LABORATORY INVESTIGATION
Vol. 16, No. 1

Printed in U.S.A.

Human Renal Transplants

I. Glomerular Changes*

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Thickening of the glomerular capillary basement membranes has frequently been seen by light microscopy in renal allografts transplanted into patients whose immune responses have been modified by chronic uremia, ionizing radiation, various immunosuppressive drugs, or by some combination of these. In most instances the changes have been minor.[3, 5, 9, 14, 18, 35, 41, 43, 44, 56] When one such kidney was examined electron microscopically, the basement membrane thickening was found to be focal and accompanied by fusion of the epithelial foot processes.[58] Although two patients were reported as showing more pronounced changes,[22, 23] it was Hamburger and his colleagues[11-13, 15] who first emphasized the severity of the glomerular lesions that may occur in long-surviving renal allografts. They described six patients, three of whom developed proteinuria and renal insufficiency 3 months to 1 year after transplantation; no particular clinical signs were present in the other three patients when they were diagnosed on biopsy of the graft at 3, 6, and 31 months respectively. One of these patients died suddenly from renal infarction 1 month later, while another slowly deteriorated and perished 10 months after the onset of proteinuria. The other four patients remained well with normal renal function 1 month to 2 years 10 months after diagnosis. Ultrastructurally in the glomeruli of all these cases there was "proliferation of endocapillary cells with subendothelial and intercellular hyaline deposits."[11] Stress is also laid on glomerular lesions in renal transplants in a recent light microscopy report from another center.[36]

Little is known about the incidence, etiology, and pathogenesis of these glomerular lesions and the prognosis for the patient. In this paper an attempt is made to answer some of these questions on the basis of the light and electron microscopic glomerular changes encountered in 50 renal allografts when they were examined 43 days to 2 years 3 months after transplantation. As controls a renal isograft and a donor's remaining kidney were studied.

A preliminary account of the changes in some of these kidneys has already been given.[42, 49, 50]

Accepted for publication September 22, 1966.

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* This investigation was supported by grants from the Medical Research Council of Great Britain, British Heart Foundation, and St. Mary's Hospital Research Fund; by grants from the Hartford Foundation and the Medical Research Council of Canada; and by Grants AM 06283, AM 06344, AM 07772, AM 02375, AM 7513, AI 04152, AI 04444, FR 00051, and HE 07735 from the United States Public Health Service. The work was further facilitated by the award of a Wellcome Research Travelling Grant to Dr. Porter.

MATERIALS AND METHODS

Patients with Renal Allografts

Living Donors. The 35 patients from the University of Colorado Medical Center, Denver, Colorado, received kidneys from living donors, and each recipient is denoted by a number prefixed by the letters LD (Tables 1 and 2). The same code has been used previously[47, 48, 50] and further details of any of the patients can be obtained by

TABLE 1. CLINICAL DATA ON THE 27 PATIENTS WITH MARKED GLOMERULAR LESIONS IN THEIR ALLOGRAFTS

Recipient			Donor sex, age, and relationship	No. of rejection episodes	Time biopsy taken after transplant	Renal function at time of biopsy		Outcome
Patient no.	Sex and age	Disease[a]				Ccr[b]	Urinary protein	
						ml./min.	*mg./100 ml.*	
LD1	M, 14	CPGN	F, 35, mother	1	2 yr.	100	100	Ccr 97 ml./min. at 4 yr., but still has proteinuria of about 100 mg./ml.
LD3	M, 22	CMGN	M, 22, dizygotic twin	0	2 yr.	131	100	Ccr 100 ml./min. at 3 yr. 10 mo.; now has no proteinuria
LD15	M, 23	CPGN	M, 28, brother	1	1 yr. 9 mo.	139	0	Ccr 140 ml./min. at 3 yr. 5 mo.; needs steroids and antihypertensive drugs
LD18	M, 39	CMGN	F, 44, sister	1	2 yr. 2 mo.	61	13	Died at 2 yr. 7 mo. from coronary thrombosis
LD22	F, 15	Poly	F, 41, mother	2	2 yr. 1 mo.	66	34	Ccr 141 ml./min. at 3 yr. 3 mo.; needs steroids; now has no proteinuria
LD27[c]	F, 20	CMGN	M, 22, unrelated	2	2 yr.	59	90	Deterioration of function; kidney removed at 2 yr. 7 mo.
LD30	M, 40	CMGN	M, 28, unrelated	2	1 yr. 11 mo.	70	310	Ccr 58 ml./min. at 3 yr. 2 mo.; proteinuria of 300 mg./100 ml.; needs steroids and antihypertensive drugs
LD36	M, 48	CPyN	F, 41, wife	1	1 yr. 10 mo.	30	18	Deterioration of function; died at 1 yr. 11 mo. from infection.
LD40	F, 21	CMGN	F, 57, mother	1	1 yr. 10 mo.	62	18	Ccr 48 ml./min. at 3 yr. 1 mo.; needs steroids and antihypertensive drugs
LD41	M, 3	CPGN	F, 37, mother	2	1 yr. 10 mo.	33	11	Ccr 41 ml./min. at 3 yr. 1 mo.; proteinuria of 60 mg./100 ml.; needs steroids and antihypertensive drugs
LD45	M, 35	CPGN	F, 29, sister	1	2 yr. 1 mo.	65	20	Ccr 41 ml./min. at 3 yr.; needs steroids and antihypertensive drugs
LD47	M, 37	CPGN	M, 27, brother	4	1 yr. 10 mo.	10	350	Deterioration of function; died at 1 yr. 10 mo. from infection.
LD48	M, 34	CPGN	F, 29, sister	2	2 yr.	58	105	Ccr 74 ml./min. at 2 yr. 11 mo.; needs steroids and antihypertensive drugs
LD51	M, 18	CPGN	F, 56, aunt	1	1 yr. 11 mo.	92	4	Ccr 81 ml./min. at 2 yr. 10 mo.; needs steroids; proteinuria of 20 mg./100 ml.
LD54[c]	M, 21	CPGN	M, 38, unrelated	3	1 yr. 10 mo.	89	200	Deterioration of function; kidney removed at 2 yr. 5 mo.
LD55	M, 21	CPGN	M, 49, father	2	1 yr. 11 mo.	82	14	Ccr 74 ml./min. at 2 yr. 10 mo.; proteinuria of 20 mg./100 ml.; needs steroids
LD60	M, 21	CPGN	M, 23, cousin	1	1 yr. 10 mo.	90	5	Ccr 41 ml./min. at 2 yr. 9 mo.; proteinuria of 30 mg./100 ml.; needs steroids and antihypertensive drugs
LD63	M, 35	CPGN	M, 33, unrelated	0	1 yr. 10 mo.	95	16	Ccr 99 ml./min. at 2 yr. 9 mo.; needs steroids and antihypertensive drugs
LDBS	M, 16	CPGN	F, 40, mother	2	2 yr. 5 mo.	10	200	Deterioration of function; kidney removed at 2 yr. 5 mo.
M13	M, 28	CPyN	F, 29, cadaver	4	10 mo.	50	0	Ccr 30 ml./min. at 2 yr. 1 mo.; proteinuria of 70 mg./100 ml.; needs steroids and antihypertensive drugs
M17	M, 18	RPGN	M, 8, cadaver	3	7 mo.	45	0	Ccr 75 ml./min. at 1 yr. 10 mo.; proteinuria of 300 mg./100 ml.; needs antihypertensive drugs
M30	M, 29	CPGN	M, 57, cadaver	2	4 mo.	60	70	Ccr 74 ml./min. at 1 yr. 7 mo.; proteinuria of 65 mg./100 ml.; needs steroids
DB	M, 37	Poly	M, 43, cadaver	6	1 yr. 7 mo.	73	150	Ccr 133 ml./min. at 2 yr. 8 mo.; proteinuria of 200 mg./100 ml.
EN	F, 24	CPyN	M, 21, cadaver	1	1 yr. 5 mo.	112	30	Ccr 107 ml./min. at 2 yr. 6 mo.; proteinuria of 5 mg./100 ml.
AR[c]	M, 37	CPGN	F, 41	2	43 da.	4	200	Irreversible rejection; transplant removed at 3 mo.
RT	M, 14	RPGN	F, 34, cadaver	5	1 yr.	30	10	Deterioration of function; died at 1 yr. from infection
SL1	M, 30	CPGN	F, 17, cadaver	1	8 mo.	50	200	Deterioration of function; kidney removed at 1 yr. 3 mo.

[a] CPGN, Chronic proliferative glomerulonephritis; RPGN, rapidly progressive acute glomerulonephritis; CMGN, chronic membranous glomerulonephritis; CPyN, chronic pyelonephritis; and Poly, bilateral polycystic disease.

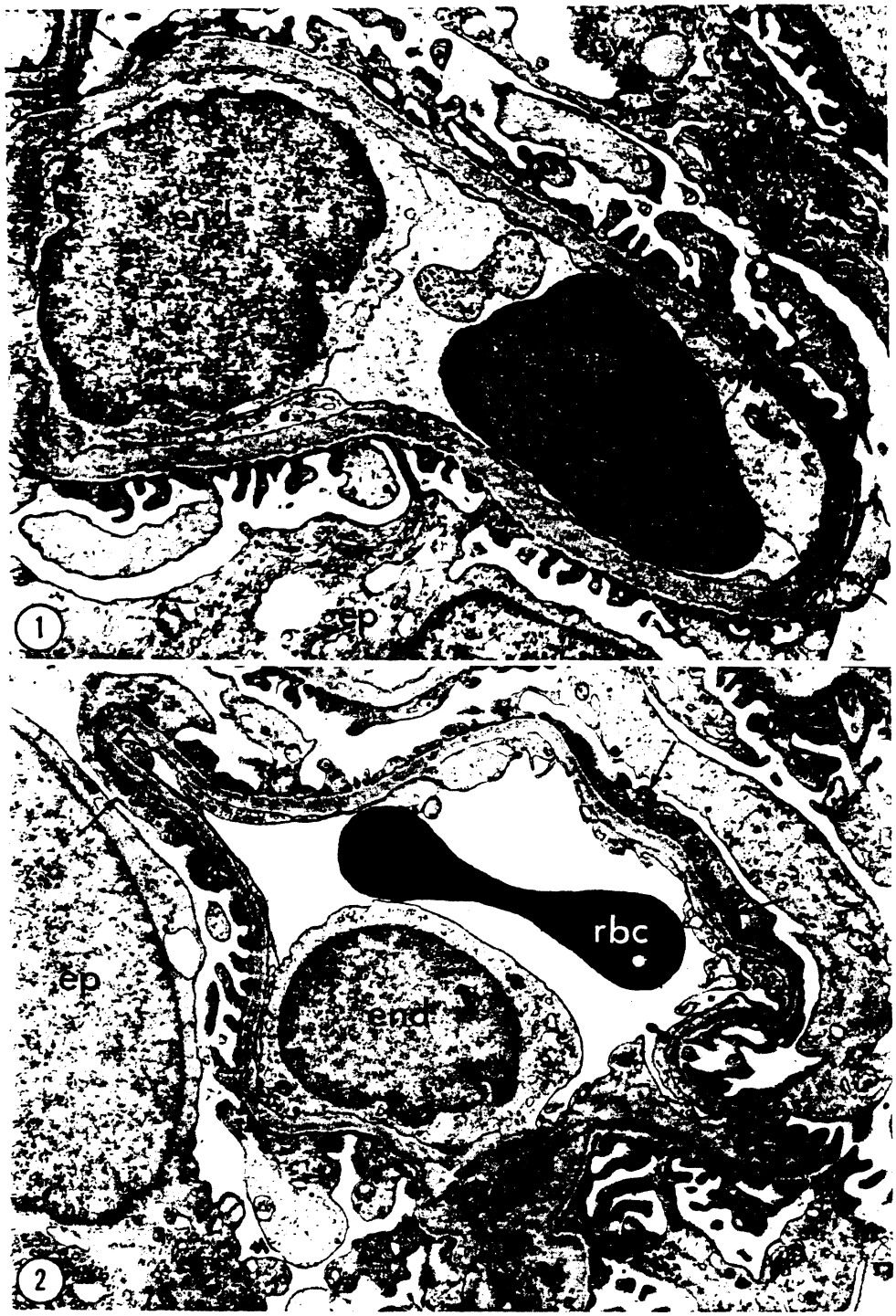
[b] Ccr, Creatinine clearance.

[c] Biopsied during a rejection episode.

