Histologic Changes in Preserved Cadaveric Renal Transplants

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The histologic changes in human cadaveric kidneys preserved by either simple hypothermia or continuous pulsatile perfusion were studied by light and electron microscopy and by immunofluorescence in biopsies obtained immediately before (15 kidneys) and approximately 1 hour after (41 kidneys) transplantation. The changes observed in pretransplantation biopsies were largely similar in kidneys preserved by either method with the exception that foreign material was found in the vasculature of perfused kidneys only. Endothelial edema was more frequent and more severe in nonperfused kidneys. In the 1 hour posttransplantation biopsies, a lesion resembling intravascular coagulation (IVC) was noted in 18 of the 25 perfused kidneys but was absent from all 16 nonperfused kidneys. Transplants with severe IVC lesions failed to function and had extensive cortical necrosis when removed by the end of the first month. In follow-up biopsies, milder lesions appeared to heal fast and were compatible with transplant function. The IVC lesion is probably due to endothelial cell damage during perfusion of the kidneys. (Am J Pathol 88:403–428, 1977)

AT THE PRESENT TIME, approximately 65% of human renal transplants originate from unrelated cadaver donors.[1] Some of these cadaveric organs require several hours of extracorporeal preservation to provide enough time for adequate compatibility testing and for transportation to their prospective recipients. Two major methods of kidney preservation are currently employed: a) simple hypothermia involving placement of the organ in a slush of ice after initial flushing with Ringer's lactate, Collin's solution, or similar preparations [2-6] or b) continuous perfusion of the organ, at low temperature, with plasma or a solution containing human albumin.[7,8]

Several studies have supported the view that continuous perfusion is more effective in preserving such kidneys than is simple hypothermia.[7-11] The theoretical basis for this alleged superiority is not well understood and whether or not this particular method of preservation is successful in prolonging *in vivo* transplant survival is still controversial.[12]

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In the present study, cadaveric kidneys subjected to these methods of extracorporeal preservation were examined for morphologic changes prior to actual transplantation and/or shortly after reestablishment of the circulation. The purpose of this study was to document any morphologic alterations which may have resulted from either of these preparatory procedures and to compare them with those generally attributed to the homograft rejection process itself. A lesion morphologically identical to intravascular coagulation was observed in a number of the transplanted perfused kidneys in which biopsies had been obtained within 1 hour of reestablishment of the renal circulation. This lesion was absent in kidneys preserved by hypothermia alone, and its occurrence could not be predicted consistently on the basis of light microscopy, electron microscopy, or immunofluorescence patterns in pretransplant biopsies of the isolated kidneys.

Materials and Methods

Fifteen biopsies were obtained from isolated cadaveric kidneys immediately prior to transplantation; ten were from perfused kidneys and five from nonperfused kidneys (Table 1). In two instances, one of a pair of kidneys was not transplanted and the entire nontransplanted organ was available for study. Each of the transplanted kidneys was subsequently rebiopsied approximately 1 hour following reestablishment of the circulation. An additional 26 kidneys in which pretransplant biopsies were unavailable were also biopsied 1 hour posttransplantation (Table 1). Of a total of 41 1-hour biopsies obtained in this series, 25 were from kidneys that had been perfused. Six of the 41 patients had further follow-up biopsies (Table 1), and in 10, the transplanted kidneys were eventually removed surgically. Autopsy tissues were available in 1 patient who died 24 hours following transplantation.

A portion of each biopsy was prepared for light microscopy. These tissues were fixed in 10% buffered formalin, processed through increasing concentrations of alcohol to xylol, and sectioned at 3 μ. Serial sections (average 18) were stained with hematoxylin and eosin, Jones' methenamine silver (13), chromotrope (14), phosphotungstic acid hematoxylin (PTAH) (15), and/or Martius scarlet blue (16). For direct immunofluorescence, the tissues were immediately frozen in a mixture of 2-methylbutane and dry ice and stored at −40 C until further processing.[17] The antisera used were obtained from commercial sources (Meloy and Behring Companies) and included anti-IgA, anti-IgG, anti-IgM, anti-β_1C-globulin, antialbumin, and antifibrinogen. Indirect immunofluorescence was performed using sera obtained before and after transplantation from 6 patients. These sera were tested against tissue from five isolated cadaveric kidneys, two perfused and three nonperfused.

