Human Renal Allografts

Interpretation of Morphologic and Immunohistochemical Observations

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PREVIOUS STUDY of the pathophysiology of renal allograft rejection in unsensitized, sensitized, and immunosuppressed inbred rats under controlled immunogenetic conditions has provided information that may be useful in delineating correlates of the morphologic features of human renal allograft rejection and in interpreting the significance of these features in a dynamic framework. These studies in the rat have demonstrated a relatively invariable pattern of rejection, and immunopathologic and physiologic parameters have been elucidated.[1-6] In the present paper, the results of morphologic and immunofluorescence study of biopsy specimens from human recipients of renal allografts are presented, and the significance of these results is interpreted on the basis of experimental experience with renal allograft rejection in the rat.

Materials and Methods

A total of 71 tissue specimens from 42 renal allografts in 41 patients were available for morphologic study. The material studied was only a portion of the renal allograft tissue available at our hospital. The material was selected at random and usually because tissue was examined by one of us (R.R.L.) at the time of biopsy or necropsy. Paraffin-embedded tissue fixed in either 10% formalin or 95% cold alcohol was used to prepare 5- to 6-μ sections which were stained with hematoxylin and eosin (H&E), and Schiff's leukofuscin after periodic acid oxidation (PAS). Mallory's trichrome connective tissue stain, Van Gieson's elastic tissue stain, and methyl green pyronine (MGP) stains were done on selected specimens. Additional tissue was occasionally fixed in osmium tetroxide, embedded in epoxy resin, and used to prepare 1-μ sections, which were then stained with toluidine blue O.

For collecting the data to be presented in this paper, all the slides from each patient were examined together, without knowledge of the name or clinical status

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Table 1. Morphologic Alterations in Human Renal Allografts

Pt. & biopsy No.		Day*	Interstitium					Vessels				Glomeruli				BUN (mg./100 ml.)	C_{cr} (ml./min.)	Subsequent course
			IC	Ed	He	MC	FI	AA	VT	OL	TA	Th	M/EH	CwT	MS			
1 V.S.D.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
	2	164	0	0	0	+2	+2	NA	0	NA	0	0	0	0	0	33	41	Died 9.5 mo. later, pancreatitis
2 M.L.S.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0			Alive; chronic rejection; stable 2.5 yr. with Ccr of 28 ml./min.
	2	270	0	0	0	0	+1	0	0	+1	0	0	0	+2	+2	24	77	
3 R.S.D.	1	25	—	—	—	—	—	+	+	—	—	—	—	—	—	144	8	Necrotic graft
	2A	40	+1	+3	+1	+1	±	?	+	†	—	0	0	+2	0	150	12	Died Day 40
4 B.S.R.	1	194	0	0	0	0	0	NA	0	NA	0	0	0	0	0	29	56	Died after 33 mo.; chronic reject.
5 R.C.A.	1	437	0	0	0	0	+1	0	0	NA	+1	0	0	+1	+1	20	66	Alive; early clin. reject.; at 3 yr., function normal
6 M.S.N.	1	22	+2	+1	0	0	0	0	0	FC	0	0	0	0	0	140	18	Died at 6 mo.; kinked renal artery
7 W.M.E.	1	30	+2	+2	0	0	0	+	0	NA	0	0	0	0	0	30	39	Alive; clin. diagnosis of reject.; at 2½ yr., function good
8 R.D.O.	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	46	50	Died at 9 wk.; sepsis
9 R.R.N.	1	30	0	0	0	0	+1	0	0	0	0	0	PMN	0	0	112	4	Alive 2¾ yr.; good function
10 J.R.G.	1	12	+1	0	0	+1	0	0	0	NA	0	0	+3	0	0	100		
	2	179	+2	0	0	+1	+2	0	0	0	+2	0	Ax	+1	+2	29	71	Alive 2 yr.; good function
11 D.R.S.	1	28	+1	+1	0	+1	0	0	0	0	0	0	0	0	0	20	56	Alive; chronic reject.; Ccr of 29 ml./min. at 2½ yr.
	2	40	0	0	0	0	0	0	0	0	0	0	0	0	0			
12 R.B.N.	1	180	0	±	0	+1	+3	0	0	NA	0	+3	0	+1	0			Died at 6 mo.
13 L.B.H.	1	303	0	0	0	+1		0	0	+2	0	0	0	+2	+2	27	77	Alive; good function; at 4 yr., Ccr of 65 ml./min.
14 W.M.X.	1	327	0	0	0	±	+4	0	0	NA	+4	±	0	+2	+2	32	23	Died at 13 mo.
15 R.H.K.	1	282	±	0	0	+3	+3	0	0	0	+3	0	0	+2	+2			Alive
16 R.G.D.	1	17	0	+3	0	0	0	0	0	0	0	0	0	0	0			Died, sepsis 1 mo.; major blood group mismatch
17 D.M.R.	1	500	0	0	0	+1	+1	0	0	NA	+1	0	0	±	0	39	38	Alive; good function; Ccr of 80 ml./min. at 4½ yr.
18 D.P.M.	1	252	+2	0	0	±	+2	0	0	+2	0	0	0	+2	0	36	39	Died on Day 350, pneumonia & pancreatitis
	2	283	±	0	0	+1	+1	0	0	+2	+1	0	0	+2	0			
	3A	350	0	+1	+1	0	0	0	0	+2	0	+2	0	±	0			

19 S.W.G.	1	303	0	0	0	+1	+1	0	0	0	+1	0	0	0	±	23	70	Alive; good function; Ccr, 78 ml./min.
	2	660	+1	0	0	+1	+1	0	0	+1	+1	0	0	Wr	0	29	87	at 3 yr.
20 H.H.T.	1	98	0	0	0	+1	+1	0	0	+1	0	0	0	+1	0			Alive; chronic reject.; kidney removed
	2	183	0	0	0	+1	+1	0	0	0	+1	0	0	+2	0			at 1 yr.
	3	355	0	0	0	+1	+1	0	0	NA	+1	0	0	+3	0			
	4	740	0	+2	0	+1	+1	+	±	+2	+1	0	0	‡	0			
21 E.L.O.	1	150	±	0	0	+1	+1	0	0	+4	+1	+1	0	0	0	42	38	Alive; good function; Ccr, 70 ml./min. at 2½ yr.
22 J.W.E.	1	210	0	0	0	+1	+2	0	0	0	0	0	0	§	0			Alive; good function; Ccr, 80 ml./min. at 3 yr.
23 H.R.E.	1	49	+4	+1	+2	0	0	0	0	0	0	0	0	0	0	45	29	Alive
	2	542	0	0	0	+2	+3	0	0	+2	+1	0	0	0	+1	30	57	Chronic reject. with nephrotic syn-
	3	2½*	0	0	0	+1	+3	0	0	+2	+2	0	0	+2	0	50	30	drome; kidney removed, 2½ yr.; no
	4	3*	0	0	0	+1	+3	0	0	+2	+2	0	0	+2	+1			immunosuppression 1 mo. prior to
	5	3½*	+3	0	0	+1	+3	0	0	+3	+2	0	0	+4	+4			removal of kidney
24 C.S.I.	1	4	+2	0	0	0	0	0	0	0	0	0	0	0	0	78		Alive; chronic reject. with nephrotic
	2	274	0	0	0	±	±	0	0	0	±	0	0	+1	0	24	87	syndrome; kidney functioned poorly
	3	660	0	0	0	+1	+1	0	0	0	+1	+1	0	+3	0	20	90	at 3 yr.
	4	3*	0	0	0	+1	+1	0	+	0	+1	+2	0	+3	0	87	32	
25 T.G.I.	1A	160	0	0	0	±	+3	0	0	+3	+1	0	0	0	0			Died, CVA, 5½ mo.
26 S.K.F.	1	60	+1	‡	?	0	?	?	+	Or	0	+1	0	0	+2	100	16	Died; chronic reject. 2½ mo.
	2A	70	+1	+2	+	0		+	?	+4	0	+4	0	0	0	110	9	
27 P.G.S.	1	12	+2	+3	+	0	0	+	0	0	0	0	+2	0	0	6		Died, 1 mo.
	2	28	+1	+4	0	0	0	0	0	Ea	0	+2	0	0	0	84		
28 G.J.L.	1	5	+1	0	0	0	0	0	0	0	0	+2	0	0	0	49		Died, cryptococcal meningitis, sepsis,
	2A	79	0	+3	0	+1	Ea	±	0	0	0	0	0	0	0	52	12	& hepatitis, 2½ mo.
29 J.J.S.	1	10	+1	+1	0	0	0	+	+	0	0	0	0	0	0	174		Died; chronic reject. 1 yr.
	2	22	+2	+1	Fo	0	0	0	0	0	0	0	+4	0	0	91		

Key to abbreviations in table heads:

Interstitium:
- IC immature cells
- Ed edema
- He hemorrhage
- MC mature cells
- Fi fibrosis

Vessels:
- AA acute arteritis
- VT venous thrombosis
- OL obliterative lesions
- TA tubular atrophy

Glomeruli:
- Th thrombi
- M/EH mesangial and/or endothelial hypercellularity
- Cwt capillary wall thickening
- MS mesangial sclerosis

Key to abbreviation in body of table:
- A autopsy
- NA arteries not present in biopsy
- FC foam cell
- Fo focal
- Ea early
- Or organization of fibrin
- Wr wrinkled glomerular capillary walls
- PMN polymorphonuclear leukocyte accumulation
- Ax axial hypercellularity

* Figures marked with asterisk denote years. † +3 to +4. ‡ +2 to +3. § +1 to +2.