TABLE 2. CLINICAL DATA ON THE 25 PATIENTS WITH MINOR GLOMERULAR CHANGES OR NORMAL GLOMERULI

Recipient			Donor, sex, age, and relationship	No. of rejection episodes	Time biopsy taken after transplant	Renal function at time of biopsy		Outcome
Patient no.	Sex and age	Disease[a]				Ccr[b]	Urinary protein	
						ml./min.	*mg./100 ml.*	
LD2	M, 38	CPGN	F, 32, sister	1	2 yr.	129	0	Ccr 73 ml./min. at 3 yr. 10 mo.
LD6	M, 23	CPGN	M, 22, brother	1	1 yr. 11 mo.	130	0	Ccr 104 ml./min. at 3 yr. 8 mo.; now has proteinuria of 10 mg./100 ml.
LD12	M, 49	RPGN	M, 53, brother	2	1 yr. 10 mo.	65	0	Ccr 32 ml./min. at 3 yr. 6 mo.; needs steroids and antihypertensive drugs
LD13	M, 17	CPGN	F, 35, mother	2	1 yr. 9 mo.	105	0	Ccr 154 ml./min. at 3 yr. 5 mo.; needs steroids
LD14	M, 44	CPyN	M, 37, brother	0	1 yr. 9 mo.	102	0	Ccr 103 ml./min. at 3 yr. 5 mo.; now has proteinuria of 10 mg./100 ml.
LD17	F, 15	CPyN	F, 44, mother	1	2 yr. 1 mo.	71	8	Ccr 70 ml./min. at 3 yr. 3 mo.; proteinuria of 10 mg./100 ml.; needs steroids
LD25	M, 33	CPGN	M, 29, brother	1	2 yr.	84	5	Ccr 111 ml./min. at 3 yr. 3 mo.; needs steroids
LD33	F, 18	CPGN	F, 42, mother	1	1 yr. 11 mo.	184	0	Ccr 91 ml./min. at 3 yr. 3 mo.
LD34	F, 8	CPGN	F, 30, mother	1	1 yr. 11 mo.	59	0	Ccr 72 ml./min. at 3 yr. 3 mo.; now has proteinuria of 5 mg./100 ml.
LD37	M, 21	CPGN	F, 43, mother	1	1 yr. 10 mo.	159	22	Ccr 130 ml./min. at 3 yr. 2 mo.; needs steroids; now has no proteinuria
LD39	M, 17	CPGN	F, 48, mother	1	1 yr. 10 mo.	87	20	Ccr 93 ml./min. at 3 yr. 1 mo.; needs steroids; proteinuria of 10 mg./100 ml.
LD42	F, 19	CPGN	M, 47, father	1	1 yr. 9 mo.	84	9	Ccr 78 ml./min. at 3 yr.; now has no proteinuria; needs steroids
LD49	M, 32	CMGN	F, 41, sister	0	1 yr. 10 mo.	70	4	Ccr 75 ml./min. at 2 yr. 11 mo.; needs steroids
LD50	F, 16	CPGN	M, 38, father	1	2 yr.	121	2	Ccr 63 ml./min. at 2 yr. 11 mo.; needs steroids
LD52	F, 15	CPGN	M, 56, father	0	1 yr. 11 mo.	103	8	Ccr 82 ml./min. at 2 yr. 10 mo.; needs steroids and antihypertensive drugs
LD53	M, 15	CPGN	M, 35, uncle	1	1 yr. 11 mo.	101	3	Ccr 79 ml./min. at 2 yr. 10 mo.; needs steroids
LD58	M, 27	CPGN	F, 23, sister	0	1 yr. 9 mo.	114	4	Ccr 61 ml./min. at 2 yr. 9 mo.; now has no proteinuria; needs steroids and antihypertensive drugs
M8	M, 24	Hydr	F, 53, cadaver	3	1 yr. 2 mo.	50	100	Ccr 51 ml./min. at 2 yr. 5 mo.; now has no proteinuria; needs steroids and antihypertensive drugs
M15	M, 22	CPGN	M, 34, cadaver	2	8 mo.	35	80	Ccr 65 ml./min. at 1 yr. 11 mo.; proteinuria of 40 mg./100 ml.; needs steroids
M21	M, 37	CPGN	M, 22, cadaver	1	7 mo.	80	80	Ccr 106 ml./min. at 1 yr. 10 mo.; now has no proteinuria; needs steroids and antihypertensive drugs
M29	M, 25	CPGN	M, 17, cadaver	2	79 da.	45	40	Ccr 38 ml./min. at 1 yr. 5 mo.; proteinuria of 70 mg./100 ml.; needs steroids and antihypertensive drugs
CB[c]	M, 25	CPGN	M, 43, cadaver	5	11 mo.	33	0	Reversal of rejection episode and restoration of function, but later further rejection and deterioration. Ccr 20 ml./min. at 2 yr.; now has proteinuria of 50 mg./100 ml.; needs steroids
KF	M, 17	CPyN	F, 41, cadaver	3	1 yr. 10 mo.	124	0	Ccr 139 ml./min. at 3 yr.
IDT2	M, 18	CPGN	M, 18, identical twin	0	2 yr. 3 mo.	115	37	Ccr 110 ml./min. at 3 yr. 6 mo.; now has no proteinuria
Donor to LD15	M, 32					130	0	Killed in accident 2 yr. 4 mo. after donating kidney to his brother LD15

[a] CPGN, Chronic proliferative glomerulonephritis; RPGN, rapidly progressive acute glomerulonephritis; CPyN, chronic pyelonephritis; CMGN, chronic membranous glomerulonephritis; and Hydr, hydronephrosis.
[b] Ccr, Creatinine clearance.
[c] Biopsied during a rejection episode.



FIGS. 1 AND 2

referring to these publications. The patient LDBS received a renal transplant at the Peter Bent Brigham Hospital, Boston, Massachusetts, and the allograft was eventually removed at Denver. In all but one of the patients, splenectomy and bilateral nephrectomy were performed at or before the time of transplantation: the exception was LD2 who retained his right kidney. The thymus was removed from 12 of the patients, four before and eight from 250 to 520 days after transplantation.

Cadaveric Donors. The seven patients from the Royal Victoria Hospital, Montreal, Canada, the six patients from St. Mary's Hospital, London, England, and the one patient from the John J. Cochran Veterans Hospital, St. Louis, Missouri, all received kidneys from cadavers (Tables 1 and 2). The Montreal recipients are denoted by a number prefixed by the letter M, the London patients by their initials, and the St. Louis patient by SL1. The St. Mary's patients were subjected to splenectomy and bilateral nephrectomy at the time of transplantation; the others were not. Thymectomy was not performed.

In 48 of these cases the tissue was obtained at open biopsy; in two it was collected at autopsy. All the patients had been receiving daily azathioprine; 36 were receiving 2.5 to 25 mg. per day of prednisone; and 26 were being treated with one or more of the antihypertensive drugs chlorothiazide, hydralazine, reserpine, and methyldopa.

Control Patients

Renal Isograft (IDT2). This patient had undergone bilateral nephrectomy, but retained his spleen and thymus. At the time of open biopsy he was receiving no azathioprine or antihypertensive drugs.

Remaining Kidney from a Donor. A 32-year-old man was killed in an accident 2 years 4 months after donating a kidney to his brother (LD15). His remaining hypertrophied kidney (weight 300 gm.) was used as a control in this study.

Lymphocyte Typing

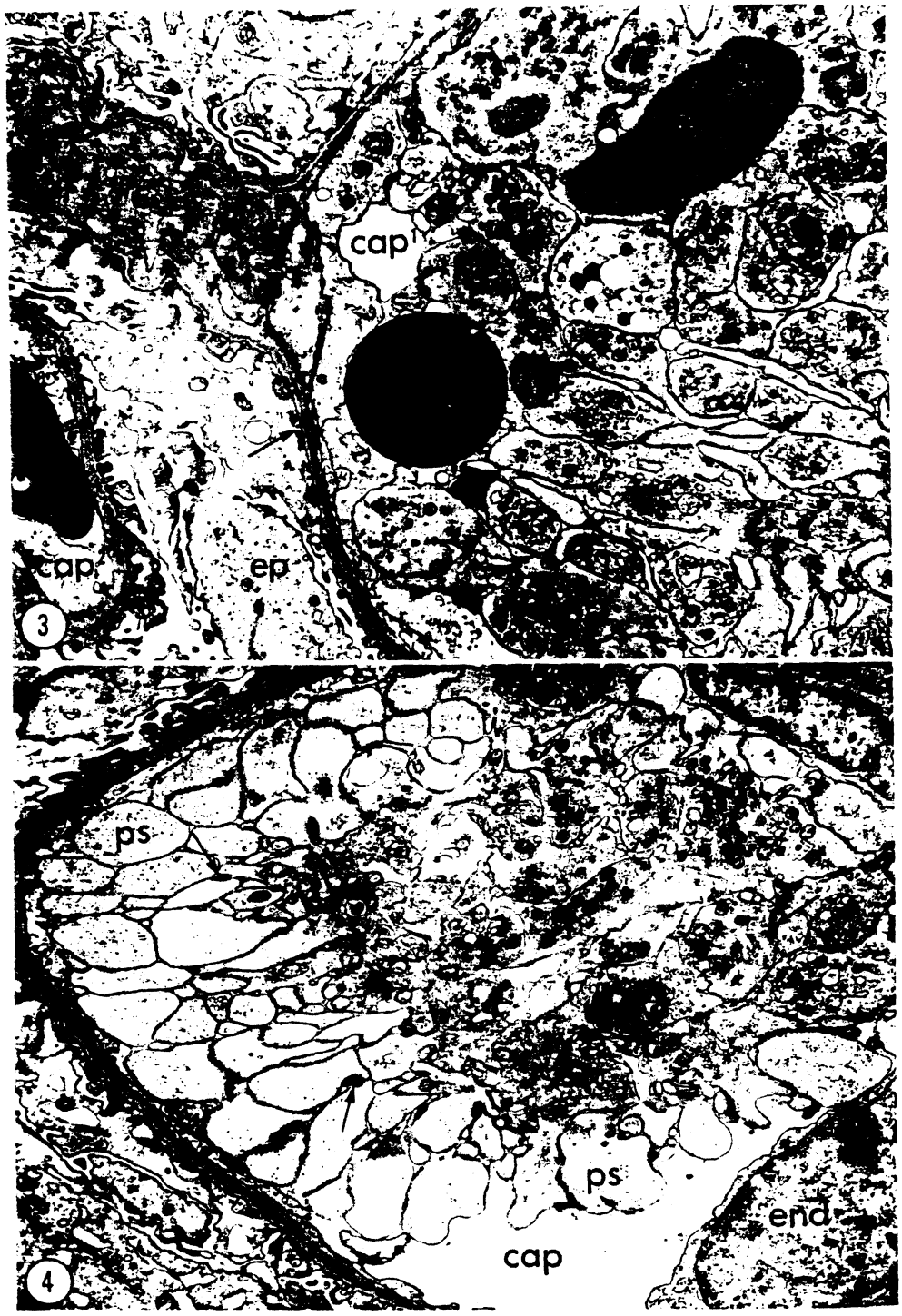
In 36 of the 50 patients retrospective typing of the lymphocytes of the donor and the recipient was undertaken. Blood lymphocytes obtained from the patients and from their donors were tested against a panel of 65 to 121 different cytotoxic antisera in the presence of rabbit complement.[52] Those antisera 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.[53] Incompatibilities between donors and recipients were then noted.

Glomerular Permeability Studies

In those patients with moderate to heavy proteinuria, the selectivity of the protein loss was measured by Dr. J. S. Cameron of Guy's Hospital, London, England, who used a modification of the method first described by Soothill as mentioned by Joachim, Cameron, Schwartz, and Becker.[20] The urinary clearance of five to seven plasma proteins, ranging in molecular weight from 40,000 to 1,300,000, was assessed by immunochemical means, and the clearance of each protein was then expressed as a percentage of the clearance of transferrin. The regression line of log clearance upon log molecular weight was calculated and the angle of this line, called θ, was taken as the index of selectivity.

FIG. 1. Part of a glomerular capillary loop from the left kidney of a normal 32-year-old man. The visceral epithelial cells (*ep*) contain cytosomes (*cy*), and there are a few tiny foci of fusion of the foot processes (*arrows*). This individual's right kidney had been transplanted to patient LD15 2 years 4 months previously. *end,* Endothelial cell; *rbc,* erythrocyte. ×10,300.

FIG. 2. Glomerular capillary loop from a renal isograft (IDT2) 2 years 3 months after transplantation. The basement membrane is normal. There are focal areas of fusion of epithelial (*ep*) foot processes (*arrows*), and the epithelial cytoplasm adjacent to the capillary basement membranes contains aggregates of a dense, finely granular material. ×8300.