For ultrastructural studies, specimens were immediately fixed in 2.5% buffered glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon. Toluidine blue–stained 1-μ sections were examined for identification of representative glomeruli and vessels. Ultrathin sections from at least two representative blocks were stained with uranyl acetate and lead citrate and studied using an RCA EMU-3 electron microscope. Data on the histologic material are summarized in Table 2.

All recipients had been examined monthly for lymphocytotoxic antibodies using a 50-donor lymphocyte panel. The kidneys had been obtained through the Southeastern Regional Organ Procurement Foundation and had been initially flushed with one of the

Table 1—Summary of Data

Case No.	Tissue examined: Pretransplant	Tissue examined: Posttransplant	IVC lesion	Pulsatile perfusion
1	Yes	1 hr	No	No
2	Yes	1 hr	No	No
3	Yes	1 hr	No	No
4	Yes	1 hr, 5 d nephrectomy	No	No
5	Yes	1 hr, 30 d, 60 d	No	No
6	No	1 hr, 20 d nephrectomy	No	No
7	No	1 hr	No	No
8	No	1 hr	No	No
9	No	1 hr	No	No
10	No	1 hr	No	No
11	No	1 hr	No	No
12	No	1 hr	No	No
13	No	1 hr	No	No
14	No	1 hr	No	No
15	No	1 hr	No	No
16	No	1 hr	No	No
17	Yes	1 hr, 28 d nephrectomy	Severe	Yes
18	Yes	1 hr	No	Yes
19	Yes	1 hr, 14 d	Mild	Yes
20	Yes	1 hr, 24 hrs autopsy	No	Yes
21	Yes	1 hr	Moderate	Yes
22	Yes	1 hr, 60 d nephrectomy	Moderate	Yes
23	Yes	1 hr	No	Yes
24	Yes	1 hr	Mild	Yes
25*	Yes	1 hr, 2 hr nephrectomy	No	Yes
26*	Yes	1 hr, 24 d nephrectomy	Severe	Yes
27	No	1 hr, 5 d, 18 d nephrectomy	Severe	Yes
28	No	1 hr, 2 hr nephrectomy	Severe	Yes
29	No	1 hr, 5 d, 20 d	Mild	Yes
30	No	1 hr, 10 d nephrectomy	Severe	Yes
31	No	1 hr	No	Yes
32	No	1 hr, 10 d, 12 d nephrectomy	Severe	Yes
33	No	1 hr, 45 d	Moderate	Yes
34	No	1 hr	Moderate	Yes
35	No	1 hr	Mild	Yes
36	No	1 hr	Mild	Yes
37	No	1 hr	No	Yes
38	No	1 hr	Mild	Yes
39	No	1 hr	No	Yes
40	No	1 hr	Mild	Yes
41	No	1 hr	Moderate	Yes

IVC = intravascular coagulation.

* In addition to the transplanted kidney, the entire nontransplanted kidney of the same pair was studied.

following solutions: Ringer's lactate, Collins' solution, or a solution containing 6% dextran in normal saline and heparin. Kidneys which had been further preserved with continuous perfusion had been attached either to a Waters MOX-100 or MOX-100 T perfusion apparatus. The perfusate had consisted of either cryoprecipitated AB plasma or a modified Ringer's albumin solution. The latter had consisted of 400 to 700 ml Ringer's lactate, 100 to 200 ml 25% albumin, 25 ml mannitol, 5 ml 1% lidocaine, 5000 IU heparin, 125 mg Solu-

Table 2—Histologic Data

Tissues obtained	Tissue examined by light microscopy	Immuno-fluorescence	Electron microscopy
Pretransplant biopsies	15	7	9
One hour posttransplant biopsies	41	18	12
Posttransplant follow-up biopsies	8	4	5
Nephrectomy/autopsy	11	10	7
Total	75	39	33

Medrol, 1 million IU penicillin, and 6 to 8 ml sodium bicarbonate. The pH of this solution ranged from 7.2 to 7.3, and the average osmolarity was 430 mOsm/liter. All kidneys used for transplantation in this study had acceptable perfusion characteristics [7,11] with an average perfusion pressure of 50/24 mm Hg and a flow of 160 ml/min (Table 3).