(Continued)

Table 1. Continued

Pt. & biopsy No.		Day*	Interstitium					Vessels				Glomeruli				BUN (mg./ 100 ml.)	C_{cr} (ml./ min.)	Subsequent course
			IC	Ed	He	MC	FI	AA	VT	OL	TA	Th	M/EH	CwT	MS			
30 C.M.R.	1	4	+2	+1	+4	0	0	0	0	0	0	+1	+3	0	0			No function; on dialysis; graft removed
31 J.A.O.	1	60	+1	+4	+4	+1	0	0	+	+2	0	0	0	+1	0			Died, 74 days
32 R.T.A.	1	6	+1	0	0	0	0	0	0	0	0	0	+3	0	0	124	3	Died, septicemia at 35 days with improving function
33 J.P.A.	1	40	+2	+3	0	0	0	0	+	NA	0	0	0	0	0			Alive; developed metastatic carcinoma
	2	228	0	0	+1	±	+2	0	Or	+4	+2	0	0	+2	+2			of lung in kidney transplant; im-
	3	590	±	+2	+4	±	±	+	+	+2	+2	+2	0	+1	+2			munosuppression withdrawn & kid-
	4	21¶	+3	±	0	+2	+2	0	0	+2	+2	0	0	0	+1			ney & tumor rejected at 19 mo.
34 M.J.N.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0			Alive; chronic reject. 2½ yr.; severe
		150	0	+3	0	+	0	0	0	0	0	0	0	0	+2	52	34	renal failure
35 R.H.N.	1	11	0	+2	+1	0	0	0	0	0	0	0	0	0	0	54	9	Died; subdural hematoma, 15 days
36 B.T.N.	1	264	0	0	0	±	±	0	0	0	0	0	0	0	0	26	70	Alive; good function, 4 yr.
37 K.R.H.	1	297	0	0	0	+1	+1	0	0	0	0	0	0	±	0			Alive; good function, 4.3 yr.
38 P.D.K.	1	46	0	+2	0	±	0	0	0	0	0	0	0	0	0	96	20	Died, 2.5 mo., sepsis
	2A	81	0	+3	+1	+	0	0	0	0	0	+1	0	0	0	195	8	
39 A.H.N.	1	195	±	+4	0	0	0	+	+	0	0	0	0	0	0	15	28	Died, 12 mo., sepsis
	2A	373	0	+3	0	0	Ea	0	0	+2	0	0	0	+1	0	23	52	
40 F.C.S.	1		0	0	0	+1	±	0	0	0	+1	0	0	0	+1			
	2	742	0	0	0	+1	+1	0	0	0	+1	0	0	+2	+2			
	3A	3	0	0	0	0	+2	0	0	+3	+2	0	0	+3	+2	37	19	Died, cryptococcosis
41 R.R.S.	1	93	0	0	0	±	+2	0	0	+3	0	0	0	+2	0	37	19	Died, 16 mo.; chronic reject.

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* Figures marked with asterisk denote years. † +3 to +4. ‡ +2 to +3. § +1 to +2. ¶Second graft.

of the patient. Semiquantitation was attempted in evaluating some of the morphologic alterations by grading 70 specimens on a +1 to +4 scale: +1 corresponding to mild alteration and +4 to severe alteration. Although this attempt at quantitation is not absolute, the values are probably valid as a measure of relative severity within the group. No attempt at quantitation was made for tissue from patients with acute arteritis or venous thrombosis. In computing the incidence of a morphologic alteration, ± and +1 alterations were regarded as no alteration. It is realized that sampling errors can occur when evaluation is of a small amount of biopsy material, and this error is occasionally reflected in some inconsistencies of the data.

Frozen tissue of 33 biopsies from 21 patients was available for localization of immunoglobulin G (IgG), β_{1c}-globulin (C'3), and fibrin by the fluorescent antibody technique,[7] using methods previously described in detail.[2]

Results

The morphologic features of renal allograft rejection and their incidence, as well as data on the functional status of the graft at the time of biopsy and its subsequent course, are presented in Table 1.

Interstitium

The principal interstitial alterations occurring in human renal allografts examined are (1) infiltration by immature mononuclear cells, (2) infiltration by mature mononuclear cells, (3) edema, (4) hemorrhage, and (5) fibrosis.

The immature cells infiltrating the renal interstitium are large, with basophilic and pyronophilic cytoplasm, and a large central or slightly eccentric vesicular nucleus with a coarsely outlined nuclear membrane and containing one or two large, irregularly shaped nucleoli (Fig. 1 and 2). Mitotic figures in these large mononuclear cells are not infrequent. When the cellular infiltration is sparse, these mononuclear cells show a predilection for perivascular areas. Even when the infiltration is diffuse, the cells are usually more dense in perivascular, and frequently periglomerular, areas. Only rare cells contain IgG demonstrable by fluorescein-labeled rabbit anti-human IgG staining. The large mononuclear cells are occasionally seen in peritubular capillaries, and some cells in peritubular capillaries are adherent to vascular endothelium.

The renal interstitium also may be infiltrated by mature lymphocytes and plasma cells. The mature cells usually accumulate in focal collections around vessels or in areas of interstitial fibrosis and tubular atrophy. IgG is demonstrable in some mature cells by immunofluorescence methods.

Interstitial edema occurs both with (Fig. 6) and without interstitial hemorrhage (Fig. 5). Mononuclear cells usually are relatively sparse in areas of interstitial edema. The edema fluid contains abundant IgG,

globulin, and fibrin, as demonstrated by immunofluorescence techniques and is represented by diffuse fluorescence.

When rejection is expressed by interstitial hemorrhage (Fig. 6–8), extensive tubular necrosis is frequent. Rupture of peritubular capillaries with extravasation of erythrocytes into the interstitium is seen infrequently. Thrombosis of small- and medium-sized veins is a frequent finding associated with interstitial hemorrhage (Fig. 13–15). Immature and mature mononuclear cells are usually uncommon in areas of interstitial hemorrhage. Polymorphonuclear leukocytes may be scattered throughout these areas of hemorrhage.

Interstitial fibrosis, when present, may be patchy or diffuse (Fig. 3). Atrophic tubules and tubules with thickened basement membranes are frequently found in areas of interstitial fibrosis (Fig. 4). These areas usually contain a variable number of mature lymphocytes and plasma cells.

Vessels

Acute necrotizing arteritis was seen in eight of the examined renal allograft specimens. Arteries of various sizes contain eosinophilic, PAS-positive, and slightly granular material—i.e. fibrinoid, deposited in and not infrequently around vessel walls (Fig. 9 and 10). Smooth-muscle-cell nuclei of the arterial media are frequently pyknotic or karyorrhexic. Immunofluorescence methods reveal deposition of IgG, β_{1C}-globulin, and fibrin in the walls of vessels exhibiting necrotizing arteritis. IgG and β_{1C}-globulin are localized as semidiscrete deposits in the vessel wall (Fig. 11), and fibrin is located diffusely throughout the vessel wall, frequently extending into the surrounding perivascular area (Fig. 12).

Arteries in long-surviving renal allografts frequently show obliterative lesions with intimal thickening and luminal narrowing (Fig. 16–21). The intimal thickening is composed of either dense or loose connective tissue. Lipid and occasionally fibrin may be seen in the intimal thickening (Fig. 17 and 20). The medial layer of arteries with obliterative lesions may show atrophy and loss of smooth-muscle cells; disruption and reduplication of the internal elastic lamellas is usual (Fig. 19). Immunofluorescence study infrequently shows some focal IgG localization in the wall of these vessels or on the endothelium (Fig. 22).

The deposition of hyaline material in the walls of arterioles is also frequent in long-surviving renal allografts. IgG fibrin with β_{1C}-globulin is occasionally seen in this benign hyalinosis. Similar immunofluores-

cence findings, however, are seen in nontransplanted kidneys with hyaline deposits within arteriolar walls.

Glomeruli

Although swelling of glomerular endothelial and mesangial cells is seen frequently in renal allografts, glomerular hypercellularity with proliferation of endothelial and mesangial cells is less frequent. Figure 25 illustrates a proliferative glomerulopathy developing 22 days after renal allotransplantation. Immunofluorescence examination of this biopsy did not reveal IgG or β_{1C}-globulin localization at this time. A second glomerular alteration, also interpreted as an acute phenomenon, seen in renal allografts consists of platelet and fibrin thrombi within glomerular capillaries (Fig. 23 and 24). These alterations usually are local and focal, involving only a portion of some glomerular tufts.

Glomerular alterations are common in long-surviving renal allografts, and several morphologically different alterations are seen (Fig. 27–37). Least frequent are glomerular capillary walls with a wrinkled or wavy appearance (Fig. 31 and 33). Usually only some portions of the glomeruli show this alteration, and associated mesangial matrix increase and capillary wall thickening is usual. The glomeruli exhibiting these capillary wall undulations not infrequently are partially collapsed. A more common glomerular alteration seen in renal allografts consists of a diffuse membranous glomerulopathy characterized by diffuse thickening of glomerular capillary walls (Fig. 27 and 28). In some cases capillary wall thickening is marked, and it severely compromises the patency of glomerular capillaries. Focal membranous and mesangial glomerulopathy with irregular thickening of glomerular capillary walls and increase in mesangial matrix is also a common glomerular alteration in long-surviving renal allografts (Fig. 30).

The different morphologic types of glomerular alterations cannot be separated by immunofluorescence examination. Several patterns of IgG, β_{1C}-globulin, and fibrinogen localization are seen by immunofluorescence methods. The most frequent pattern consists of slight-to-moderate amounts of IgG and β_{1C}-globulin localization on glomerular capillary walls in a granular and linear fashion (Fig. 32). Mesangial areas frequently also contain IgG and β_{1C}-globulin. Only slight-to-moderate amounts of fibrinogen, more often in mesangial areas then on capillary walls, is detectable in this immunofluorescence pattern. A second general pattern of immunofluorescence consists of slight IgG and β_{1C}-globulin localization on capillary walls, associated with pronounced fibrin localization on capillary walls and mesangial regions (Fig. 34 and 36). The

third and least frequent immunofluorescence pattern is abundant IgG and β_{1C}-globulin localization on glomerular capillary walls in a pronounced linear fashion (Fig. 35 and 37). Some glomeruli show no evidence of host protein localization.