FIGS. 3 AND 4

Tissue Processing

Tissue for light microscopy was fixed in 10 per cent neutral formalin or buffered formol saline, embedded in paraffin wax, and serially sectioned. The stains used routinely were hematoxylin and eosin, periodic acid-Schiff reagent (PAS), Weigert's for elastic tissue, picro-Mallory 5, Martius scarlet blue, Mallory's phosphotungstic acid-hematoxylin, and methyl green-pyronin. Other special stains were used when indicated. For electron microscopy small fragments of tissue were fixed in Palade's buffered osmium tetroxide (pH 7.4). After fixation at 4° C. for 1½ to 2 hours, the tissues were washed and then dehydrated in a graded series of ethanol solutions and embedded in an epoxy resin, usually Epon 812 but sometimes Araldite. Sections, 0.5 μ thick, were cut on a Huxley ultramicrotome with glass knives and then stained with azur II. After identification of suitable glomeruli under the light microscope, ultrathin sections were cut and mounted both on naked and carbon-coated copper grids. After treatment with lead hydroxide, lead citrate, phosphotungstic acid, or a combination of lead hydroxide and uranyl acetate, the ultrathin sections were examined in a Siemens Elmiskop IA or an AEI 6B electron microscope operating at 80 kv. Some tissue from each biopsy was used for immunofluorescent study, the results of which will be reported separately.

RESULTS

Contralateral Donor Kidney

By light microscopy the only unusual glomerular feature of the remaining donor kidney was hypertrophy. Ultrastructurally the visceral epithelial cells contained several cytosomes and cytosegresomes,[6] and there were rare, tiny, focal areas of fusion of the foot processes (Fig. 1). These changes in fine structure were regarded as being within normal limits.

Renal Isograft

Apart from hypertrophy the glomeruli appeared to be normal by light microscopy. Ultrastructurally, small focal areas of fusion of the visceral epithelial foot processes were more common and larger than in the control donor kidney (Fig. 2). The epithelial cytoplasm that abutted against the capillary basement membranes contained aggregates of a dense, finely granular material. These areas of density were more pronounced than in normal glomeruli. The capillary basement membrane was of normal thickness in most places, the width being 3300 Å (mean of 250 measurements; range, 1600 to 5200 Å; standard deviation, 540). There were, however, several small subendothelial thickenings and irregularities. These were mostly of the same density as the normal lamina densa; a few were less dense. The endothelial cells were normal and the capillary lumina were patent and contained many red cells and an occasional platelet. There was a slight increase of mesangial matrix.

Renal Allografts

Biopsied during Rejection. Four of the 50 patients (LD27, LD54, AR, and CB) were biopsied during a rejection episode. This was a clinical diagnosis based upon the occurrence shortly before the biopsy of oliguria and a fall in creatinine clearance in a patient previously doing well. In two of the cases there was also an outpouring of lymphoid and tubular cells in the urine. The rejection episode was rapidly reversed in one of the patients (CB) with

Fig. 3. Biopsy of a renal allograft taken during a rejection episode which was later reversed by treatment with return of normal function to the graft (patient CB). A glomerular capillary loop (*cap*[1]) is occluded by a large aggregate of intact platelets together with two erythrocytes (*rbc*). The absence of much interdigitation of pseudopods suggests that the platelets are only loosely adherent. An adjacent capilary loop (*cap*[2]) is unobstructed. There is fusion of epithelial (*ep*) foot processes (*arrow*). ×7680.

Fig. 4. Same renal allograft as was shown in Figure 3. A glomerular capillary loop (*cap*) is almost occluded by aggregated platelets. At the periphery of the platelet mass, there are many bulbous pseudopods (*ps*). The platelet lysosomes and plasma membranes are mostly intact. There are a few fragments of fibrin (*arrows*). *end*, Endothelial cell. ×7500.

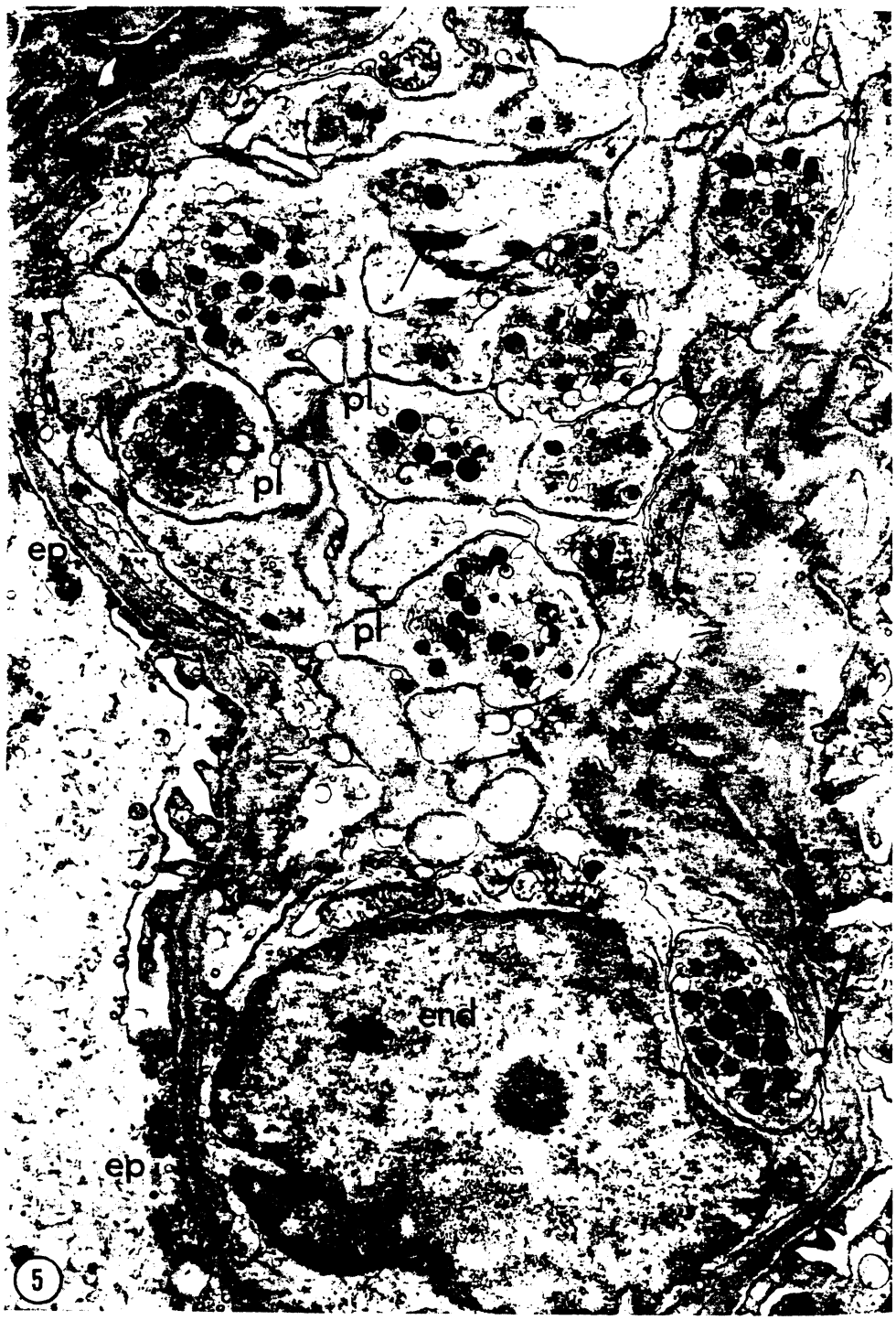


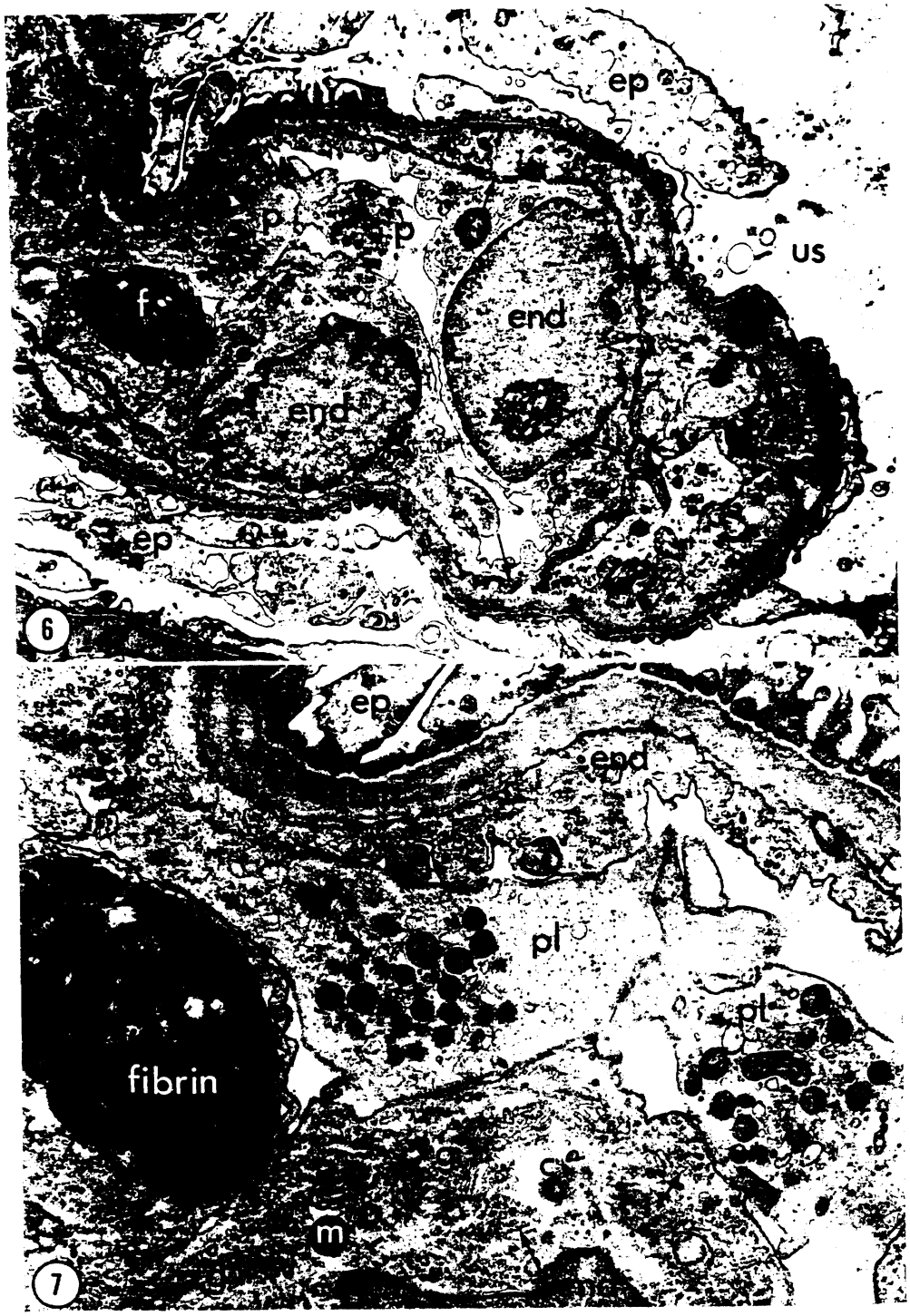
FIG. 5

restoration of normal function, but the other three patients failed to respond to treatment and their renal allografts had ultimately to be removed.

Reversible Rejection. By light microscopy, in the case where intensified treatment with steroids and azathioprine produced recovery of renal function, there were pale, homogeneous masses occluding the capillary lumina in several of the glomeruli. There were also a few small areas in which the glomeruli showed periglomerular fibrosis and some increase of mesangial matrix. In the electron microscope the affected capillary loops were found to be blocked by aggregated masses of platelets (Fig. 3). At the periphery of the clumps many of the platelets had bulbous pseudopods which were pale and lacked lysosomes and mitochondria (Fig. 4). In most of the aggregates the platelet lysosomes and plasma membranes were still intact and there was not much interdigitation of pseudopods, which suggested that the platelets were only loosely adherent. Tiny amounts of fibrin were present in several of the clumps. In many of the capillaries there were small, focal, subendothelial accumulations of amorphous material on the basement membrane. A few platelets had inserted pseudopods between adjacent endothelial cells and were in contact with this altered membrane (Fig. 5). Both the endothelial and epithelial cells were enlarged, and their content of cytosomes, cytosegresomes, multivesicular bodies, and ribosomes was increased. The epithelial cells possessed microvilli, and there were areas of fusion of the foot processes.