Results

The histologic findings in the pretransplant biopsies are summarized in Table 4; those for the 1 hour posttransplant biopsies in Table 5. Of the 41 biopsies obtained approximately 1 hour following reestablishment of circulation, 18 showed a lesion morphologically consistent with intravascular coagulation. All 18 biopsies had been derived from kidneys preserved by continuous perfusion. For purposes of comparison, the results of the pretransplant and 1 hour posttransplant biopsies are tabulated and presented in two parts: a) pretransplant, and b) posttransplant, with each divided into 1) nonperfused kidneys, 2) perfused kidneys that did not subsequently develop the intravascular coagulation (IVC) lesions, and 3) perfused kidneys that did develop IVC within 1 hour of transplantation.

Pretransplantation Biopsies

Nonperfused Kidneys

Five pretransplant biopsies from kidneys preserved by simple hypothermia were studied (Table 4). By light microscopy, most of the glomerular

Table 3—Perfusion Characteristics

	Kidneys preserved by pulsatile perfusion*	
	IVC absent (N = 8)	IVC present (N = 13)
Flow rate (ml/min)		
Beginning	148 (80–240)	150 (60–240)
End	179 (110–200)	159 (100–240)
Pressure (mm Hg)		
Beginning	53/31	51/26
End	49/25	48/22

* Mean values, with range in parentheses. IVC = intravascular coagulation.

Table 4—Findings in Pretransplantation Biopsies

		Pulsatile perfusion	
	Simple hypothermia	Without IVC	With IVC
Number of kidneys	5	4	6
Glomeruli			
Endothelial edema	Moderate to severe	Absent to mild	Absent to mild
Endothelial disruption	Focal	Focal	Focal
Foot-process fusion	Patchy	Patchy	Patchy
Capillary loop collapse	Focal, segmental	Focal, segmental	Focal, segmental
Foreign material	None	1/4	2/6
Arterioles—small arteries			
Endothelial disruption	Focal	Focal	Focal
Deposits by immunofluorescence	0/2	0/2	1/3
Tubules			
Degenerative changes	Frequent	Frequent	Frequent
Dilatation	Mild	Often marked	Often marked

IVC = intravascular coagulation.

loops were patent, some containing rare red blood cells and occasional polymorphonuclear leukocytes. Mild, segmental collapse of capillary loops was observed in a few glomeruli.

Four biopsies examined with the electron microscope showed moderate

Table 5—Findings 1 Hour After Transplantation

		Pulsatile perfusion	
	Simple hypothermia	IVC absent	IVC present
Number of kidneys	16	7	18
Glomeruli			
Endothelial edema	Moderate/marked	Mild/moderate	Mild/moderate
Endothelial disruption	Focal	Focal	Focal/diffuse
Foot-process fusion	Patchy	Patchy	Patchy
Capillary loop collapse	Focal	Focal	Focal
PMN clusters	None	Focal, occasional	Focal, frequent
Platelet clusters	None	Focal, occasional	Focal, frequent
Fibrin	None	None	Focal, diffuse
Foreign material	None	Frequent (4)	Frequent (12)
Arterioles—small arteries			
Endothelial disruption	Focal (EM, 1/5)	Focal (EM, 1/3)	Focal (EM, 2/4)
Deposits by immunofluorescence	0/7	0/3	IgM, β_1C, fibrin. (3/8)
Tubules			
Degenerative changes	Frequent	Frequent	Frequent
Dilatation	Frequent	Frequent	Frequent

to striking edema of capillary endothelium (Figure 1). In two of these biopsies, the endothelium appeared focally disrupted and the nuclei were pyknotic (Figure 2). Focal collapse of capillary loops, associated with corrugation of the basement membrane, was seen in all biopsies. The lumina were empty except for rare erythrocytes and polymorphonuclear leukocytes; in two biopsies, there were intraluminal subcellular organelles, in particular mitochondria. The visceral epithelium exhibited focal fusion of the foot processes, edema, and occasional fatty droplets and large dense bodies. Arterioles appeared normal except in two biopsies in which they contained small, discrete dense deposits. One of these biopsies also showed thickened arterioles by light microscopy. In three biopsies, a small artery was available for ultrastructural examination; all these biopsies exhibited areas of endothelial distortion and two showed patchy detachment of endothelium (Figure 3). The tubular epithelium showed variable degrees of cytoplasmic edema, swelling of mitochondria, and in one, large membrane-bounded vacuoles containing material of low electron density.

Direct immunofluorescence was performed on two biopsies. No reactivity was noted, although minute speckled fluorescent material was seen in arteriolar walls in 1 biopsy after incubation with anti-β_1C globulin.