Tubules

Acute tubular alterations in renal allografts are varied and common, and consist of hyaline droplet formation, cytoplasmic vacuolation, isolated necrosis of tubular cells, and extensive tubular necrosis. Chronic alterations include tubular atrophy, thickening of tubular basement membranes, flattening of tubular epithelial cells, loss of brush borders, tubular casts, and multinucleated epithelial cells. Host proteins are located by immunofluorescence methods in tubular casts, in tubular epithelial cells along the free border of these cells, within epithelial cells diffusely, and as discrete granules within epithelial cells.

Discussion

Examination of human renal allograft rejection reveals several distinctive morphologic features that can serve as the basis for a tenative morphologic classification of renal allograft rejection. This classification is as follows:

- Acute allograft rejection characterized by
  - Interstitial infiltration by immature mononuclear cells
  - Interstitial edema
  - Interstitial hemorrhage
  - Acute necrotizing vasculitis
  - Acute glomerulopathy
    - Proliferative glomerulopathy
    - Thrombotic glomerulopathy
- Chronic allograft rejection characterized by
  - Interstitial infiltration by mature mononuclear cells
  - Interstitial fibrosis and tubular atrophy
  - Chronic obliterative arteritis
  - Chronic glomerulopathy
    - Diffuse membranous glomerulopathy
    - Membranous and mesangial glomerulopathy
    - Undulating membranous glomerulopathy

It should be emphasized, however, that a single allograft may contain several features of rejection. By using the experience gained from immunogenetically controlled renal transplantation in sensitized, unsensitized, and immunosuppressed inbred rats, the significance of some of these morphologic patterns of rejection can be postulated.

Acute Renal Allograft Rejection

Characterized by Interstitial Immature Mononuclear Cell Infiltration. This corresponds to rejection seen in the unsensitized nonimmunosuppressed inbred rat. In man, then, this pattern is probably an expression of rejection in an inadequately immunosuppressed, unsensitized host. The mononuclear cells with abundant cytoplasmic RNA and free ribosomes, large vesicular nuclei, and one or more prominent nucleoli accumulate first in perivascular areas and later infiltrate the cortex diffusely so that they surround individual tubules and glomeruli. They probably reach the renal interstitium initially by way of peritubular thin-walled vessels from intravascular mononuclear cells, as well as originating from donor cells contained in the interstitium. From studies with labeled cells, it appears that at least some of these mononuclear cells are seen within peritubular capillaries and transversing peritubular capillaries to reach the renal interstitium in rejecting rat renal allografts where they actively proliferate.[8-11] The results of the present study suggest a similar series of events in human renal allografts. Although these cells may be engaged in protein synthesis, as indicated by abundant free ribosomes, the presented immunohistochemical data suggest that they are not engaged in antibody synthesis. The rarity of IgG in these human cells confirms previous experience with rejecting rat renal allografts where no IgG or IgM was detected immunohistochemically in the immature mononuclear cells during rejection.[2] We have previously proposed that these cells are engaged in protein synthesis necessary for growth and replication. The in-vitro studies of Sell, Rowe, and Gell[12] on lymphocytes transformed following exposure to phytohemagglutinin, staphylococcal filtrate, antiallotype serum, or heterologous antiserums to homologous serum showed blast forms characterized by morphologic features similar to the mononuclear cells seen in rejecting renal allografts supports this view; for although the cells studied by these authors synthesize abundant protein, no IgG synthesis was demonstrated using autoradioimmunoelectrophoresis, and no IgM was found using an autoradio-Ouchterlony test. The possibility of these mononuclear cells infiltrating the renal interstitium producing nonconventional immunologic graft damage—i.e., nonantibody-mediated damage analogous to that seen in target cell cytotoxicity exhibited by phytohemagglutinin-stimulated nonimmune allogeneic lymphocytes[13-15]—seems less likely in view of the absence of intimate cell contact between the infiltrating cells and parenchymal cell of the graft.

The complete reversal of rejection, characterized by interstitial infiltration by immature mononuclear cells in dogs after retransplantation of rejecting renal allografts back into the original donor,[16] suggests that the cellular infiltrates are reversible and not necessarily harmful to the graft. However, it must be remembered that in the reported experiments the recipient's cells infiltrating the graft become an allograft after retransplantation to the original donor and can themselves be rejected. Even though we do not consider the infiltrating cells necessarily harmful to the parenchymal portion of the organ graft in a conventional immunologic sense, their simple presence may add to increased resistance to blood flow by plugging and/or compressing vessels, thus leading to renal ischemia. The electron microscopic studies of these cells in the rejecting rat renal allografts [17] leads us to suggest that some of these cells are actively engaged in collagen production and produce eventual interstitial fibrosis. Thus, the potential for these large blast cells to become fibroblasts and acquire another function is suggested. Patient 23 provides support for this speculation. Biopsy 1, done on the forty-ninth post-transplant day, shows a very extensive interstitial infiltration by immature mononuclear cells which disappear after appropriate therapy, leaving normal renal tubules surrounded by a moderately extensive interstitial fibrosis. While some interstitial fibrosis seen in human renal allografts probably can be explained on the basis of renal ischemia secondary to obliterative arteritis, in this patient interstitial fibrosis was out of proportion to moderate obliterative arteritis, and renal arteriograms showed only moderate irregular narrowing.[18]

Characterized by Acute Proliferative Glomerulopathy. This is a feature of renal allograft rejection in the unsensitized rat. Immunohistochemical studies in the rats suggests that this glomerulopathy is initiated by the nonimmunologically directed deposition of immune complexes consisting of possible organ-specific and transplant antigens and antibodies with bound complement in the glomerulus. Although one of the human biopsy specimens with proliferative glomerulopathy, examined by immunohistochemical techniques, did not show IgG localization, a subsequent biopsy specimen from this patient did show fine granular deposits of IgG throughout the glomerulus, suggesting immune deposits.

The functional significance of proliferative glomerulopathy is reflected in the poor renal function of all 5 patients showing this glomerular alteration. A second biopsy specimen from Patient 10 showed resolution of the proliferative glomerulopathy, with the return of good

renal function. Patient 29, however, had slowly progressive uremia with a creatinine clearance (C_{cr}) of 5 ml./min.

Characterized by Platelet and/or Fibrin Plugging of Glomerular Capillary Loops. This is seen in both presensitized and unsensitized acute rat allograft rejection. It is not known if clumping of platelets and fibrin deposition is due to contact with antibody-antigen complexes, to contact with damaged endothelium, or for hemodynamic reasons. Although the platelet clumping may be reversible by administration of corticosteroids and perhaps by anticoagulation, the electron microscopic study of Porter *et al.*[19] indicates that when fibrin is deposited along with platelets, the process is probably irreversible, and this material becomes incorporated into glomerular capillary walls. The repeated glomerular deposition of platelets and fibrin, and their incorporation into glomerular capillary walls, may be a pathogenetic mechanism leading to the chronic membranous glomerulopathy seen in long-surviving renal allografts. Further evidence for a pathogenetic role of fibrin and its degradation products in chronic glomerular lesions has been suggested.[20,21]

Characterized by Interstitial Hemorrhage and Edema. These rejection patterns are seen in previously sensitized rats [5] and are considered as examples of accelerated rejection in man. Since most transplant recipients received numerous blood transfusions prior to transplantation, presensitization of human kidney recipients to donor tissue may occur by contact with leukocytes [22] or platelets [23] in blood transfusions or during prior pregnancies. In addition, the work of Chase and Rapaport [24] and Rapaport and Chase [25] suggest that certain bacterial antigens may sensitize the host to subsequent tissue allografts. The relatively high incidence of previous sensitization of renal transplant recipients has been emphasized recently by Terazaki, Thresher, and Hauber,[26] who found preformed antibodies which were cytotoxic to donor cells in 23% of male and 40% of female recipients. Marked interstitial edema with relatively few infiltrating mononuclear cells is one of the two major distinctive morphologic patterns of rejection seen in presensitized rats and, if experience with renal allografts in the inbred rat is applicable to man, this pattern in man indicates an accelerated rejection. Abundant IgG is demonstrated in the interstitial edema and could be interpreted as antibody directed against the graft. However, since fibrinogen and β_{1C}-globulin are also present, increased vascular permeability to serum proteins with their nonspecific accumulation is suggested, rather than an immunologically specific event. A similar nonspecific interstitial accumulation of serum proteins is seen in the inbred rat renal allograft. The mechanism of edema formation in the unsensitized rat has been dis-

cussed previously.[2,27] In rat renal allografts, cell-bound, and probably free, humoral antibodies likely react with antigens residing on the endothelium of the graft, bind complement, and lead to increased vascular permeability. Evidence supporting the existence of host antibody directed against renal graft endothelium also has been provided from investigation of canine renal allografts.[28] Qualitatively similar, but quantitatively more extensive, antibody-antigen interaction may take place in grafts in the presensitized host and may lead to more pronounced interstitial edema. The possible significance of venous thrombosis as a mechanism leading to interstitial edema is considered later.

Acute rejection characterized by interstitial hemorrhage is the other major distinctive morphologic rejection pattern seen in presensitized rats. In humans this morphologic pattern of rejection carries a poor prognosis, as indicated by the clinical course of patients exhibiting such a pattern. The grafts of 5 of 6 patients with this pattern failed soon after biopsy. In the sixth patient the interstitial hemorrhage was focal. Interstitial hemorrhage may originate by rupture of peritubular capillaries. Evidence of such rupture during rejection of canine renal allografts in unsensitized hosts has been presented.[10,29,30] In the rat we find rupture of peritubular capillary infrequently, and interstitial hemorrhage is rare in unsensitized recipients, in contrast to rejection in the previously sensitized rat where interstitial hemorrhage is common and rupture of peritubular capillaries more frequent, but not common.