Irreversible Rejection. In two patients these were second rejection episodes, beginning 1 month and 2 years respectively after transplantation; in the third patient a third rejection episode began at 1 year 10 months. Under light microscopy the majority of the glomeruli in each transplant showed thickening of the capillary basement membranes by PAS-positive material. Deposits of similar material were present in the mesangium of many tufts. A few of the glomeruli were hypercellular, either focally or diffusely. Several of the glomerular capillaries contained trapped neutrophils, small plugs of amorphous material, and fragments of phosphotungstic acid-hematoxylin-positive fibrin. A few showed adhesions between tuft and capsule. Rare cellular capsular crescents were present. Ultrastructurally the epithelial cells were enlarged and their cytoplasm contained increased numbers of ribosomes, cytosomes, cytosegresomes, and multivesicular bodies. The Golgi bodies were enlarged. Fusion of foot processes, although focal, was extensive (Fig. 6), and that part of the epithelial cytoplasm in contact with the capillary basement membranes was dark and granular. Almost all the capillary basement membranes were greatly thickened by subendothelial accumulations of an amorphous, finely granular material which had an electron density similar to normal lamina densa (Figs. 6 to 8). The lamina interna rara was lost, and there were some extensions of the amorphous material between endothelial cells. A few oval, much denser deposits lay in the lamina densa (Figs. 6 and 8). In some areas cytoplasmic fragments and organelles, together with fragments of fibrin and lipid particles, were embedded in the subendothelial deposit (Figs. 6 and 8). In a few places larger fibrin masses, which showed the characteristic periodicity of about 200 Å, were incorporated into the thickened wall and covered by a thin layer of endothelium (Figs. 6 and 7). The endothelial cells were greatly enlarged and contained increased numbers of free ribosomes, cytosomes, multivesicular bodies, and vacuoles. The amount of rough endo-

FIG. 5. Same renal allograft as was shown in Figures 3 and 4. A glomerular capillary loop is obstructed by an aggregation of intact platelets (*pl*) and rare fibrin fragments (*fine arrows*). One platelet has inserted a pseudopod (*broad arrow*) between two of the endothelial cells lining the capillary and is making contact with the basement membrane. Focal deposits of homogeneous material (*x*) are present on the subendothelial aspect of this membrane and there is fusion of some of the epithelial foot processes (*ep*). *end*, Endothelial cell. ×10,300.



FIGS. 6 AND 7

plasmic reticulum in these cells was greater than normal, and the Golgi bodies and centrioles were prominent (Fig. 7). The capillary lumina were narrowed and contained erythrocytes, lymphoid cells, platelets, and occasional neutrophil polymorphonuclear leukocytes. The platelets, several of which showed loss of lysosomes and rupture of cell membranes, were usually in clumps intermixed with fibrin (Fig. 9). Neutrophils were found in association with the platelet-fibrin aggregates, completely occluding some capillary loops. Occasionally there appeared to be loss of the plasma membranes between the endothelial cells and the polymorphonuclear leukocytes (Fig. 9). The mesangial matrix was increased in amount and in several glomeruli contained small strands of fibrin. There were increased numbers of cytosomes, cytosegresomes, and ribosomes in the mesangial cells.

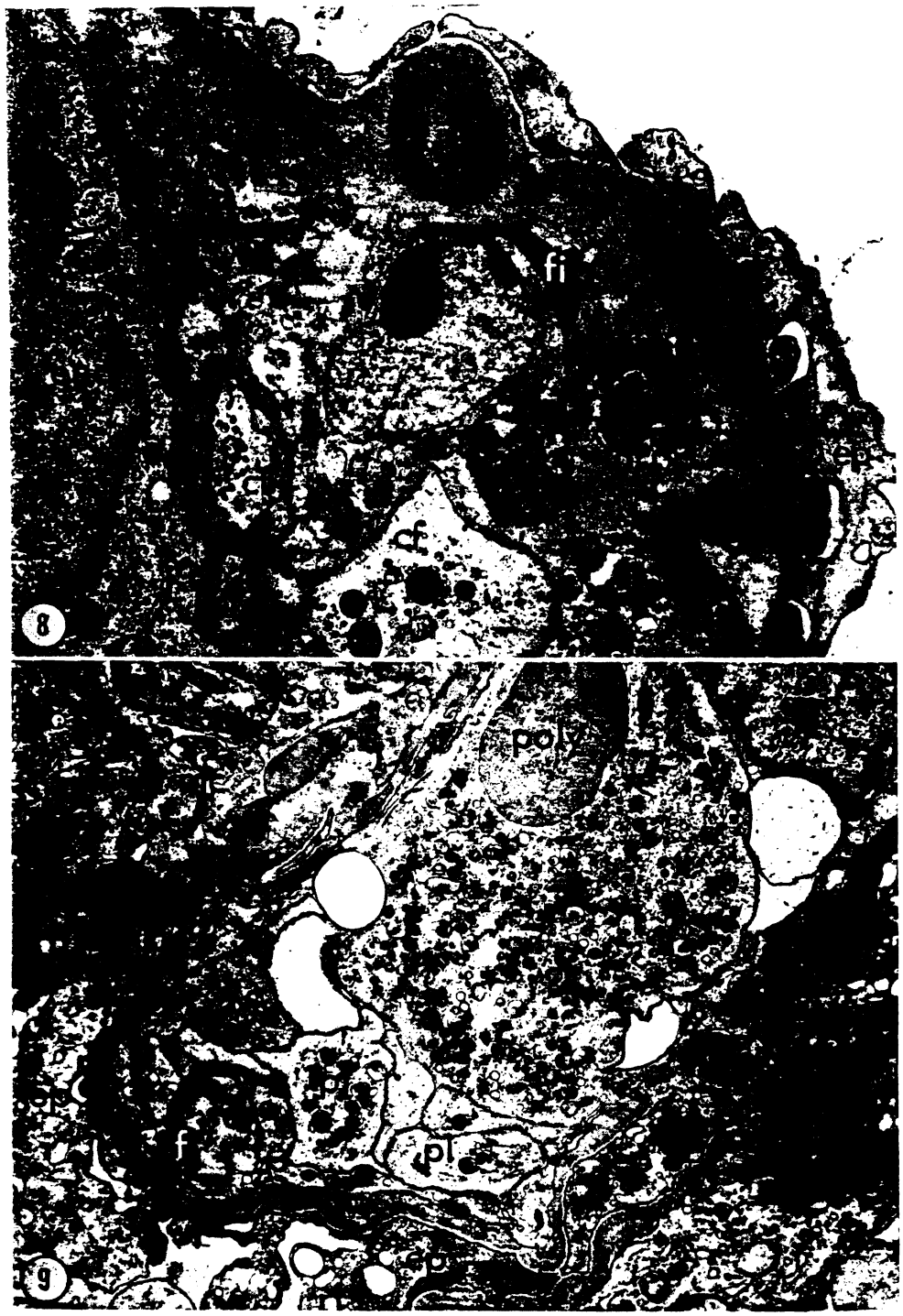
BIOPSIED WHEN NOT IN REJECTION. Forty-six of the 50 patients were biopsied at a time when there was no clinical evidence of rejection. In 24 of the renal allografts, light microscopy of ordinary sections 4 μ thick showed that the glomerular capillary basement membranes were thickened. The other 22 kidneys appeared normal.

Renal Allografts with Glomerular Capillary Basement Membrane Thickening Obvious in 4-μ Sections (Group A). In the majority (63 per cent) of the 24 renal allografts, the PAS-positive thickening of the capillary walls seen by light microscopy was focal within the glomerular tufts and associated with large mesangial deposits of similar material producing a picture resembling lobular glomerulonephritis. Less frequently this was a diffuse process resembling chronic membranous glomerulonephritis. These changes were sometimes accompanied by localized areas of increased tuft cellularity (21 per cent), and adhesions between tuft and Bowman's capsule (37 per cent). A few glomeruli contained cribriform areas in the tuft resembling those seen in radiation nephritis. Capsular crescents and lobular lesions containing trapped erythrocytes and polymorphonuclear leukocytes were rare except in two cases: RT where the former lesions were so common as to give the picture of a rapidly progressive acute glomerulonephritis, and M17 where the latter lesions were common. Examination of plastic-embedded sections 0.5 μ thick revealed another feature which distinguished the renal allografts of M17 and RT from the other 22 kidneys in this group. Only these two kidneys showed large hemispheric nodules, or "humps,"[21] on the epithelial side of the capillary basement membrane.

The electron microscopic appearances of the glomeruli are summarized in Table 3. In all cases the capillary basement membrane was altered by subendothelial accumulations of amorphous material similar to that seen in the three transplants that were biopsied during irreversible rejection (Fig. 10). The deposits obliterated the lamina interna rara, but the lamina densa was generally normal. The density and compactness of the deposits varied; in many capillary loops, rarefied areas were present (Figs. 11 and 12). In a few of the tufts it was difficult to distinguish deposit from lamina densa, and the over-all effect was of a greatly thickened basement membrane (Fig. 13). In

FIG. 6. Biopsy of a renal allograft taken during a rejection episode which later failed to respond to treatment (patient AR). A glomerular capillary loop is greatly narrowed. The lumen contains two platelets (*p*); the lining endothelial cells (*end*) are hypertrophied; a mass of fibrin (*f*) lies just beneath the endothelium; and the capillary basement membrane is irregularly thickened by large deposits. The majority of the deposits lie beneath the endothelium (*arrows*), but a few (*d*) are within the lamina densa. *ep*, Epithelial cell; *us*, urinary space. ×4400.

FIG. 7. Enlargement of part of Figure 6. The hypertrophied endothelial cell (*end*) contains an increased amount of rough endoplasmic reticulum (*arrows*), a prominent centriole (*c*), and enlarged Golgi bodies (*g*). A large *fibrin* mass is incorporated into the thickened capillary wall and covered by a thin layer of endothelium. There are two disintegrating platelets (*pl*) in the narrowed capillary lumen. The basement membrane (*bm*) is thickened by subendothelial deposits (*x*). *m*, Multivesicular body. ×16,400.



FIGS. 8 AND 9

TABLE 3. GLOMERULAR ULTRASTRUCTURAL CHANGES IN 50 RENAL ALLOGRAFTS FROM PATIENTS TREATED WITH AZATHIOPRINE AND PREDNISONE

Type of change	Kidneys showing various ultrastructural features[a]		
	Group A	Group B	In whole series
	%	%	%
Visceral epithelial cells			
Enlarged	75	45	62
Fusion of foot processes	100	100	100
Microvilli	50	9	32
Increased number of organelles	67	64	66
Enlarged Golgi bodies	67	55	60
Capillary basement membranes			
Subendothelial deposits	100	41	74
Subepithelial "humps"	8	0	4
Deposits in lamina densa	17	0	12
Extension of deposit between endothelial cells	96	14	58
Incorporation of cell fragments in deposit	88	5	50
Endothelial cells			
Increased numbers	21	0	10
Enlarged	75	14	50
Increased number of organelles	71	59	68
Enlarged Golgi bodies	33	5	24
Capillary lumina			
Narrowed	71	9	44
Obstructed	29	0	20
Containing impacted polymorphonuclear leukocytes	17	0	10
Containing monocytes	42	32	34
Containing lymphoid cells	54	32	44
Mesangium			
Increased numbers of cells	42	18	28
Increased amount of matrix	92	45	70
Increased number of organelles in cells	63	41	54

[a] The 46 kidneys in groups A and B were biopsied at a time when the patient was not in a clinical rejection episode. Group A consists of 24 renal allografts which by light microscopy showed obvious glomerular capillary basement membrane thickening. Group B is composed of 22 renal allografts with normal glomerular capillary basement membranes by light microscopy. The whole series comprises 50 renal allografts and includes four which were examined during a clinical rejection episode.

37 per cent of the allografts, the deposits affected all the capillaries examined, but in the majority the change was focal, and normal loops were present. The deposits bulged into the capillary lumina and were covered by an intact endothelial layer (Figs. 11, 14, and 15). The material also extended between endothelial cells (Fig. 14). Really dense deposits in the lamina densa or in the subendothelial layer were

FIG. 8. Enlargement of part of Figure 6. The lamina densa (*ld*) contains an oval, dense deposit (*d*). Between the endothelial cell (*end*) and the lamina densa there is an accumulation of cell fragments (*cf*), organelles (*or*), and fibrin (*fi*) embedded in an amorphous, finely granular material. The cytoplasm of the endothelial cell contains increased numbers of ribosomes (*arrow*). *ep*, Epithelial cell. ×17,700.