Perfused Kidneys Without Subsequent Intravascular Coagulation

Four perfused kidneys which did not develop IVC within 1 hour of transplantation were biopsied before being connected to the donor (Table 4). Foreign bodies consisting of starch and minute birefringent fibers measuring 2 to 4 μ were observed in glomerular loops in one of the four biopsies. The glomeruli showed focal segmental collapse of loops as in nonperfused kidneys. By electron microscopy (three biopsies), however, there was less endothelial swelling in the capillary loops and a more prominent subendothelial electron-lucent zone. Patchy fusion of footprocesses was noted. In one biopsy, pyknotic, apparently detached endothelium, was observed in a small artery and focally in the glomeruli of another biopsy. A few glomerular loops contained polymorphonuclear leukocytes and occasionally, subcellular organelles. The tubules were markedly dilated in 2 cases. Direct immunofluorescence, applied to two biopsies, was negative except for traces of complement in the hilar arterioles and within a few glomerular loops.

Perfused Kidneys That Subsequently Developed Intravascular Coagulation

Six adequate pretransplant biopsies were obtained from kidneys which at 1 hour showed an intravascular coagulation lesion (Table 4). These

exhibited glomerular, tubular, and interstitial changes essentially the same as kidneys that did not develop IVC. Foreign bodies, consisting of starch and birefringent fibers, were identified in two biopsies. In one biopsy (No. 17), a peculiar homogeneous eosinophilic material was observed in an arteriolar wall. This latter kidney subsequently developed very severe intravascular coagulation. Samples from a pair of kidneys (No. 26) consisting of one entire nontransplanted kidney and a pretransplant biopsy from its mate showed diffuse loss of glomerular capillary endothelium. These kidneys had been preserved for almost 40 hours; 11 hours on ice and 29 hours in a perfusion machine using AB plasma.

Direct immunofluorescence was performed on three biopsies: one was negative with all reagents and one showed only spotty β_1C-globulin in arterioles. In a third biopsy (No. 26) fluorescence was observed with all reagents (IgG, IgM, IgA, β_1C-globulin, fibrinogen) in various patterns in both glomeruli and small vessels.

In summary: Although obvious renal damage was recognized in a few pretransplant biopsies from perfused kidneys, in most instances it was not possible to distinguish perfused from nonperfused kidneys using the morphologic methods described. An exception to this statement was the frequent observation of refractile foreign material in the vasculature of perfused kidneys.

One Hour Posttransplant Biopsies

Nonperfused Kidneys

Sixteen nonperfused kidneys were biopsied approximately 1 hour following completion of the vascular anastomosis (Table 5). The changes observed by light microscopy were essentially the same as those seen in pretransplant biopsies of nonperfused kidneys. The glomeruli showed patchy collapse of capillary loops; the lumina contained a few red blood cells and occasional leukocytes. There was significant dilatation of the tubules in six biopsies.

Ultrastructural studies (5 biopsies) confirmed the presence of focal glomerular capillary collapse associated with corrugation of the basement membrane. The great majority of loops were "empty" except for a few neutrophils, occasional red blood cells, and rare platelets. The endothelium was variably swollen. Focal to patchy epithelial foot-process fusion was a common finding. A small, discrete subepithelial electron-dense deposit was identified in one biopsy. Another (No. 6) showed rather extensive mesangial and paramesangial subendothelial electron-dense deposits and subendothelial low-density granular deposits. Pyknotic endo-

thelium, apparently detached from the basement membrane, was noted in one small artery. Large, membrane-bounded vacuoles were present in desquamated tubular epithelial cells.

Direct immunofluorescence was performed on seven biopsies. They were negative except for the biopsy (No. 6) that had displayed electron-dense mesangial deposits in the glomeruli; it showed anti-β_1C-globulin, anti-IgA and very weak IgG reactivity. Occasionally, as in the pretransplant biopsies, weak β_1C-globulin reactivity was also present in the arteriolar walls.

Perfused Kidneys Without Intravascular Coagulation

Seven perfused kidneys showed no IVC in the biopsies obtained 1 hour posttransplant (Table 5). In four biopsies, foreign bodies (starch and refractile fibers) were present in the capillary loops. Focal collapse of loops was frequently observed (5/7), while in two biopsies small focal neutrophil aggregates were seen in glomerular capillaries. The ultrastructural changes were similar to those observed in pretransplant specimens from perfused kidneys. These biopsies were negative for immunoglobulins by direct immunofluorescence; fibrinogen was identified as small focal deposits in a few loops in only one biopsy (No. 20).