In renal allograft rejection in the presensitized rat, thrombosis of small- and medium-sized veins is frequently associated with interstitial hemorrhage and edema, and suggests possible participation of increased venous pressure as a mechanism leading to interstitial hemorrhage and edema. In the present human series, about half of the patients with interstitial hemorrhage had associated venous thrombosis, and 6 of 8 patients with venous thrombosis had associated interstitial hemorrhage or edema, suggesting a possible causal relationship between venous thrombosis, and interstitial edema and hemorrhage.

Characterized by Necrotizing Vasculitis. This condition may be an example of accelerated rejection. In rat renal allografts, necrotizing vasculitis is seen both in sensitized and unsensitized rats. In the unsensitized rat, however, necrotizing vasculitis is only seen *after* the kidney is completely rejected and the recipient kept alive with one of his own kidneys in place. This could suggest the importance of excess antibody or other nonspecific factors. Although immunofluorescence examination of the rat kidney gives no indication of the pathogenesis of the necrotizing vasculitis, immunohistochemical examination of the

necrotizing vasculitis in humans may. The IgG and β_{1C}-globulin discretely deposited in the walls of vessels with acute necrotizing arteritis suggests deposition of antibody or immune complexes as a pathogenic mechanism in the induction of acute necrotizing arteritis. Similar immunohistochemical findings and interpretation have been reported in canine renal allografts.[31] The gravity of acute necrotizing arteritis on the survival of the renal allograft in humans is indicated by graft failure in all 5 patients showing acute necrotizing vasculitis. Fibrin deposits, however, without evidence of necrosis of the arterial wall is not necessarily a poor prognostic finding.

Chronic Renal Allograft Rejection

Characterized by Obliterative Vascular Lesions. These lesions are seen in 50% of the grafts reviewed that survived 3 months or more. Even though obliterative vasculitis has not been studied in the inbred rat model, some of the morphologic and immunofluorescence findings in human renal allografts provide a basis for suggesting a possible mechanism leading to the intimal thickening. The occasional presence of fibrin in these lesions and the morphologic similarity between the intimal thickening and organizing thrombus suggests organization of deposited fibrin as a possible mechanism leading to obliterative arteritis. The frequent disruption of elastic lamellas seen in those vessels suggests a prior episode of acute arteritis and, possibly, that healing of this arteritis leads to the obliterative lesions. Patient 27 illustrates this possibility. Acute necrotizing arteritis was present in the Day 12 biopsy, and by Day 28 early obliterative lesions were unmistakable. This does not exclude the significance of recurrent vascular deposition of fibrin secondary to immunologic damage to endothelium and organization of fibrin into intimal fibrosis and thickening. The poor prognosis of grafts showing necrotizing arteritis, however, indicates that some grafts may not survive long enough for necrotizing arteritis to be significant in the development of chronic obliterative vascular lesions.

Characterized by Chronic Glomerulopathy. This also is a frequent finding in long-surviving human renal allografts. About half of the grafts surviving 2 months or longer show evidence of glomerular alterations. The multiplicity of the morphologic and immunofluorescence features of the chronic glomerular lesions seen in renal allografts suggests participation of several pathogenetic mechanisms, rather than a single pathogenesis. The first pathogenesis to be considered is redevelopment of the host's original renal disease in the graft. The significance of this mechanism is emphasized by the course of renal transplants in identical

twins. Of 18 patients, with renal failure secondary to glomerulonephritis, who received isografts from their identical twin, 11 redeveloped clinical evidence of glomerulonephritis, and 7 died with recurrent glomerulonephritis.[32] Data pertinent to the pathogenesis of and recurrence of glomerulonephritis have been obtained by finding circulating antiglomerular basement membrane antibody in some anephric patients awaiting renal transplantation.[33] Placement of a renal graft in one of these patients was associated with decrease in circulating serum antiglomerular basement membrane antibody and the binding of host IgG to glomerular basement membrane of the grafted kidney. The nephritogenicity of the antibody fixed to kidney has been demonstrated in these studies. It was possible to elute antibody from about half of the glomerulonephritic kidneys studied, and some of this eluted antibody produced glomerulonephritis when injected into monkeys. While fixation of circulating antiglomerular basement membrane antibody may initiate some of the glomerular lesions seen in allografts—a possibility supported by finding linear localization of IgG and β_{1C}-globulin on glomerular walls in some allografts—preformed antiglomerular antibody does not explain all of the glomerular lesions seen in long-surviving renal allografts, since glomerular alterations are seen also in patients whose original kidney disease was other than glomerulonephritis. Chronic glomerular alterations also are seen in long-surviving canine renal allografts.[34] The chronic deposition of circulating immune complexes may be another mechanism to be considered in the pathogenesis of the chronic glomerulopathies developing in long-surviving human renal allografts. Although the antigens of such complexes await demonstration, consideration should be given to transplantation antigens.

The immunohistochemical finding of abundant fibrin and relatively little IgG in some glomerular lesions suggests a significant role for fibrin in the pathogenesis of some glomerular lesions. Fibrin has been implicated in the development of nontransplant glomerular lesions.[20,21] The electron microscopic observations made on renal allografts suggests that platelet and fibrin thrombi of glomerular capillaries become incorporated into capillary walls.[19] Repeated episodes of capillary thrombosis and incorporation into capillary walls may lead to capillary wall thickening. The possible factors initiating capillary thrombosis have deen discussed (*vide supra*).

The least frequent chronic glomerulopathy seen in human renal allografts is characterized by undulating capillary walls. The resemblance of this glomerular alteration to obsolescent glomeruli seen in renal vascular disease may be more than coincidence. A similar glomerular alteration is seen late in renal allograft rejection in the unsensitized, nonimmuno-

suppressed rat at a time when effective renal blood flow is markedly reduced and the renal microvasculature no longer fills with injected Silastic.[4] This supports the speculation that the wrinkled appearance of glomerular capillary walls may be related to glomerular ischemia.

Summary

Light microscopic examination of 71 tissue samples from 42 human renal allografts and immunoflorescence examination of 33 tissue samples from 21 allografts were performed and the results presented.

Renal interstitial alterations consisted of infiltration by immature and mature mononuclear cells and interstitial hemorrhage, edema, and fibrosis. Immunoglobulin G (IgG) was rarely demonstrated in the immature mononuclear cells, but was seen in mature mononuclear cells. IgG, β_{1C} globulin (β_{1C}) and fibrinogen were abundant in edematous interstitium. Glomerular changes consisted of proliferation of endothelial and/or mesangial cells, occlusion of glomerular capillaries by fibrin and/or platelet thrombi, thickening of glomerular capillary walls, increase in mesangial matrix, and undulation of glomerular capillary walls. Several patterns of glomerular localization of IgG, β_{1C}, and fibrinogen were seen: slight to moderate granular and linear localization of IgG and β_{1C} with slight fibrinogen; slight IgG and β_{1C} with pronounced fibrinogen localization on capillary walls and in mesangial areas; and abundant localization of IgG and β_{1C} on capillary walls in a pronounced linear fashion. Vascular alterations consisted of acute necrotizing arteritis with semidiscrete IgG and β_{1C} globulin deposits in the vessel walls, obliterative endarteritis with occasional IgG deposits on the vessel's endothelium, and thrombosis of veins. Tubular alterations were frequent and varied.

These results provided a basis for a tentative morphologic classification of human renal allograft rejection. The significance of the various morphologic features of human allograft rejection was interpreted on the basis of previous experience with renal allograft rejection under the controlled immunogenetic conditions provided by using inbred strains of rats.

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Miss Linda Krasinski and Nancy Jones provided skillful technical assistance.

[*Illustrations follow*]

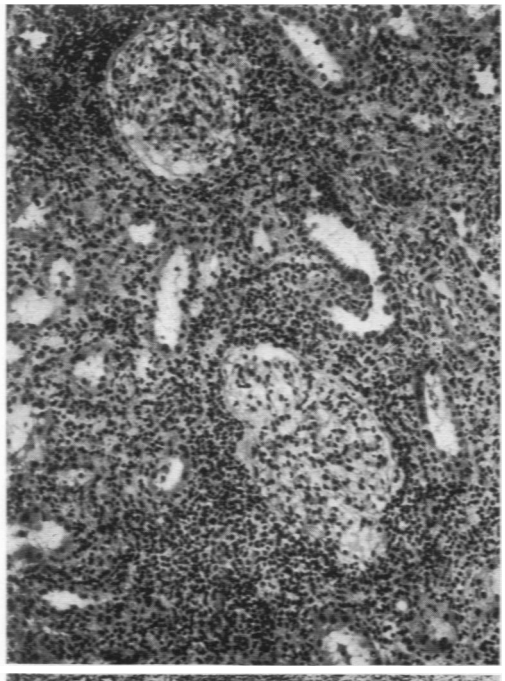
Legends for Figures

Fig. 1. Renal allograft rejection characterized by infiltration of interstitium by large mononuclear cells. Hematoxylin and eosin. × 100.

Fig. 2. Higher magnification of large mononuclear cells which infiltrate interstitium. Toluidine blue O. × 540.

Fig. 3. Renal allograft rejection characterized by infiltration of interstitium by mature mononuclear cells. The accumulation of mature mononuclear cells is patchy; interstitial fibrosis is also present. Hematoxylin and eosin. × 35.

Fig. 4. Higher magnification of mature interstitial cellular infiltrate. Small lymphocytes and plasma cells are evident. Basement membranes surrounding tubules are thickened. Hematoxylin and eosin. × 250.