FIG. 9. Renal allograft biopsied during a rejection episode which later proved irreversible (patient LD27). A glomerular capillary loop is obstructed by an aggregate of platelets (*pl*) and fibrin (*f*), and by a neutrophil polymorphonuclear leukocyte (*poly*). There appears to be breakdown of the plasma membranes separating the polymorphonuclear leukocyte and the endothelium lining the capillary at the point marked with an *arrow*. There is fusion of the epithelial foot processes (*ep*). *mc*, Mesangial cell. ×7180.

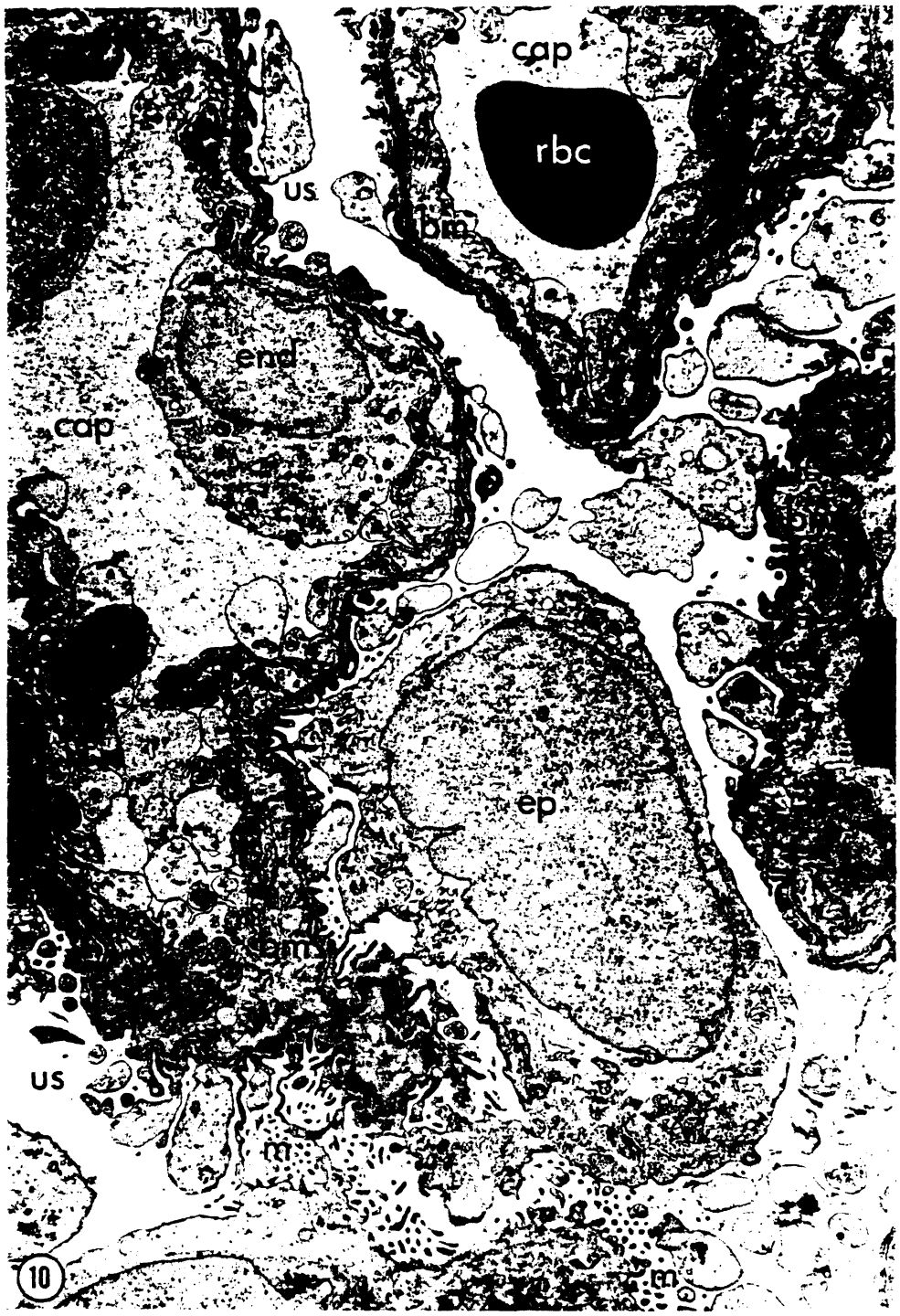


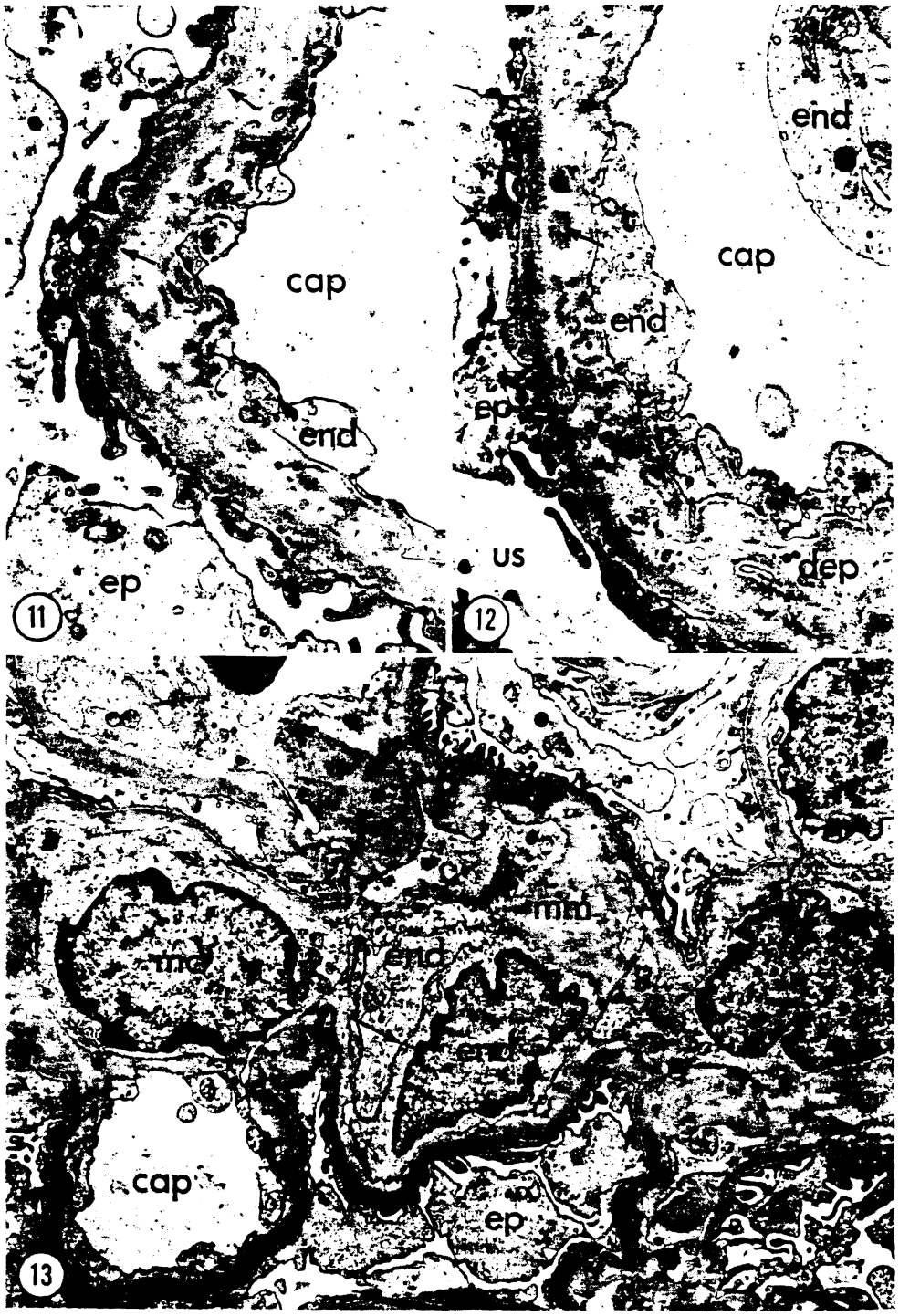
FIG. 10. Biopsy of a renal allograft 1 year 10 months after transplantation (LD41). The basement membranes (*bm*) of three glomerular capillary loops (*cap*) are diffusely thickened by a subendothelial accumulation of amorphous material. The epithelial cells (*ep*) are enlarged and possess microvilli (*m*). *rbc*, Erythrocyte; *us*, urinary space; *end*, endothelial cell. ×6100.

unusual (Fig. 15). Sometimes it was as though the lamina densa had been split and a broad band of loosely arranged material inserted between the two layers (Fig. 16). Fragments of cell cytoplasm and isolated organelles were commonly incorporated in the subendothelial accumulations. In a few allografts the cell fragments were large and, in serial sections, some could be shown to be extensions from mesangial cells; others appeared to be endothelial, and a few resembled platelets (Fig. 17). Subepithelial deposits were rare being present in only three of the 24 kidneys; they were always combined with subendothelial collections. In one patient (SL1) they consisted of small foci with prominent rarefied areas, but in the other two kidneys (M17 and RT) there were dense hemispheric humps on the epithelial side of the capillary basement membranes (Figs. 18 and 19). In almost all of the kidneys the mesangial matrix was increased (Figs. 13 and 20), and in 42 per cent there was hyperplasia of the mesangial cells. The endothelial cells were enlarged in 75 per cent of the transplants; in the five most severely affected kidneys, they were also increased in number. Their cytoplasm was voluminous and was the cause of narrowing of many and obliteration of a few of the capillary lumina (Fig. 13). A few kidneys showed capillary obstruction as a result of impaction and disintegration of neutrophil polymorphonuclear leukocytes; this was particularly a feature of the two kidneys with subepithelial humps (Figs. 21 and 22). Monocytes and lymphoid cells were frequently found in groups filling the capillary lumina. Platelets were sometimes present, but not clumped together. The visceral epithelial cells were enlarged in most of the transplants (Fig. 23); occasionally trinucleate forms were encountered (Fig. 24). In all cases there were areas in which the epithelial foot processes were fused producing a continuous sheet of cytoplasm over the capillary basement membranes (Fig. 25), but only in a few allografts was this change widespread. Epithelial microvilli (Fig. 10) were present in several of the kidneys. In many of the transplants the epithelial, endothelial, and mesangial cells possessed increased numbers of cytosomes, cytosegresomes, and free ribosomes, increased amounts of rough endoplasmic reticulum, and enlarged Golgi bodies. Multivesicular bodies were more common than usual in the endothelial and epithelial cells.

Renal Allografts with Normal Glomerular Capillary Basement Membranes by Light Microscopy (Group B). Of the 22 renal allografts that appeared normal on light microscopy, all were abnormal on electron microscopy in that they showed fusion of their epithelial foot processes (Table 3). In many this was of slight degree and, although more than was seen in the control donor kidney, it was comparable to the amount of change found in the renal isograft. Only 13 (59 per cent) of these kidneys had normal capillary basement membranes; the others all showed small, focal, subendothelial deposits of the same nature as were encountered on a much larger scale in group A (Fig. 26). An appreciable number of the epithelial cells were enlarged; these, together with some of the endothelial and mesangial cells, showed increased numbers of ribosomes, cytosomes, and cytosegresomes. Monocytes and lymphoid cells were more common in the capillaries of these transplants than in those of the isograft or the control donor kidney. Several of the kidneys showed more mesangial matrix than normal.

Correlation of Glomerular Changes with Degree of Compatibility of Donor and Recipient as Shown by Lymphocyte Typing

In 36 of the 50 patients retrospective typing of the lymphocytes of donor and recipient was undertaken. It will be seen from Table 4 that in general the allografts with normal glomeruli came from patients who were regarded as being compatible with their donors by lymphocyte typing, while the majority of grafts with glomerular capillary basement membrane thickening came from patients who were incompatible with their donors in one or more major lymphocyte groups. This association of lymphocyte incompatibility with glomerular damage in the renal graft was statistically significant ($p = 0.02$).