Perfused Kidneys With Intravascular Coagulation

Eighteen of 25 perfused kidneys showed an intravascular coagulation lesion in the 1-hour posttransplant biopsy (Table 5). By light microscopy, this lesion was characterized by obliteration of glomerular capillary loops with a granular fibrillar eosinophilic material which usually yielded a positive fibrin reaction with either PTAH or Martius scarlet blue, especially in the periphery of the "plug." Fragmented red blood cells were often trapped in a meshwork of this material. The inner luminal surface of each involved glomerular capillary wall was fuzzy and ill-defined, while other glomeruli in the same biopsy showed severe congestion and occasional clusters of neutrophils within capillary loops. The extent of these changes varied and an approximate quantitation was estimated from the total number of glomeruli, and percentage of each glomerulus, involved. According to these criteria, the lesions were classified as mild in five biopsies (less than 15% of glomerular loops involved, Figure 4), moderate in seven biopsies (between 15 and 50%), and severe in six (more than 50% of glomerular loops involved, Figure 5). Foreign bodies consisting of refractile fibers and/or starch were seen in twelve biopsies. In the six kidneys with severe lesions and in three with moderate lesions, eosinophilic material within arteriolar walls prevented clear visualization of the smooth muscle and endothelium (Figure 6). Intraarteriolar thrombi were

present in one biopsy. Red blood cells were sometimes seen within the walls of a damaged vessel.

The Jones' silver methenamine stain demonstrated preserved glomerular basement membranes, although in severely involved glomeruli there was degeneration and pyknosis of the visceral epithelium. The interstitium was often edematous, the degree of edema being proportional to the extent of glomerular and vascular damage. Cloudy swelling, vacuolar change, and desquamation of tubular epithelium were often seen. In one kidney, biopsies were obtained at 30 and 60 minutes. Damage was more widespread, and arteriolar lesions had developed in the later biopsy.

Electron microscopy (four biopsies) revealed damage to capillary endothelium and fibrin at various stages of evolution (Figures 7 and 8). Fibrin tactoids apposed to the basement membrane were located adjacent to markedly enlarged endothelial pores and interendothelial spaces. Other loops contained aggregates of partially degranulated platelets but little or no fibrin. Still others contained closely packed red blood cells, often of markedly decreased density. Normal appearing capillary loops were often seen in the same or a neighboring glomerulus. The epithelial foot processes were fused.

Fibrinogen was demonstrated within glomerular capillary loops and/or along the inner aspect of the loops in all eight biopsies examined by direct immunofluorescence (Figures 9 and 10). Kidneys with mild lesions showed only focal intercapillary fibrin and traces of β_1C-globulin in hilar arterioles. Fibrin was also present focally in the vessel walls of kidneys with moderate and severe intravascular coagulation lesions. Focal IgM and β_1C-globulin were observed only in the more severely affected kidneys. In these biopsies, IgM was localized mainly within small vessel walls and focally within glomerular capillaries. The reaction for β_1C-globulin was weaker and followed a distribution similar to that of IgM. Vascular IgG was seen only occasionally and was very weak.

In summary: Morphologic alterations in the 1 hour posttransplant biopsies of nonperfused kidneys were not significantly different from those in the pretransplant biopsy material. Eighteen of 25 posttransplant biopsies from perfused kidneys displayed varying degrees of intravascular coagulation. With the exception of refractile intravascular foreign material, the perfused kidneys which did not display intravascular coagulation 1 hour posttransplantation could not be distinguished from their nonperfused counterparts.

Follow-up Biopsies

Biopsies at different time intervals following transplantation were obtained from six kidneys. Of these, one (No. 5) had been preserved by

simple hypothermia and had been biopsied on the 30th and 60th days. In both biopsies, the glomeruli were normal and the tubular changes were consistent with previous tubular necrosis and regeneration.