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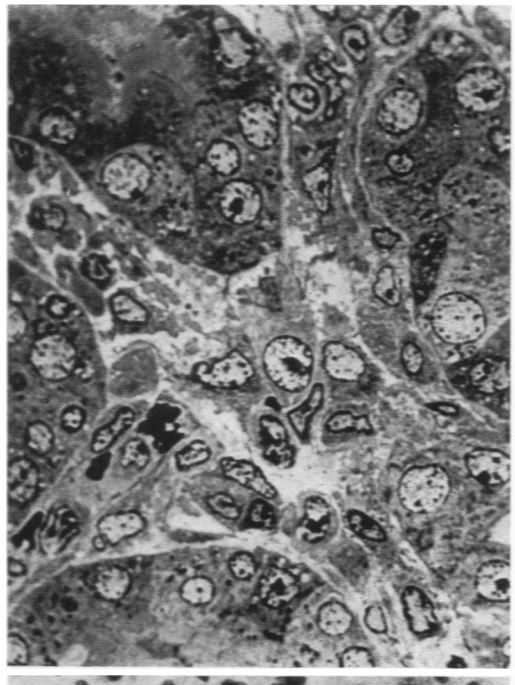

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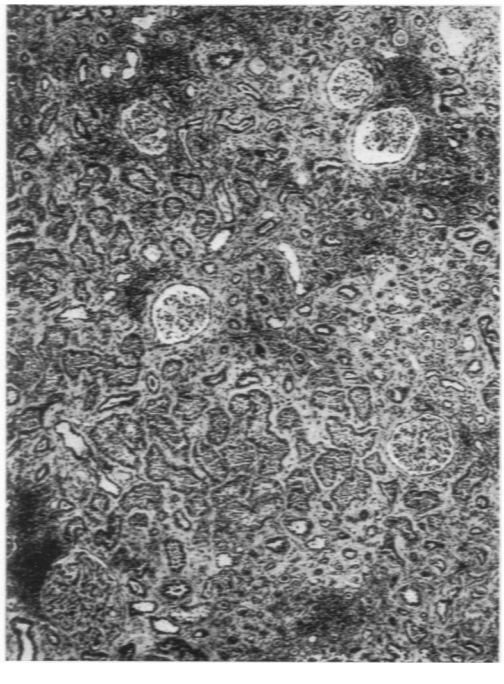

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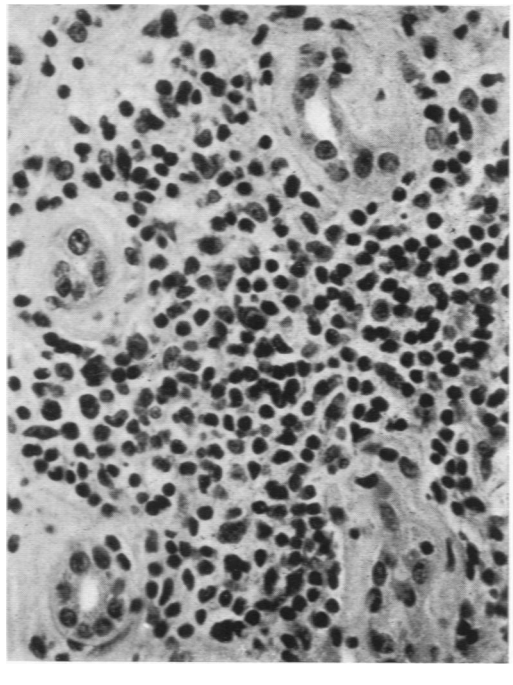

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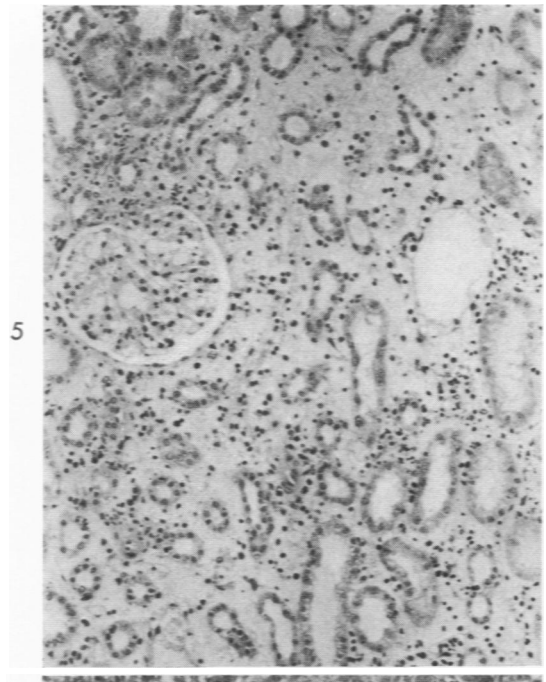
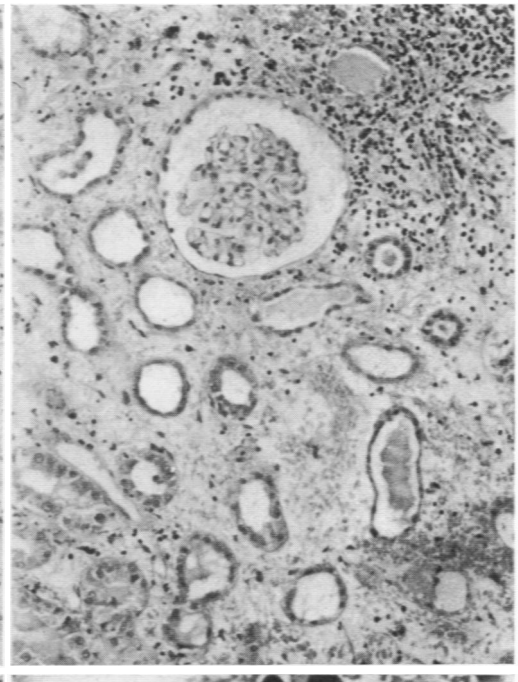
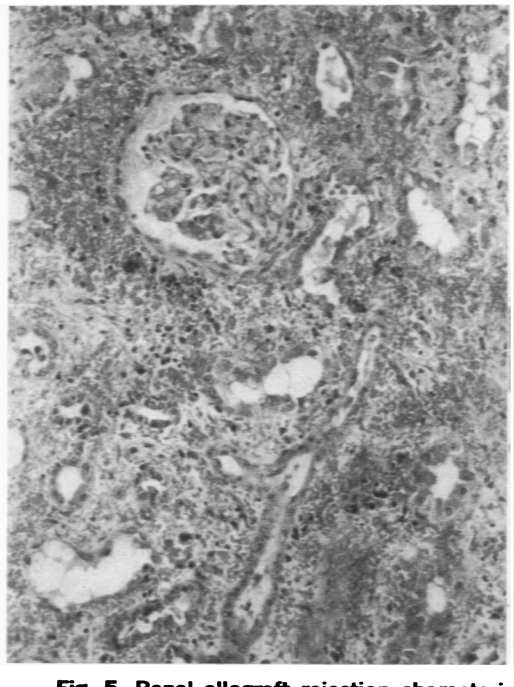






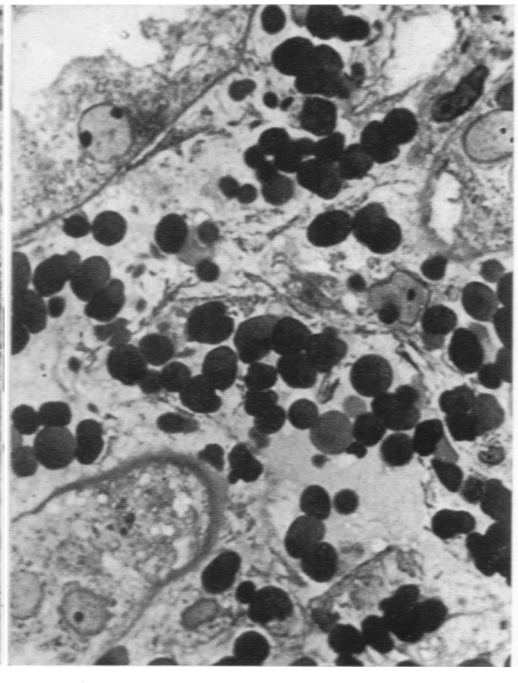
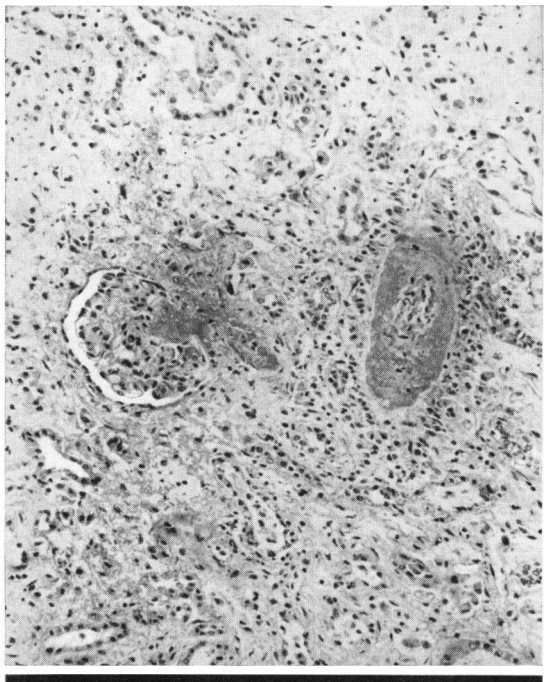


Fig. 5. Renal allograft rejection characterized by interstitial edema. Hematoxylin and eosin. × 100.

Fig. 6. Renal allograft rejection characterized by interstitial edema and hemorrhage. Hematoxylin and eosin. × 100.

Fig. 7. Renal allograft rejection characterized by interstitial hemorrhage. Hematoxylin and eosin. × 100.

Fig. 8. Renal allograft rejection characterized by interstitial hemorrhage. Periodic acid-Schiff. × 540.