FIGS. 11 TO 13

Association of Glomerular Damage with Alterations in Renal Function

The mean creatinine clearance in the 27 patients with marked glomerular lesions in their allografts was 65.4 ml. per minute with a range of 4 to 139 ml. per minute, compared with a mean of 92.8 ml. per minute with a range of 33 to 184 ml. per minute for the 23 patients without obvious glomerular lesions (Tables 1 and 2). The urine produced by the allografts with glomerular capillary basement membrane thickening contained more protein (mean 84 mg. per 100 ml; range 0 to 350 mg. per 100 ml.) than that excreted by the transplants with normal glomeruli (mean 16.7 mg. per 100 ml.; range 0 to 100 mg. per 100 ml.). The patients with proteinuria over 100 mg. per 100 ml. showed moderately unselective protein loss with an index of selectivity (θ) in no instance lower than 62.4°. There was no obvious correlation between this value and the degree or type of ultrastructural change present in the glomerular capillary basement membranes.

Table 4. Association of Lymphocyte Incompatibility between Donor and Recipient, with Presence of Marked Glomerular Damage in the Renal Allograft[a]

Histopathology	No. of allografts by lymphocyte typing		Total
	Compatible	Incompatible in one or more major groups of antisera	
Allografts showing obvious glomerular capillary basement membrane thickening under light microscopy	4	15	19
Allografts without significant glomerular capillary basement membrane changes	11	6	17
Total	15	21	36

[a] $\chi^2 = 5.45$; $p = 0.02$.

DISCUSSION

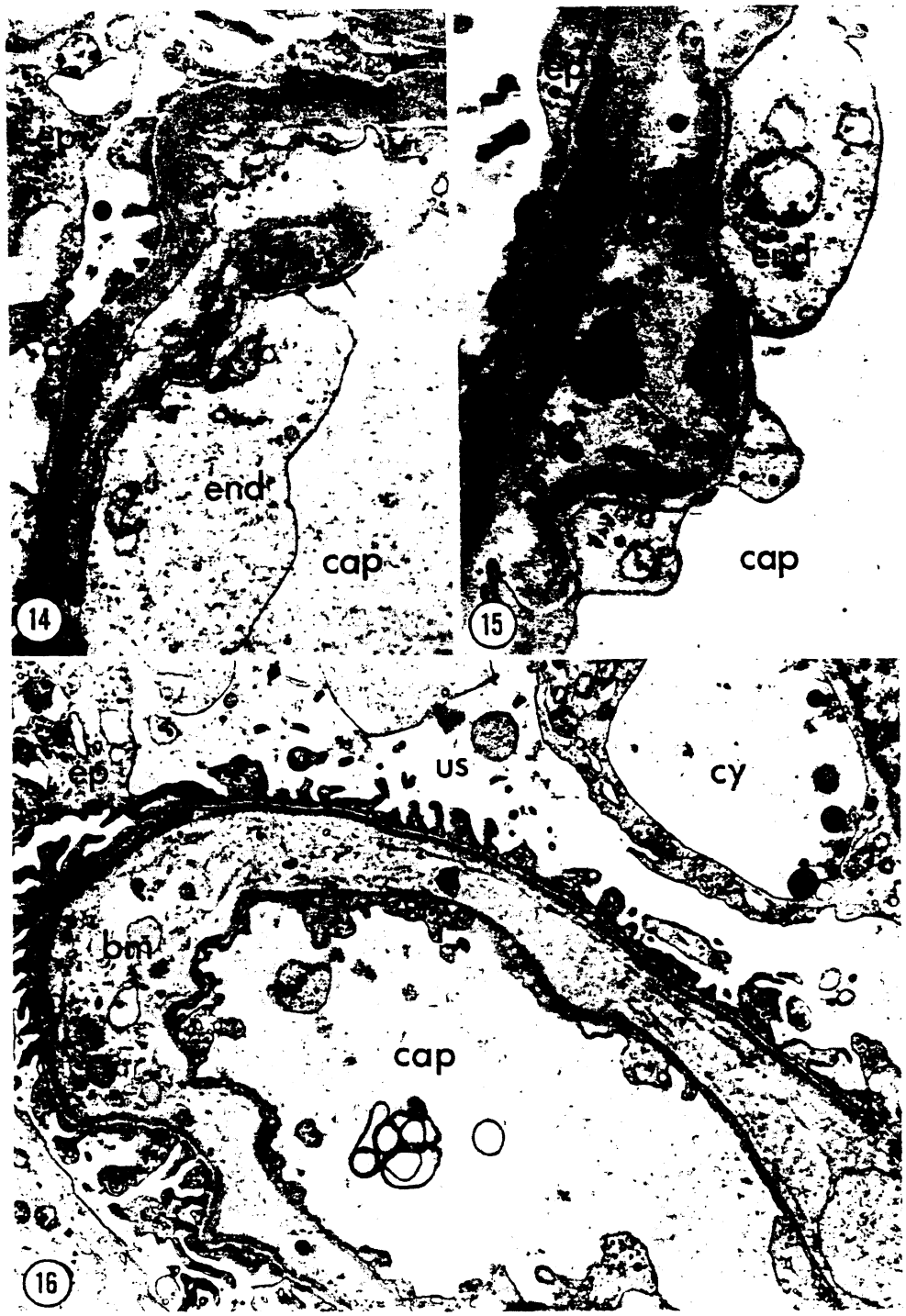
Our findings fully justify the stress that Hamburger and associates[11-13, 15] have placed upon the glomerular changes that may occur in long-surviving renal allografts. Marked lesions were present in many of the glomeruli in 27 of the 50 allogenic kidneys we examined. In the majority the most striking abnormality was an accumulation of amorphous material on the subendothelial aspect of the capillary basement membranes. When present in appreciable amounts the deposit was easily detectable by light microscopy as a PAS-positive thickening of the glomerular capillary wall. This change was accompanied by increased amounts of basement membrane-like material in the mesangium, by hypertrophy of the epithelial and endothelial cells, by fusion of some of the epithelial foot processes, and sometimes by hyperplasia of the mesangial cells.

At present the incidence of these glomerular lesions is unknown. Some indication is given by considering the first Denver series of renal allografts in which 70 kidneys were transplanted into 64 patients. In the following 1¼ years, 34 of these kidneys were examined, either after

Fig. 11. Renal allograft 1 year 9 months after transplantation (LD18). Segment of glomerular capillary wall in which the basement membrane is thickened by a subendothelial deposit of mixed density and compactness. The inner border of the lamina densa is indicated by *arrows*. The lamina interna rara has disappeared. *cap*, Capillary lumen; *end*, endothelial cell; *ep*, epithelial cell. ×13,800.

Fig. 12. Part of a glomerular capillary loop from the same renal allograft as was shown in Figure 10. The subendothelial amorphous deposit (*dep*) varies in compactness; dense areas (*arrow*) are intermixed with paler areas. *us*, Urinary space. ×9200.

Fig. 13. Renal allograft 1 year 10 months after transplantation (LD40). In this kidney it is difficult to distinguish deposit from lamina densa, and the over-all effect is of greatly thickened capillary basement membranes. One capillary (*arrow*) is occluded by enlarged endothelial cells (*end*). The amount of mesangial matrix (*mm*) is increased. *mc*, Mesangial cell. ×3680.



FIGS. 14 TO 16

surgical removal or at autopsy; only two showed glomerular capillary basement membrane thickening.[41] However, 28 of these kidneys had functioned for less than 3 months. Of the 35 long-surviving patients, 34 were biopsied at about 2 years after transplantation and are reported in detail in the present paper. Eighteen biopsies showed glomerular basement membrane changes obvious on light microscopy, an incidence of 53 per cent, and a further six biopsies, 17 per cent, which appeared normal on light microscopy showed minor subendothelial deposits on electron microscopy.

In most patients the glomerular lesions were accompanied by mild proteinuria (Table 1) which usually commenced 6 months or longer after renal transplantation. In a few cases the protein loss was moderately heavy and unselective in pattern, but this was not associated with edema. Microscopic hematuria and hypertension were not features of the syndrome in our patients, but were described by Hamburger *et al.*[11] in three of his patients. One of the French cases (patient 7) also developed hypergammaglobulinemia, splenomegaly, and marked hyperplasia of the Kupffer cells of the liver.[12] In several of our patients there were no accompanying signs or symptoms, and the diagnosis was made only after microscopic examination of a biopsy of the graft. Generally the glomerular filtration rate of these patients was impaired, but there was much individual variation and the values were greatly influenced by the presence or absence of accompanying vascular lesions.

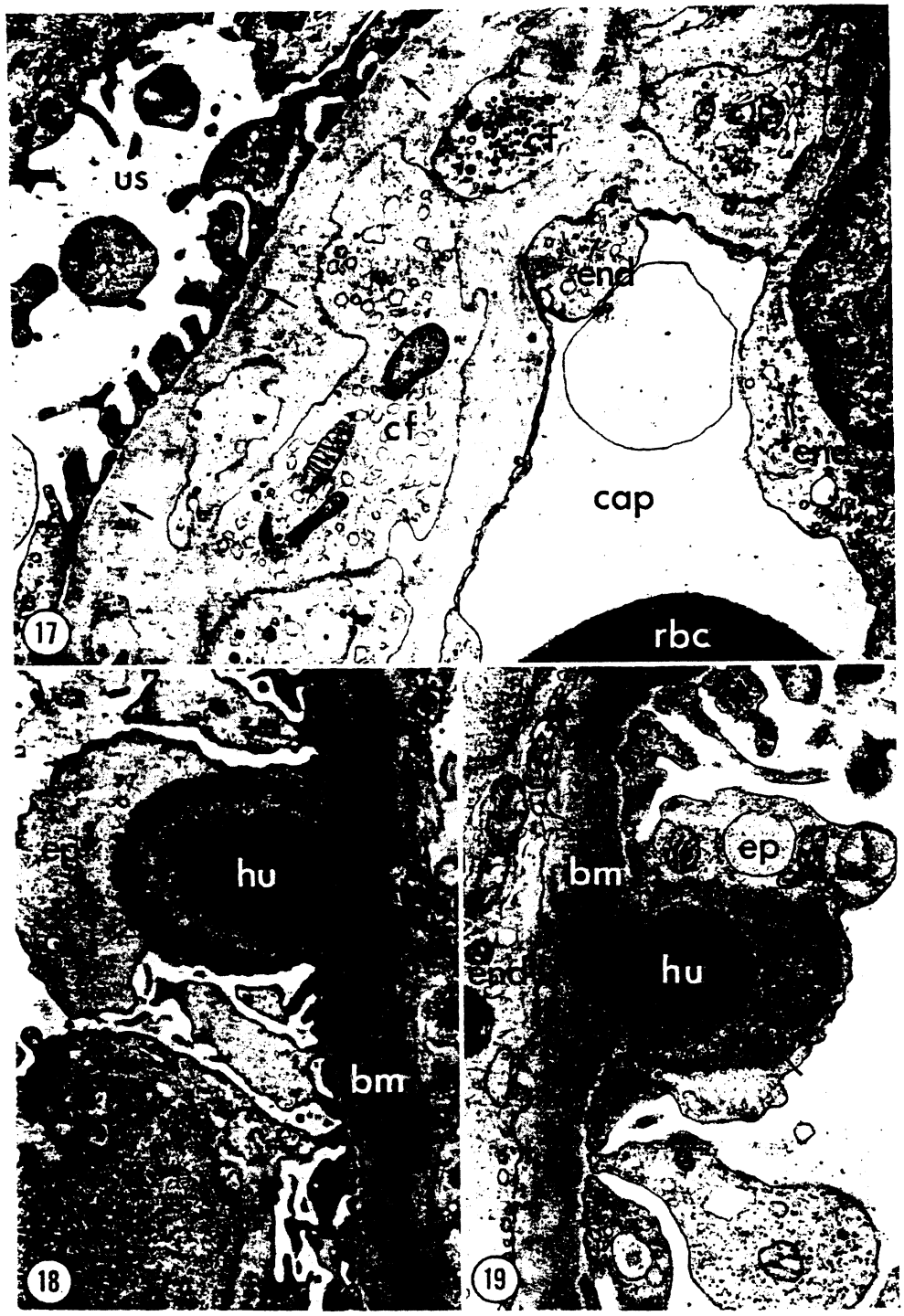
The etiology of the glomerular changes is undetermined. It is tempting to think that they result from the production by the host of antibody which becomes deposited on the glomerular capillary basement membranes of the graft. In support of this hypothesis is the finding of a close correlation between incompatibility of host and donor as shown by lymphocyte typing and the occurrence of severe glomerular lesions. The development of splenomegaly and hypergammaglobulinemia in the patients of Hamburger *et al.*[12] together with reports of rises in the immunoglobulin G levels of other patients[19, 24] and of dogs with renal allografts[4] suggest increased antibody formation by the recipient. Also, subendothelial accumulations of amorphous material, much like those seen in our cases, can be produced in the glomeruli of experimental animals by the injection of nephrotoxic serum,[7, 25] and they occur in the glomeruli of patients suffering from acute glomerulonephritis,[8, 17, 27, 32, 51, 54] the childhood form of lipoid nephrosis,[28] and lupus nephritis.[2] In all these situations the deposits have been shown to contain γ-globulin. However, the fact that similar lesions can be induced in animals by the administration of Freund's adjuvant,[57] synthetic polysaccharides,[34] and ethionine and carbon tetrachloride,[46] and in man by hepatic disease[45] indicates a need for caution in regarding these collections of γ-globulin as necessarily indicating immune injury.