Kidneys with mild to moderate IVC lesions in the 1 hour biopsy showed similar lesions persisting to the fifth day (No. 29). Small vessels displayed hyperplastic endothelium, enlarged smooth muscle cells, and focal intramural deposits of β_1C-globulin and IgM. By Day 14 (No. 19), no fibrin could be demonstrated, but the glomeruli appeared focally consolidated, with increased mesangial matrix and enlarged cellular components. Signs of regeneration included the finding of increased numbers of organelles in all the glomerular cells. Patchy foot process fusion and villous transformation of podocytes were also observed. An acute inflammatory reaction was noted in the wall of an arteriole containing a refractile foreign body. No immunoglobulin or complement was identified. In addition, mild cellular rejection and tubular regeneration were present. By the 20th day (No. 29), there was patchy collapse of the glomerular capillaries and expansion of the mesangium, resulting in segmental tuft consolidation. Again, the vascular endothelium and smooth muscle were enlarged and exhibited prominent organelles. Fibrin was absent, and mild cellular rejection was evident. By Day 45 (No. 33), mild glomerular changes characterized by focal tuft consolidation were observed. Again, this focal "scarring" was associated with capillary loop collapse and mesangial expansion. The vessels were lined by prominent enlarged endothelial cells.

A transplant showing severe IVC and vascular lesions 1 hour post-transplant was biopsied on the fifth day (No. 27). In many glomeruli, fibrin, either lining or obstructing glomerular capillaries, was identified by light microscopy, electron microscopy, and immunofluorescence. Several loops lacked endothelium (Figure 11), while in others an electron-lucent space was interposed between the basement membrane and the endothelium. These subendothelial "gaps" were associated with corrugation of basement membranes; the endothelial cells, when preserved, were large and contained increased numbers of organelles (Figure 12). Direct immunofluorescence revealed, in addition to fibrin, focal IgM and minimal IgG deposition in glomerular tufts. The walls of the vessels were thickened due to enlarged endothelial and smooth muscle cells and reacted strongly for IgM (Figure 13); sparce deposits of IgG and β_1C-globulin were focally present in the walls of small vessels. The interstitium contained patchy infiltrates of neutrophils, plasma cells, and a few lymphocytes, particularly numerous at the corticomedullary junction. One transplant with severe IVC at 1 hour was biopsied on the tenth day (No. 32); the findings were similar to those just described except that necrosis was more widespread.

Nephrectomy Specimens

Two nonperfused transplants (Nos. 4 and 6) were removed at 5 and 20 days posttransplantation respectively. One (No. 4), removed on the fifth day because of kidney rupture, showed acute tubular necrosis and evidence of vascular rejection (endothelial and smooth muscle enlargement with focal deposition of IgM, β_1C-globulin, and fibrinogen). The other kidney (No. 6), never functioned adequately in the recipient and was removed on Day 20 because of hemorrhage. It showed acute rejection and focal necrosis of small arteries. The electron-dense deposits seen in the 1 hour biopsy were no longer evident.

One perfused kidney (No. 25) was removed immediately because of surgical difficulties with the vascular anastomosis. Also, seven of the transplants with IVC at 1 hour were removed subsequently; six (Nos. 32, 17, 26, 27, 28, 30) had shown severe, and one (No. 22), moderate IVC lesions. The latter kidney, when removed 2 months posttransplantation, showed proliferative vascular changes and infiltrates representing cellular rejection. None of the severely damaged kidneys ever functioned. One kidney (No. 28), removed 2 hours following connection to the recipient, had changes similar to those in the 1 hour biopsy. Four of the remaining five kidneys showed extensive cortical necrosis, and one (No. 26) had complete generalized necrosis. Direct immunofluorescence demonstrated widespread linear deposition of fibrinogen in glomeruli and massive IgM in small and medium-sized vessels. β_1C-Globulin was weakly positive in vessel walls; IgA and IgG were absent. Interstitial plasmacytoid cells reacted with anti-IgM. Strongly reactive birefringent foreign bodies were occasionally observed in arteries that showed medial hemorrhage and fibrin deposition.

Indirect Immunofluorescence

Using recipient serum that was obtained before and after transplantation and following removal of the transplant, indirect immunofluorescence on kidney tissues (perfused and nonperfused) did not reveal positive reactions for any of the 6 patients tested. Four of these recipients had developed intravascular coagulation within 1 hour of transplantation.

