (Lever *et al.*, 1967). In those transplants with widespread obliterative arterial lesions, the juxta-glomerular hyperplasia was probably enhanced by ischemia. For example, patient LD41 was only three years old at the time of renal transplantation and bilateral nephrectomy. The two kidneys of a normal child of that age weigh about 96g (Allen, 1964). The patient received a renal allograft weighing at least 115g from his mother. Compensatory hyperplasia would not be expected in this case. In fact, after two years, the juxta-glomerular bodies were enlarged. However, there was also narrowing of many interlobular arteries in the graft and, as the patient's plasma renin level was raised and antihypertensive drugs were needed to control his blood pressure, it seems probable that, in this instance, hyperplasia of the juxta-glomerular apparatus was secondary to

ischemia. A combination of severe vascular lesions and a renin content approximately three and one-half times the normal value for kidney tissue has been reported in a renal allograft removed 11 months after transplantation (Shibagaki *et al.*, 1965).

In the past, slight thickening of the glomerular capillary basement membranes has been recorded as an incidental finding in several renal allografts (Porter *et al.*, 1963; Merrill *et al.*, 1963; Calne *et al.*, 1963). In one such transplant which was examined electron microscopically, the basement membrane was found to be focally thickened and there was some fusion of the epithelial foot processes (Woodruff *et al.*, 1962). More severe lesions were described by Krieg *et al.* (1960) in a kidney transplanted only ten days previously. Glomerular basement membrane thickening has also been encountered in 36 percent of canine renal allografts in animals which survived beyond 70 days because of treatment with immunosuppressive drugs. This change was most pronounced in a dog whose renal allograft continued to function for one and one-half years after all treatment had been withdrawn (Porter *et al.*, 1964; Zukoski & Ende, 1965). However, in 1964, Hamburger *et al.* (1964) described three patients who showed proteinuria, renal insufficiency, sudden hypertension or haematuria three months to one year after successful transplantation of a renal allograft. One patient died ten months later; another spontaneously recovered over a period of six months; and the third was greatly improved after treatment with prednisone. Electron microscopic examination of a biopsy from the second of these renal allografts showed "proliferation of endocapillary cells with subendothelial and intercellular hyaline deposits." Two further cases, both of whom subsequently recovered, are mentioned in a more recent publication by the same authors (Hamburger *et al.*, 1965).

Three aspects of the glomerular lesions in the long-surviving renal transplants described in the present paper are worthy of special emphasis. The most striking of these is the very high incidence of this complication. The second is that these conspicuous glomerular abnormalities were rarely accompanied by severe proteinuria and never produced a nephrotic syndrome. The third is that, although, under light microscopy, the lesions closely resembled those of diffuse membranous glomerulonephritis, ultrastructurally, the deposits on the capillary basement membranes were almost entirely subendothelial, unlike the predominantly subepithelial accumulations in membranous glomerulonephritis (Movat *et al.*, 1961). Glomerular deposits between the endothelial cells and the capillary basement membranes also occur in lupus nephritis (Browne *et al.*, 1963), acute glomerulonephritis (Movat *et al.*, 1962), lipoid nephrosis of children (Movat *et al.*, 1961) and experimental nephrotoxic serum nephritis (Feldman *et al.*, 1963).

The nature and origin of the deposits in the renal allografts is unknown. Immunofluorescent studies indicate that they contain immunoglobulins and the third component of complement (β1C-globulin), and that the immunoglobulins can be eluted by pretreatment with acid buffers (Calder *et al.*, 1966).

These data suggest that the accumulated material may be composed of complexes of graft antigen with host antibody. The prognosis depends upon the reversibility or not of these glomerular lesions. Subendothelial deposits of similar material are removed in human acute glomerulonephritis, perhaps by phagocytosis by endothelial and mesangial cells (Neustein & Davis, 1965). The experience of Hamburger *et al.* (1964 & 1965) suggests that the glomerular lesions in renal allografts can resolve similarly.

Although the renal isograft in the present series showed no evidence of glomerulonephritis, this complication has been reported in five renal isografts where the recipient twins originally suffered from the chronic form of this disease. At biopsy or necropsy, three of the isografts showed a combination of glomerular capillary basement membrane thickening and capsular crescent formation (Pfeiffer & Merrill, 1962). However, recrudescence of an old glomerulonephritis is unlikely to explain the glomerular lesions in allografts because the original renal condition in several of the recipients was chronic pyelonephritis or bilateral polycystic disease (Hamburger *et al.*, 1964; Porter *et al.*, 1966). Experimentally, steroids can produce glomerular lesions (Ogilvie *et al.*, 1965) but not all our patients had been treated with these drugs. Moreover, when other series are taken into consideration, even azathioprine has not been used in every case showing glomerular lesions. None of the dogs with glomerulonephritic changes in their renal allografts had received steroids (Porter *et al.*, 1964).