In our patients we have no evidence to suggest that drug-induced injury played any part in the production of the renal changes. It is known that azathioprine does not damage renal autografts when administered for 1 year in doses comparable to those used in this study,[40] and although hydrocortisone-like steroids can

FIG. 14. Renal allograft 1 year 10 months after transplantation (LD63). Small segment of glomerular capillary wall. A band of amorphous material extends between two endothelial cells (*arrow*). *ep,* Epithelial cell; *end,* endothelial cell; *cap,* capillary lumen. ×11,400.

FIG. 15. Part of a glomerular capillary wall from a renal allograft biopsied 2 years after transplantation (LD48). In the amorphous subendothelial deposit there is a roughly spherical focus (*arrow*) which is denser than the lamina densa (*ld*). ×18,800.

FIG. 16. Biopsy of a renal allograft 1 year 11 months after transplantation (LD51). The basement membrane (*bm*) is thickened by a broad band of loosely arranged amorphous material which appears to be sandwiched between two layers of lamina densa. Scattered organelles are incorporated in the deposit. An epithelial cell contains a large cytosome (*cy*). *us,* Urinary space. ×7700.



FIGS. 17 TO 19

produce glomerular lesions,[33] ultrastructurally these are distinctive and bear little resemblance to the changes we have described. Furthermore, a few of our patients with glomerular changes had not been treated with steroids, and Hamburger and associates' patient 5 had not even received azathioprine.[12] Glomerular basement membrane thickening has also been encountered in the renal allografts of 36 per cent of a group of dogs which survived beyond 70 days because of treatment with various immunosuppressive agents.[39] Several of these animals did not receive azathioprine and none were given steroids. It is significant that the glomerular changes were most pronounced in an animal in which the renal allograft functioned for 1½ years after all treatment had been withdrawn.[59]

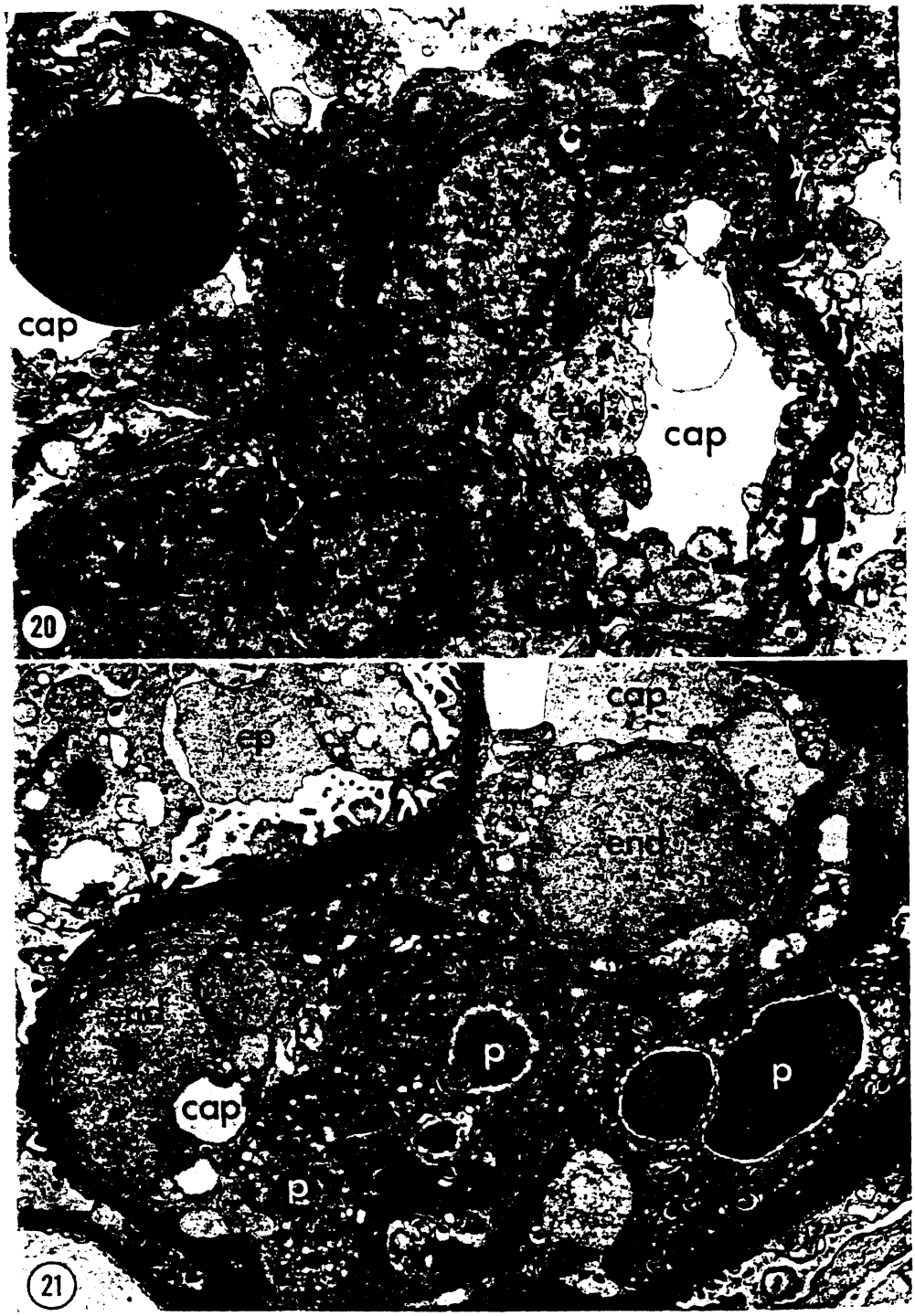
The possibility that the glomerular lesions were the result of the recipient's original disease has to be considered. Of the 27 patients who later developed pronounced basement membrane deposits in their allografts, two suffered from bilateral polycystic disease of their own kidneys and three from chronic pyelonephritis (Table 1). In these five cases it seems improbable that the initial disease contributed in any way to the changes that later occurred in the transplant. The other 22 patients all suffered from glomerulonephritis and could conceivably have subsequently developed this same condition in their allografts. Although experimentally it has not yet proved possible to transmit nephrotoxic serum nephritis to renal allografts,[55] this very complication has been reported in five renal isografts[30] with the production of glomerular capillary basement membrane thickening and capsular crescent formation.[37] If this were the explanation of the glomerular findings in our cases, it is surprising that the lesions were *subendothelial* in five of the allografts which had been transplanted into patients whose original disease was the diffuse membranous form of glomerulonephritis, a condition which would have been expected to produce predominantly *subepithelial* deposits. Because of these findings we think that transmission of glomerulonephritis from the recipient does not explain most of the cases of glomerular damage that are being encountered in long-surviving transplants and that production by the host of antibody directed specifically against the graft is more likely. Two of the 27 patients, however, were in a different category. Not only did their transplants show distinctive, large subepithelial humps, but both these patients were suffering from active glomerulonephritis at the time of renal transplantation. Subepithelial humps are characteristic of poststreptococcal glomerulonephritis,[17, 21] and as both patients developed their nephritis after a streptococcal infection, we feel that in these two cases it is very probable that the changes in the transplant were induced in some way by the recipient's original disease. In at least two other renal allografts, a similar course of events has been postulated.[10, 38]

The prognosis for patients with glomerular lesions in their transplants depends upon the reversibility or otherwise of the subendothelial deposits. It is known

FIG. 17. Biopsy of renal allograft 2 years 1 month after transplantation (LD45). Small segment of glomerular capillary wall in which the basement membrane is thickened by a subendothelial deposit containing large cell fragments (cf^1). Most of the trapped cells appear to be mesangial or endothelial, but a few (cf^2) might be platelets. The inner limit of the lamina densa is marked by *arrows*. *us*, Urinary space; *end*, endothelial cell; *cap*, capillary lumen; *rbc*, erythrocyte. ×13,500.

FIG. 18. Part of a glomerular capillary loop from a renal allograft 1 year after transplantation into a patient suffering from rapidly progressive acute glomerulonephritis (patient RT). There is a dense hemispheric hump (*hu*) on the epithelial side of the thickened basement membrane (*bm*). The foot processes of the epithelial cell (*ep*) are fused over the deposit, and the adjacent epithelial cytoplasm is dark and granular (*arrow*). ×17,000.

FIG. 19. Renal allograft 7 months after transplantation. There is a typical subepithelial hump (*hu*) on the thickened glomerular capillary basement membrane (*bm*). The epithelial cytoplasm surrounding the deposit is dark and granular (*arrow*). ×19,200.



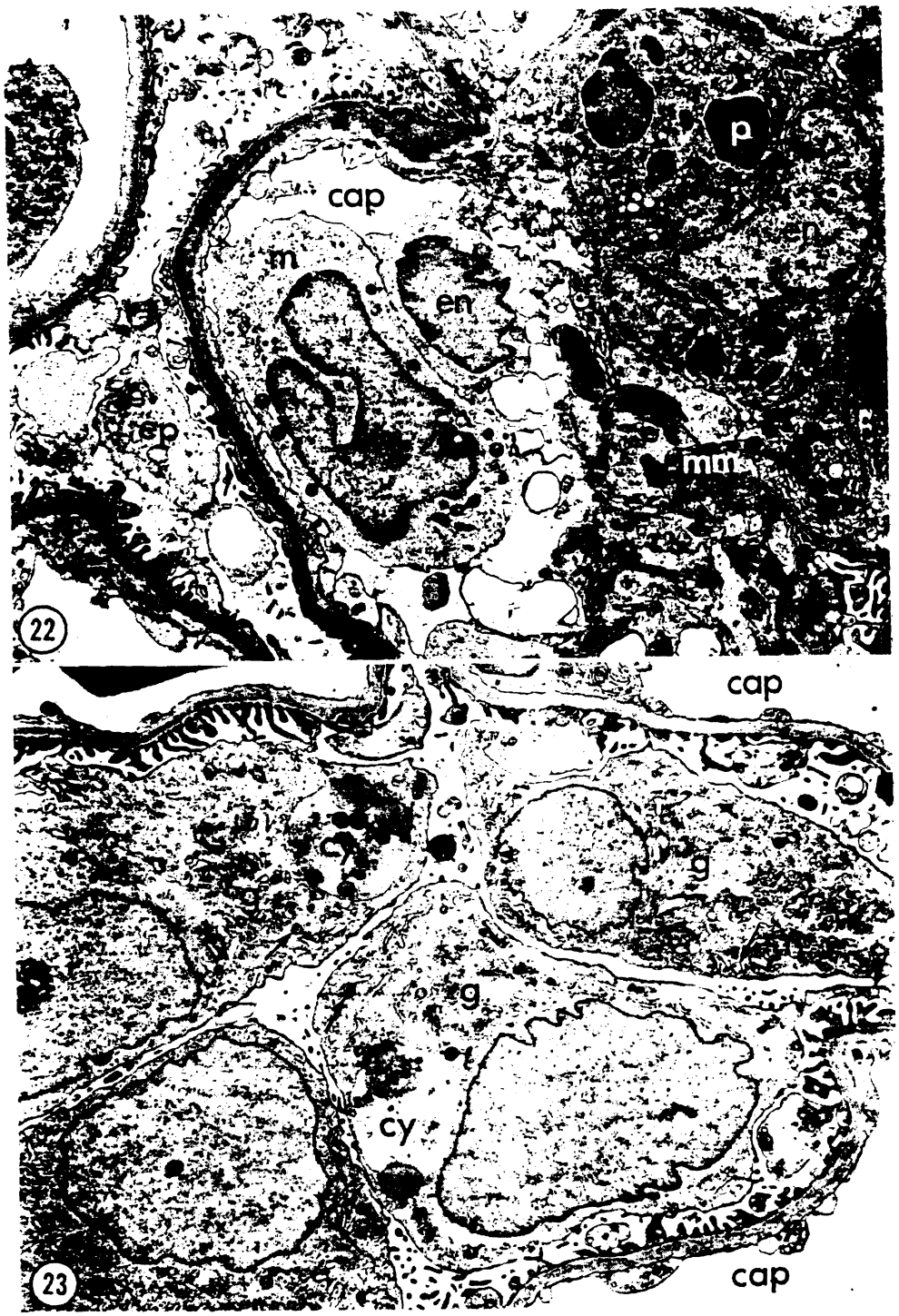
FIGS. 20 AND 21

that accumulations of similar material resolve in acute glomerulonephritis, probably by phagocytosis by endothelial and mesangial cells.[32] However, although two of the three patients with renal allografts, first described by Hamburger *et al.* in 1963,[15] later lost their proteinuria and were well 1 year 6 months and 2 years 10 months later, abnormalities persisted in the fine structure of the glomeruli in their transplants.[13] The third patient developed progressive renal insufficiency and died 10 months later. Among our 27 patients (Table 1), four (15 per cent) have died since biopsy; one of these was a case in which the allograft may have been affected by the recipient's original poststreptococcal glomerulonephritis. In two of the other three patients, progression of the glomerular lesion played a major part in causing the patient's death. A further five renal allografts (18.5 per cent) have had to be removed at periods of up to 7 months after biopsy because of progressive renal failure. Five patients (18.5 per cent) have a creatinine clearance below 50 ml. per minute, and 13 (48 per cent) have a clearance above this value 11 months to 2 years after biopsy. By contrast, none of the renal allografts with minimal glomerular changes have had to be removed in the period since biopsy; there have been no deaths in this group, and 20 (87 per cent) of the patients have a creatinine clearance above 50 ml. per minute. (Table 2). Although it is far too early to be dogmatic, these findings suggest that the prognosis for patients with marked glomerular capillary basement membrane thickening in their transplants may be worse than was first thought.