Discussion

Two major methods of preservation are employed when cadaveric kidneys are harvested for transplantation: a) simple hypothermia in which the kidney, first flushed with a cold electrolyte solution, is subsequently placed in a slush of ice,[2-6] and b) continuous hypothermic perfusion, in which oxygenated cryoprecipitated plasma or an albumin solution is

recirculated through the kidney by means of a specially designed perfusion pump.[7,8] The latter method was introduced in the hope that preservation time could be prolonged without damage to the kidneys. Clark *et al.*,[12] however, failed to demonstrate any significant improvement in immediate function or in 1-year survival for those kidneys preserved with continuous perfusion compared to those preserved with simple hypothermia. In addition, morphologic changes in renal vessels and glomeruli have been attributed to mechanical continuous perfusion.[18,19]

Factors other than the preservation method itself may alter the morphologic and functional integrity of a cadaveric kidney. These include preexisting renal pathology in the donor, circumstances of donor death, warm ischemia time, and total preservation time. All such factors must be considered and analyzed before any morphologic changes are directly attributed to the preservation method itself.

In this series, significant preexisting renal disease was presumably minimized by the selection of donors with negative clinical histories and acceptable serum creatinine levels at the time of death (mean, 1.2 mg/100 ml; range, 0.7 to 2.1 mg/100 ml). With one exception (No. 6), no morphologic evidence of significant preexistent renal disease was found in our material. The 1 hour posttransplant biopsy of Case 6 showed electron-dense mesangial and subendothelial deposits reacting with anti-IgA, anti-IgG and anti-β_1C-globulin. Clinical information about the donor was unavailable, and a pretransplant biopsy had not been obtained. The recipient had had membranous glomerulonephritis and had undergone bilateral nephrectomy 2 years prior to transplantation. The transplant was removed 3 weeks later because of an infected hematoma; the immune deposits were no longer identifiable.

Mild arteriolar sclerosis and lesions reflecting the effects of mild chronic ischemia were observed in approximately 20% of the pretransplant biopsies from both the perfused and nonperfused kidneys. In about 10%, hyalin casts were present, and an occasional biopsy contained mild focal lymphocytic infiltrates and focal tubular calcification. Focal glomerular capillary loop collapse and patchy foot process fusion were observed by electron microscopy in most pretransplant biopsies, perfused and nonperfused, but may have been present at the time the kidney was harvested or could have developed subsequently. These changes persisted in the 1 hour posttransplant biopsies.

Tubular changes (cloudy swelling, vacuolation) were seen frequently in perfused and nonperfused kidneys (73%). Sloughing of the proximal tubular epithelium was another common (65%) finding in both groups. Significant dilatation of tubules was observed in pretransplant biopsies

from perfused kidneys (five of ten) but not in the nonperfused kidneys.

It is possible that most of the degenerative changes observed in the pretransplant biopsies occurred, or at least were initiated, while the kidney still resided in the donor; that is, after the blood supply was impaired or interrupted and before the temperature was lowered to a level where enzymatic activity was practically abolished (warm ischemia). The circumstances of donor death (agonal period) could, therefore, be a major determinant in the development of these lesions. There was no difference in the frequency of vasopressor use in the donors of perfused versus nonperfused kidneys. It should be emphasized that all pretransplant biopsies were obtained at the end of the respective preservation period which on the average was approximately three times longer for the perfused kidneys (Table 6). This time difference no doubt reflected the conviction that simple hypothermia was ineffective beyond 10 hours and that more prolonged preservation required continuous perfusion. Within the group of perfused kidneys, there were no significant morphologic differences when kidneys with different preservation times were compared. The mean warm ischemia time was also significantly longer for the perfused kidneys (Table 6). The warm ischemia time for six kidneys exceeded 15 minutes, but the histology of these kidneys was not significantly different from those with shorter warm ischemia times. Obviously, one cannot predict the fate of kidneys preserved by simple hypothermia for time periods as long as those employed for some of the perfused kidneys.

At the ultrastructural level, the main differences between pretransplant biopsies from perfused and nonperfused kidneys were observed in the glomeruli. Nonperfused kidneys usually showed moderately swollen glomerular endothelium with reduced density of the cytoplasmic ground substance. In a few loops, this change appeared to compromise the lumen. The endothelium of perfused kidneys was generally flat and sometimes was associated with an increased electron-lucent subendothelial zone.

Table 6—Preservation Data

	Simple hypothermia	Pulsatile perfusion	
		IVC absent	IVC present
Total preservation time (hrs)	6.7 (1–14)	23 (7–47)	22 (6–41)
No. of kidneys	16	7	18
Warm ischemia time (mins)	6 (5–15)	15 (5–45)	11 (5–41)
No. of kidneys	18	12	18

Time values are means, with range in parentheses.