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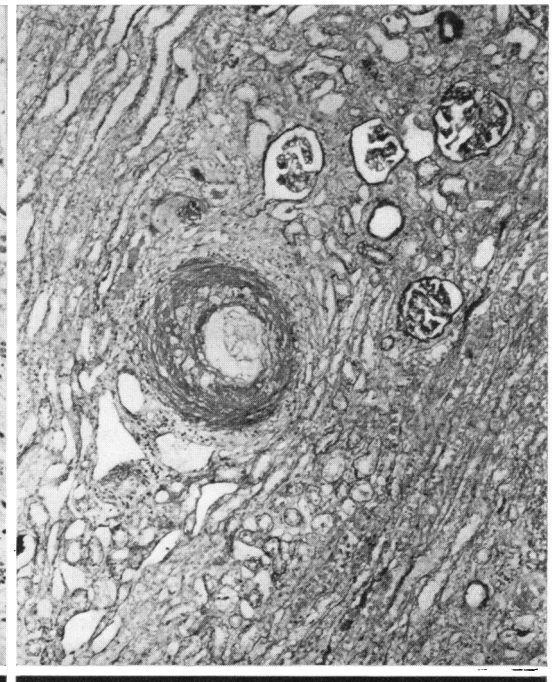

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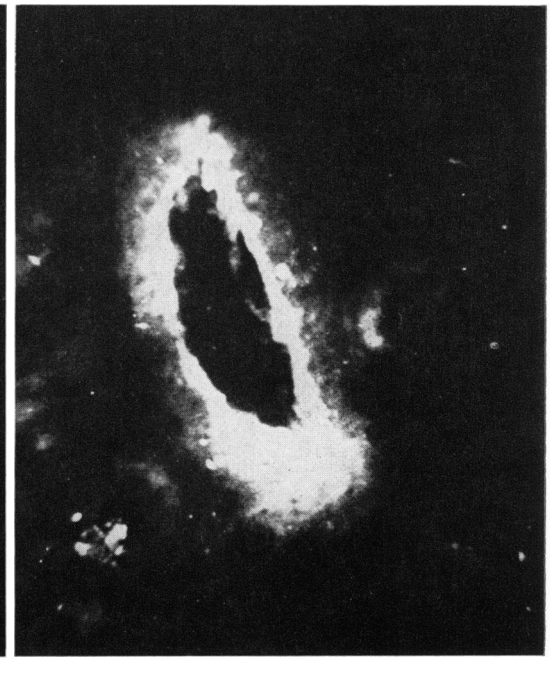

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Fig. 9. Renal allograft rejection characterized by acute arteritis. Necrotizing arteritis involves medium-sized artery and preglomerular arteriole. Fibrinoid necrosis of preglomerular arteriole extends into glomerular capillary tuft. Hematoxylin and eosin. × 100.

Fig. 10. Renal allograft rejection characterized by acute arteritis. Hematoxylin and eosin. × 35.

Fig. 11. IgG localization in artery undergoing fibrinoid necrosis. IgG localized as semidiscrete deposits. Elastic lamella is largely destroyed. Fluorescein isothiocyanate-labeled rabbit anti-human IgG. × 250.

Fig. 12. Fibrin localization in artery undergoing fibrinoid necrosis. Fibrin is located diffusely throughout entire vessel wall and extends to perivascular area. Fluorescein isothiocyanate-labeled anti-human fibrin. × 250.

Fig. 13. Thrombus with early organization occludes medium-sized vein. Hematoxylin and eosin. × 125.

Fig. 14. A small vein is partially occluded by thrombus. Hematoxylin and eosin. × 250.

Fig. 15. An organized thrombus is partially incorporated into wall of medium-sized vein. A more recent thrombus is superimposed on organized thrombus. Hematoxylin and eosin. × 250.

Fig. 16. Chronic renal allograft rejection characterized by obliterative arteritis. Arterial lumen is moderately narrowed by hypercellular intima. Periodic acid-Schiff. × 250.

Fig. 17. Chronic allograft rejection characterized by obliterative arteritis. Several areas of fibrin deposition *(arrow)* are seen in slightly thickened arterial wall. Hematoxylin and eosin. × 250.

Fig. 18. Chronic allograft rejection characterized by obliterative arteritis. Arterial lumen is severely narrowed by intima thickened by loose connective tissue. Hematoxylin and eosin. × 250.

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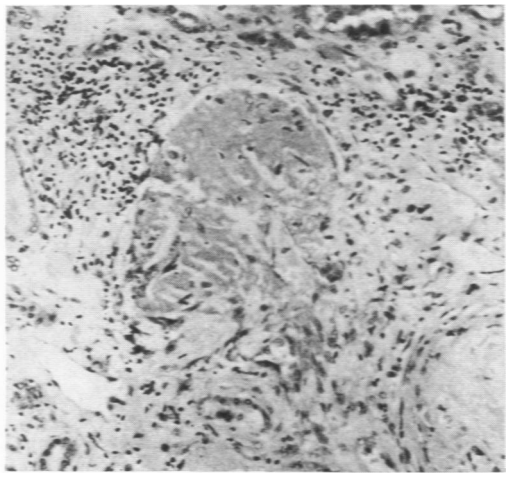

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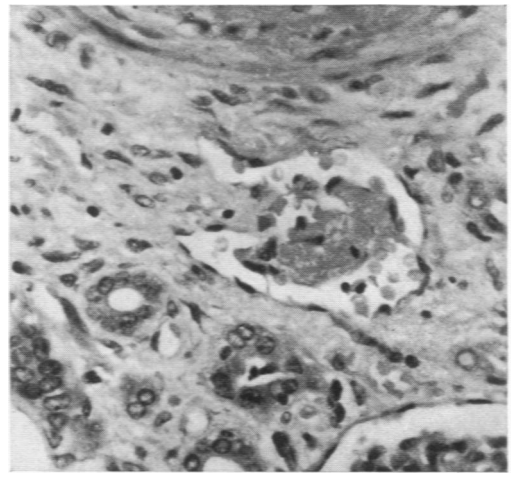

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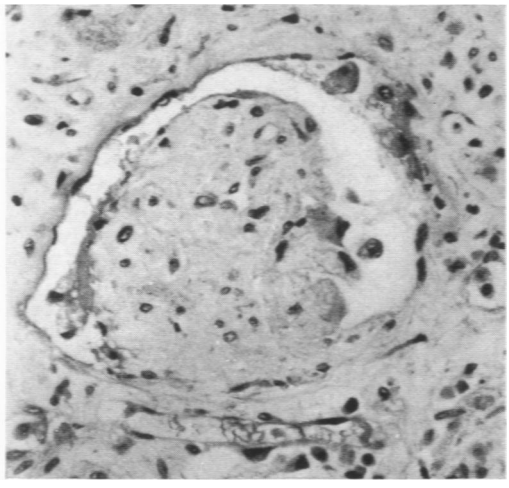

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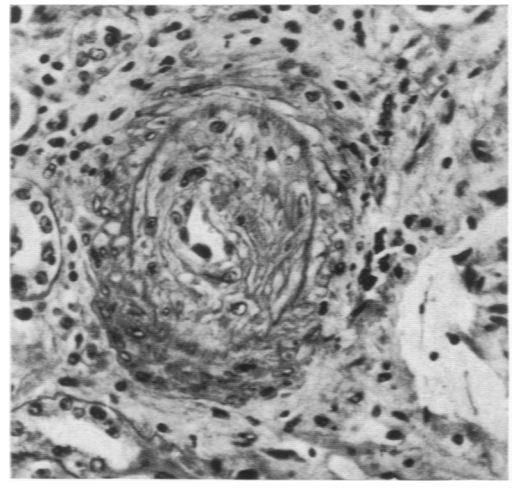

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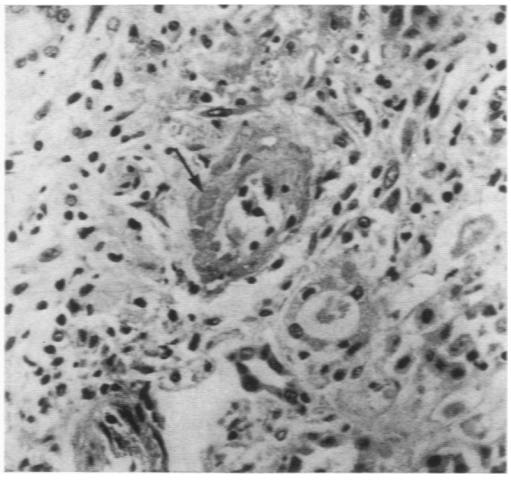

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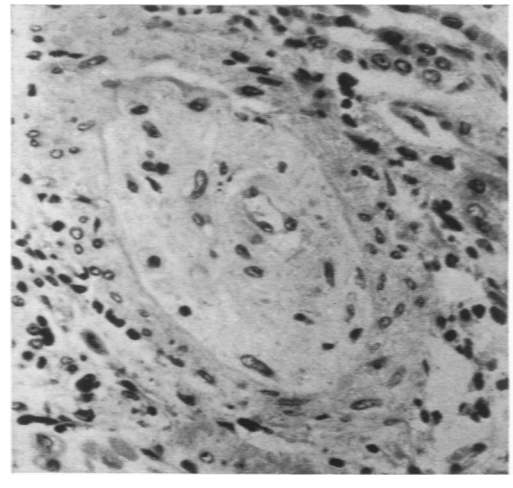
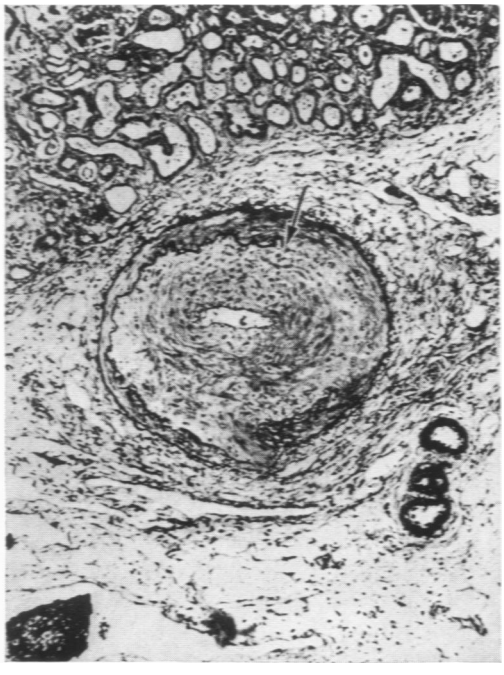


Fig. 19. Chronic allograft rejection characterized by obliterative arteritis. Marked fibrous thickening of intima narrows arterial lumen. Internal elastic lamella is interrupted *(arrow)*. Trichrome. × 35.