In this paper further light is also shed upon the mechanism of renal shutdown during clinical episodes of allograft rejection in patients receiving immunosuppressive therapy. The glomerular capillaries of four transplants biopsied during such an episode were found to be obstructed by platelet aggregates, and there was also a subendothelial deposit of amorphous material (which proved to be γ-globulin on immunofluorescence) on the basement membranes. Some platelets were also found to have inserted pseudopods between the endothelial cells onto the altered capillary basement membranes. Platelet thrombi were also present in the glomerular capillaries of Hamburger and associates' patient 17 during a rejection episode which later responded to treatment. A repeat biopsy of this kidney 4 months later no longer contained platelet aggregates.[13] As it is known that platelet aggregation can be induced by contact with antigen-antibody complexes,[1, 26] a possible explanation for these findings is that the arterioles and capillaries of the allografts became coated with antibody and that this layer induced clumping of platelets. Experimentally, it has been shown that platelet aggregates can profoundly affect renal blood flow,[16] and scattered throughout a transplanted kidney they would explain the alterations in water, electrolyte, and creatinine excretion that occur during a rejection episode.[47] *In vitro* adenosine diphosphate-induced platelet aggregation can be quickly inhibited by prednisone,[31] and this may explain why prompt treatment with steroids has been so successful in inducing a diuresis from renal allografts that have shortly before suddenly ceased to function. When aggregation is followed by platelet damage and the appearance of fibrin, then the process is probably irreversible, and our morphologic findings suggest that in rejection episodes resistant to treatment a mixture of amorphous material, probably antibody, together with platelet fragments and strands of fibrin become covered by a new layer of endothelium and incorporated

FIG. 20. Biopsy of renal allograft 1 year 5 months after transplantation (patient EN). The mesangial matrix (*mm*) is increased in amount and the mesangial cells (*mc*) are conspicuous. The capillary walls are thickened by subendothelial deposits of amorphous material (*x*). *rbc*, Erythrocyte; *cap*, capillary lumen; *end*, endothelial cell. ×5600.

FIG. 21. Same renal allograft as was shown in Figure 19. A glomerular capillary (cap^1) is obstructed by three impacted and disintegrating neutrophil polymorphonuclear leukocytes (*p*). Amorphous deposit is present between the polymorphonuclear leukocytes and on the basement membrane (*arrow*). An adjacent capillary (cap^2) is unobstructed. *ep*, Epithelial cell. ×5300.



FIGS. 22 AND 23

into the glomerular capillary wall. Radioactive labeling of platelets in patients with renal allografts has produced further evidence compatible with this hypothesis. Just before a rejection episode, there is a fall in the number of circulating platelets accompanied by evidence of their sequestration in the transplant. In those cases where the rejection yields to treatment, the platelet count returns to normal; in patients with irreversible rejection there is no evidence that the platelets ever leave the graft.[29]

SUMMARY

The light and electron microscopic appearances of the glomeruli are described in 50 renal allografts which were examined 43 days to 2 years 3 months after transplantation into patients receiving immunosuppressive treatment. A renal isograft and a donor's remaining kidney were used as controls.

The glomeruli were hypertrophied in all the kidneys. The control donor kidney showed no other significant changes. There were small focal areas of fusion of the visceral epithelial foot processes and a slight increase of mesangial matrix in the renal isograft.

In the renal allografts the epithelial and endothelial cells were hypertrophied, and there was more fusion of the epithelial foot processes than was found in the renal isograft. Subendothelial accumulations of amorphous material were present on the glomerular capillary basement membranes of 37 of the allografts. In 27 of the kidneys these deposits were large and the basement membrane thickening was obvious under light microscopy; in 10 they were small and produced no thickening visible in ordinary sections. Increased amounts of basement membrane-like material were present in the mesangium of those kidneys with subendothelial deposits, and there was sometimes hyperplasia of the mesangial cells.

These changes in the renal allografts were usually associated with mild proteinuria and impairment of glomerular filtration rate. Occasionally the proteinuria was more severe and unselective in type. A nephrotic syndrome was not produced. There was a close association between the occurrence of marked glomerular deposits and incompatibility of host and donor as shown by lymphocyte typing. Evidence is produced that these lesions are not drug-induced and in the majority are unlikely to have been caused by the patient's original disease. It is suggested that they are caused by deposition of circulating antibody directed specifically against the graft.

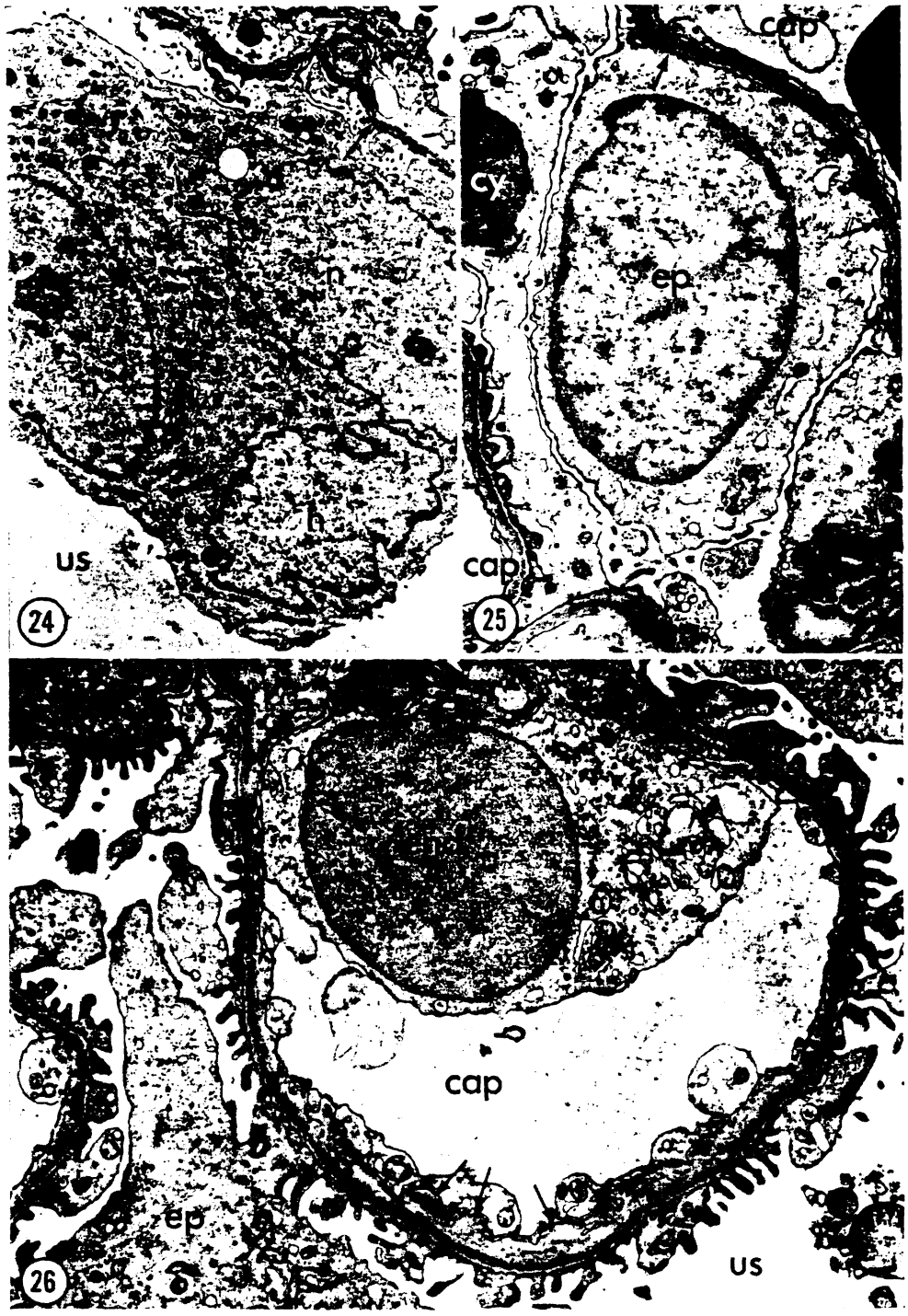
Two of the transplants were exceptions, in that they showed additional, distinctive subepithelial humps, and in these cases it was considered probable that there had been transmission of the recipient's active glomerulonephritis to the allograft.

Four of the renal allografts which were biopsied during a clinical rejection episode contained platelet aggregates in many of the glomerular capillaries. The ischemia caused by this clumping of platelets may explain the alterations in water, electrolyte, and creatinine concentration that are found in patients undergoing acute rejection.

Acknowledgments. The authors are indebted to the Anatomy Department of St. Mary's Hospital and the Nuffield Institute of Comparative Medicine for allowing them to use the electron microscopes donated by the Wellcome Trust

FIG. 22. Same renal allograft as was shown in Figure 17. One glomerular capillary is obstructed by an impacted neutrophil polymorphonuclear leukocyte (*p*); another capillary (*cap*) contains a monocyte (*m*). The basement membranes are thickened by subendothelial deposits (*x*) and the mesangial matrix (*mm*) is increased in amount. *en*, Endothelial cell; *ep*, epithelial cell. ×3980.

FIG. 23. Same renal allograft as was shown in Figure 11. Four hypertrophied epithelial cells are shown. They contain cytosomes (*cy*), enlarged Golgi bodies (*g*), and increased amounts of rough endoplasmic reticulum. Multivesicular bodies (*arrows*) are common. In this area the capillary basement membranes are normal. *cap*, Capillary lumen. ×4480.



FIGS. 24 TO 26

and the Sembal Trust, respectively, and to Dr. J. P. Merrill for permission to report the case of patient LDBS and to Dr. W. T. Newton for permission to use the case report of patient SL1. They would like to thank Dr. J. S. Cameron for undertaking the studies on glomerular permeability and Mr. P. Tyler for invaluable technical assistance.

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FIG. 24. Renal allograft previously shown in Figures 10 and 12. A trinucleate visceral epithelial cell is shown. Its cytoplasm contains many ribosomes and much rough endoplasmic reticulum. The adjacent capillary basement membrane is greatly thickened (*arrows*) by a subendothelial deposit. *n,* Nucleus; *us,* urinary space. ×4040.

FIG. 25. Biopsy of renal allograft 1 year 10 months after transplantation (LD60). There is fusion of most of the foot processes (*arrows*) of a glomerular epithelial cell (*ep*). Another epithelial cell contains a cytosome (*cy*). In this area the capillary basement membranes are normal. *cap,* Capillary lumen. ×7150.

FIG. 26. Renal allograft 1 year 10 months after transplantation (patient KF). Under light microscopy the glomerular capillary basement membranes appeared normal. In this electron micrograph small, focal, subendothelial deposits can be seen (*arrows*), which are of the same nature as those encountered on a larger scale in allografts which had thickened capillary basement membranes by light microscopy. *end,* Endothelial cell. ×7550.

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