The high incidence of obliterative changes in the interlobular arteries of long-surviving renal allografts and the association of these lesions with previous rejection episodes, suggests that some degree of permanent arterial damage is a frequent legacy of a clinically recognisable acute allograft reaction. The possible genesis of the vascular lesions has been discussed elsewhere (Porter *et al.*, 1965).

The presence of large lymphoid cells with many free cytoplasmic ribosomes has often been noted previously in human renal allografts (Galle & Montera, 1962; Hamburger *et al.*, 1965; Starzl *et al.*, 1965). These cells are characteristically found in the acute stage of the rejection of renal allografts in untreated animals (Porter *et al.*, 1964). In the long-surviving renal allografts in treated patients, they are probably indicative of a low-grade immunological attack by the host which continues between the acute exacerbations recognised clinically as rejection.

The correlation between the degree of compatibility of donor and recipient, as shown by the retrospective serotyping of their lymphocytes, and the histopathological findings in the renal allograft was surprisingly good. Of the group of ten patients whose renal allografts were either normal or only showed minimal histological damage, eight were compatible on the basis of lymphocyte typing. Conversely, only two of the group of 12 patients whose renal allografts showed severe pathological changes were considered compatible by lymphocyte typing: the other ten were incompatible in one or more of the anti-

sera groupings being used. These results are further evidence that the cytotoxicity tests used in this study are recognising histocompatibility antigens.

Generalized cytomegalic inclusion disease is a common complication in patients with renal allografts (Hill *et al.*, 1964; Rifkind, 1965; Hedley-White & Craighead, 1965). However, although the lesions are found in many tissues, the occurrence in one of our cases of abundant nuclear and intracytoplasmic inclusions in the transplant is unusual.

SUMMARY

Thirty-three human renal allografts were biopsied $1\frac{3}{4}$ to $2\frac{1}{2}$ years after transplantation. As a control, similar material was obtained at a comparable time from one renal isograft and from the remaining kidney of one of the donors.

In 23 (69.7%) of the renal allografts some of the interlobular arteries were narrowed by intimal thickening. In several kidneys these changes were slight.

Under light microscopy, glomerular lesions, resembling membranous glomerulonephritis, were present in 17 (51.5%) of the allografts. Ultrastructurally, there were subendothelial deposits of an amorphous material on the capillary basement membranes. The location of this deposit differed from the predominantly subepithelial situation of the accumulations found in membranous glomerulonephritis of adults. Subendothelial deposits of a similar material were also present in the afferent and efferent arterioles of 16 (48.5%) of the allografts.

A mononuclear cell infiltration was present in 25 (75.8%) of the allografts. Up to 60 percent of the cells possessed pyroninophilic cytoplasm and, ultrastructurally, were either large lymphoid cells with many free cytoplasmic ribosomes, or plasma cells.

There was hyperplasia of the juxta-glomerular apparatus in practically all the transplants and in the donor kidney.

Apart from hyperplasia of the juxta-glomerular apparatus and glomerular hypertrophy, ten (30.3%) of the allografts were either normal or showed minimal damage; another ten (30.3%) showed a combination of interlobular artery narrowing, glomerular lesions and cellular infiltration.

Severe lesions in the allografts were associated with two or more bouts of clinical rejection. There was also a close relationship between general histopathological damage and decreased renal function.

When the morphological findings were correlated with the results of lymphocyte typing of donor and recipient, a significant association was found between lymphocyte compatibility and minor or no histological changes in the graft and lymphocyte incompatibility and major changes in the graft.

The obliterative arterial lesions are thought to be an irreversible legacy of damage incurred in past rejection episodes. The glomerular lesions are not the result of either the recipient's original renal disease or of therapy. They may be due to deposition of antigen-antibody complexes: evidence for another

center suggests that these may sometimes resolve. The presence of large pyronophilic lymphoid cells may be indicative of a continuing host response to the allograft. Hyperplasia of the juxta-glomerular apparatus in allografts is usually thought to be a compensatory phenomenon, but sometimes it may be partly in response to ischemia.

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