Fig. 20. Foam cell projects into lumen of medium-sized artery. Hematoxylin and eosin. × 250.

Fig. 21. Chronic renal allograft rejection characterized by obliterative arteritis. Fibrous thickening of two arteries severely narrows lumens of vessels. Trichrome. × 35.

Fig. 22. IgG localization in artery with moderate intimal thickening. Intense blue autofluorescence of internal elastic lamella is evident. Green fluorescence after a tissue is exposed to isothiocyanate-labeled rabbit anti-human IgG is slight and consists of patchy fluorescence on endothelium *(arrows)*. Fluorescein isothiocyanate-labeled rabbit anti-human IgG. × 250.



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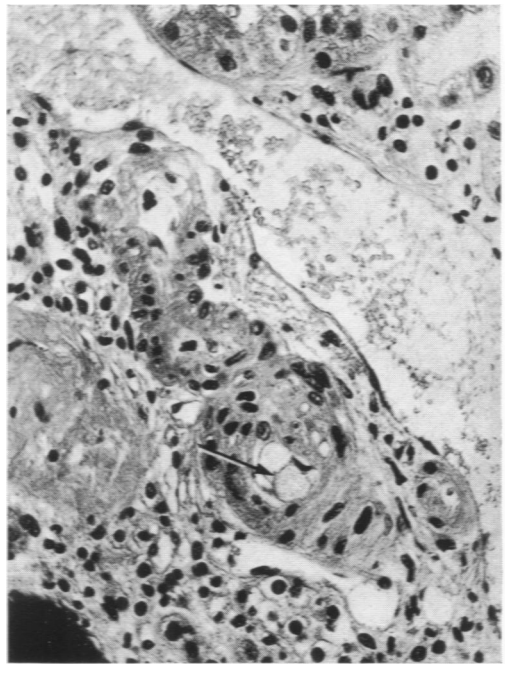

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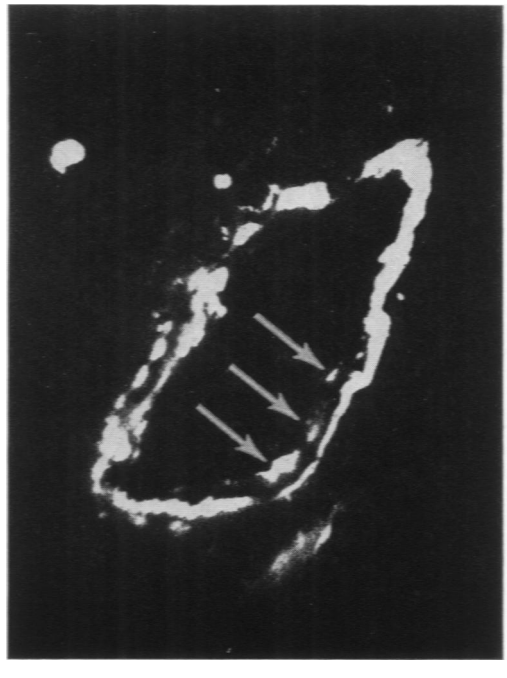

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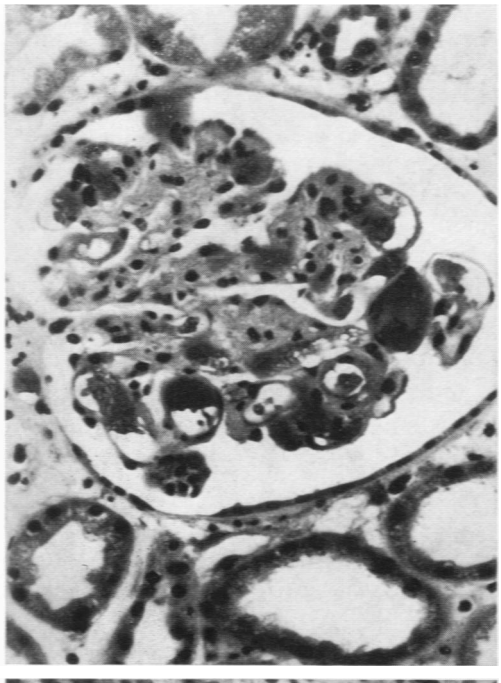
Fig. 23. Acute allograft rejection characterized by thrombotic glomerulopathy. Some glomerular capillaries are partially or completely occluded by eosinophilic material. Hematoxylin and eosin. × 250.

Fig. 24. Acute allograft rejection characterized by thrombotic glomerulopathy and interstitial hemorrhage. All glomerular capillaries are occluded. Hematoxylin and eosin. × 250.

Fig. 25. Acute allograft rejection characterized by acute proliferative glomerulopathy. Glomerulus is hypercellular with mesangial and/or endothelial cells occluding glomerular capillary lumens. A mesangial and/or endothelial cell is in mitosis *(arrow)*. Hematoxylin and eosin. × 250.

Fig. 26. Axial glomerular hypercellularity in a renal allograft. Hematoxylin and eosin. × 250.



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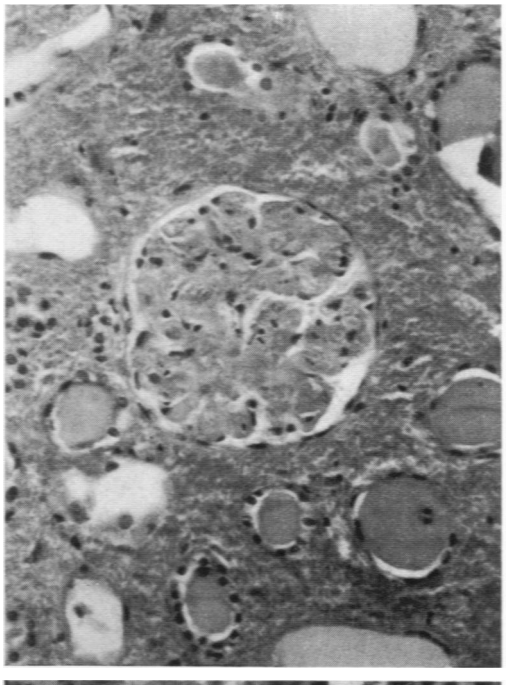

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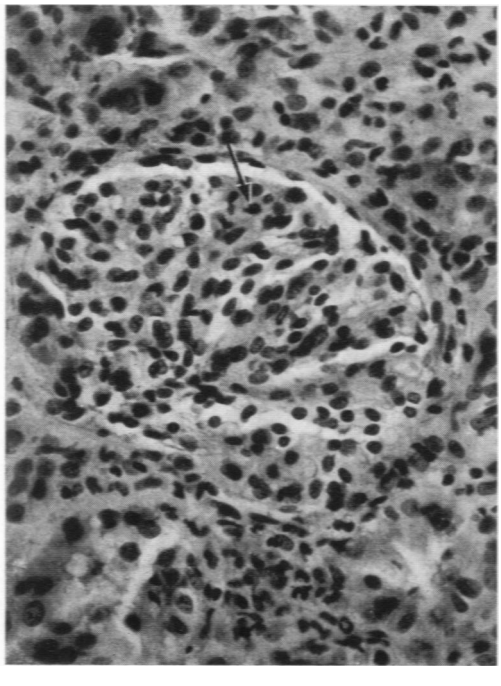

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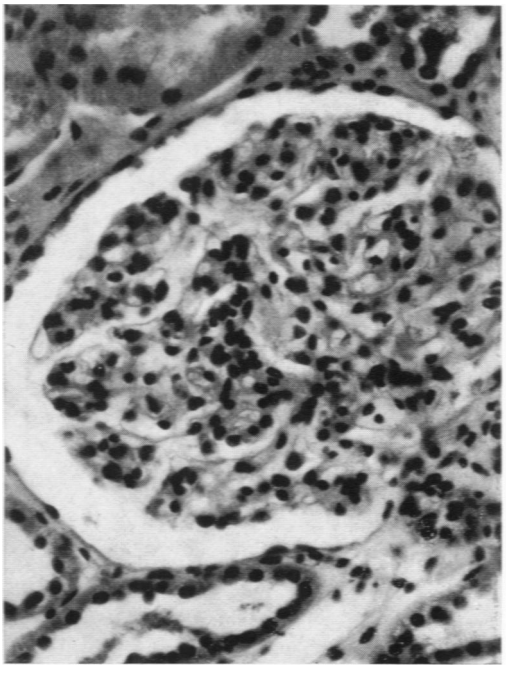

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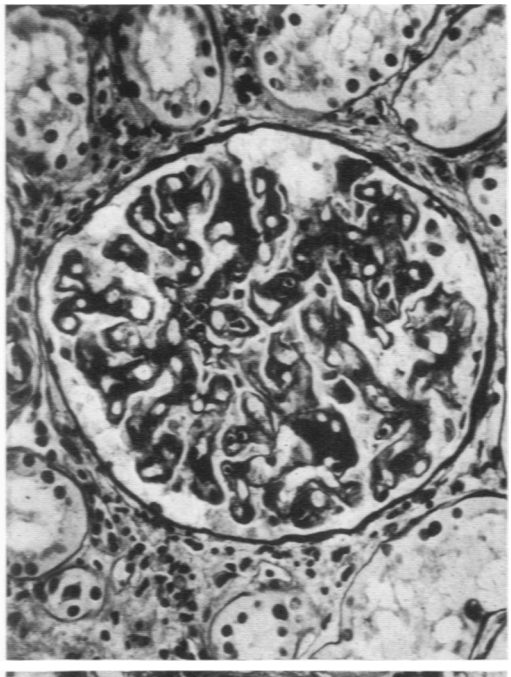
Fig. 27. Chronic allograft rejection characterized by membranous glomerulopathy. Glomerular capillary walls are moderately thickened. Periodic acid-Schiff. × 250.

Fig. 28. Thin sections of kidney in Fig. 27 show thickening of basement membranes. Mesangial matrix is also increased. Toludine blue O. × 250.

Fig. 29. Foam cells in glomerulus of renal allografts. Mesangial matrix is increased, and some capillary walls are slightly thickened. Hematoxylin and eosin. × 250.

Fig. 30. Chronic allograft rejection characterized by membranous and mesangial glomerulopathy. Hematoxylin and eosin. × 250.



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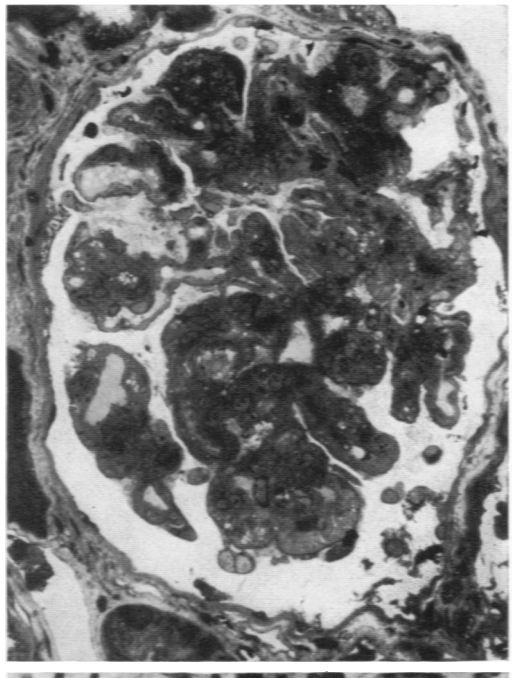

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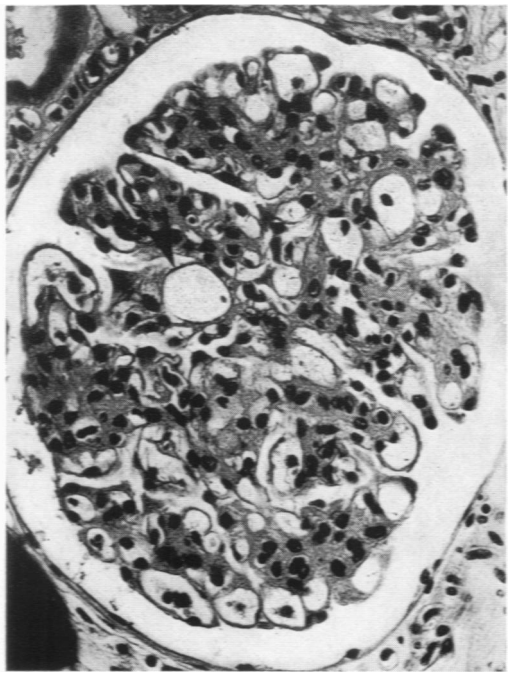

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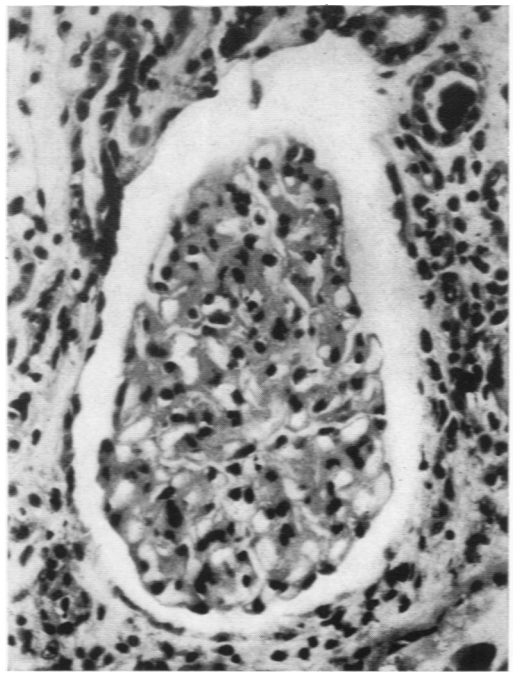

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Fig. 31. Chronic renal allograft rejection characterized by undulating glomerular capillary walls. Periodic-acid Schiff. × 250.

Fig. 32. Higher magnification of Fig. 31 shows wrinkling or undulation of basement membrane. × 1250.

Fig. 33. IgG localization in glomerulus of renal allograft. Fluorescein isothiocyanate-labeled rabbit anti-human IgG. × 150.

Fig. 34. Higher magnification of Fig. 33 illustrates the linear localization of IgG along glomerular capillary walls. × 460.

Fig. 35. IgG localization in glomerulus of renal allograft. Linear and granular localization of IgG on capillary walls and mesangial areas. Fluoroscein isothiocyanate-labeled rabbit anti-human IgG. × 250.

Fig. 36. IgG localization in glomerulus of renal allograft. Only slight fluoresence is apparent. Fluorescein isothiocyanate-labeled rabbit anti-human IgG. × 250.

Fig. 37. Fibrin localization in same kidney as seen in Fig. 36. In contrast to slight IgG localization, abundant fibrin is located on capillary walls and in mesangial areas. Fluorescein isothiocyanate-labeled rabbit anti-human fibrin. × 250.

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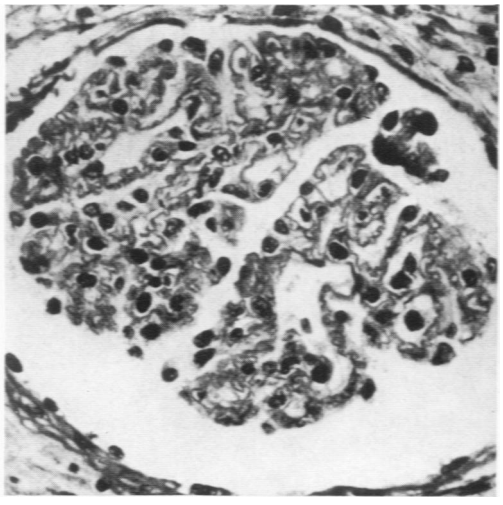

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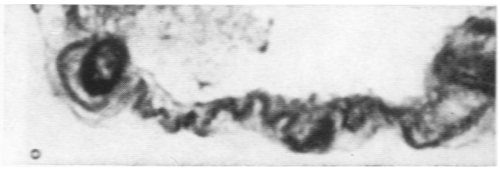

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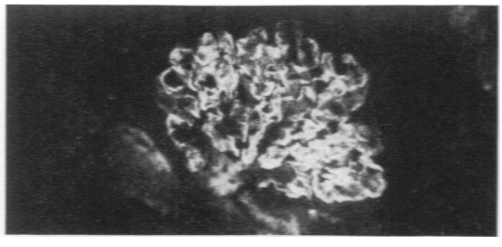

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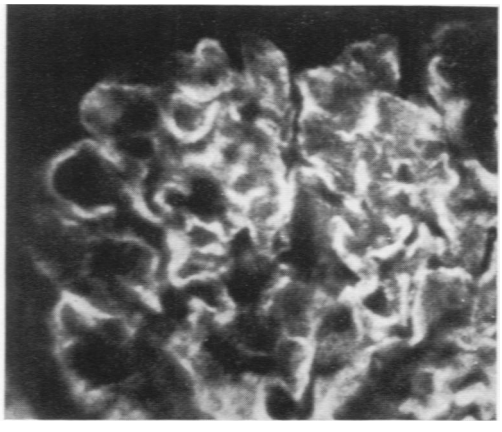

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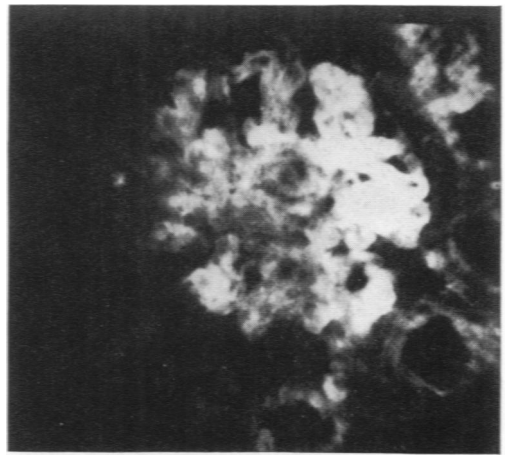

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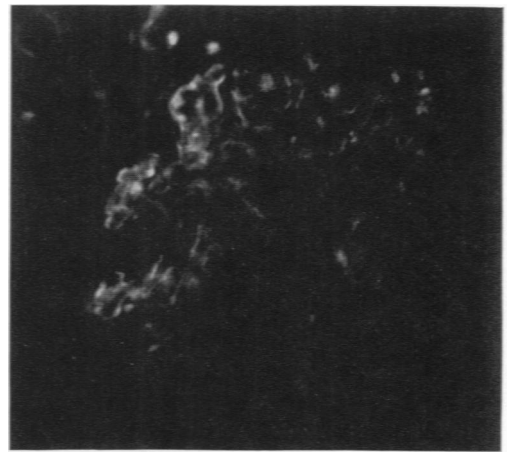

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