These differences could be attributed to variations in the osmolarity of the fluids in which the kidneys had been preserved. High osmolarity fluids have been advocated for perfusion of isolated kidneys [6] since reduction of intracellular edema theoretically might permit more rapid blood flow following connection to the recipient circulation [20] and thus effectively diminish warm ischemia time. Our findings suggest that endothelial swelling is indeed prevented by the use of hyperosmolar perfusates, although this does not necessarily guarantee integrity of cell membranes or preservation of endothelial structure and function. Focal cell disruption and nuclear pyknosis of the glomerular endothelium were noted in pretransplant biopsies of kidneys preserved by either method. These focal endothelial changes, apparent only by electron microscopy, did not correlate with subsequent development of intravascular coagulation as observed in the 1 hour posttransplant biopsies. Others [19] have reported similar endothelial changes in perfused, but not in nonperfused, kidneys and have concluded that they are the result of mechanical perfusion. According to our observations, these focal endothelial disruptions cannot be attributed solely to perfusion and do not necessarily represent the lesion which initiates intravascular coagulation. In our series, endothelial cells were focally detached from the basement membrane of small arteries in both perfused and nonperfused kidneys, supporting the notion that these changes are also not caused by mechanical perfusion, as has been previously suggested.[18]

Widespread endothelial destruction was seen in only one pair of kidneys in which both methods of preservation had been used (No. 26). Such extensive endothelial changes were obvious by light microscopy and were associated with nonspecific deposition of plasma proteins within vessel walls and glomeruli of the kidney perfused with AB plasma.

In summary: Examination of pretransplant biopsies did not reveal a specific morphologic lesion consistently associated with subsequent development of intravascular coagulation in the transplant. Whenever severe endothelial damage is detectable by the light microscope, as in Case 26, the kidney should not be transplanted since recovery of function is very unlikely. In the 1-hour biopsies of previously perfused kidneys, a frequent finding was mild to moderate intravascular coagulation, with fibrin and platelet aggregations in glomerular loops. These focal glomerular lesions were unaccompanied by arteriolar changes and did not necessarily influence subsequent transplant function; seven of twelve kidneys with these lesions functioned adequately during the six-month follow-up period. The gross appearance of these kidneys was unremarkable, both at the time of perfusion and intraoperatively. Follow-up biopsies showed

that these mild to moderate lesions resolved completely or resulted in focal collapse and scarring of glomerular loops.

In six perfused transplanted kidneys there was extensive deposition of intravascular fibrin accompanied by focal deposits of IgM and β_1C-globulin in the glomeruli and vessels. None of these kidneys functioned adequately and all were removed within 4 weeks. Three of the six developed a soft texture and patchy blue discoloration within 1 to 2 hours of transplantation. The presence of immunoglobulins, in particular IgM, in the vessel walls of these severely affected kidneys, persisted in follow-up biopsies and in the nephrectomy specimens, suggesting a possible immunologic mechanism of injury such as hyperacute rejection or a localized Shwartzman reaction.[12,22] Polymorphonuclear leukocyte infiltration of glomeruli and peritubular capillaries, a morphologic feature often seen in early biopsies of hyperacute homograft rejection [23] was neither a constant nor a particularly striking feature in any of these extensively damaged kidneys. On the other hand, intravascular coagulation is one of the major findings in hyperacute homograft rejection, and this possible pathogenetic mechanism cannot be completely excluded on the basis of morphology alone. None of the recipients had had preformed antibodies as determined by the conventional lymphototoxicity technique, and HLA matching had been similar in both groups. If hyperacute rejection was indeed the cause of these lesions, one would have expected to find them with equal frequency in both perfused and nonperfused kidneys; such was not the case in our material.

It is possible that when plasma is used as perfusate an immunologic reaction may result from antibodies present in the perfusate which are capable of reacting with antigens on the renal endothelium. Such a phenomenon has been described previously.[24,25] In the present study, the seven pretransplant kidneys (five of which were perfused) examined by direct immunofluorescence showed no deposition of immunoglobulins with the exception of one pair of kidneys (No. 26) preserved for a total of 40 hours and perfused with AB plasma. These kidneys showed endothelial damage that was obvious by light microscopy and showed deposition of immunoglobulins, fibrinogen, complement, and albumin within glomeruli and vessels. Since these kidneys also showed severe endothelial damage and a nonspecific immunofluorescence pattern, we may attribute these findings to a nonspecific increase in permeability to all plasma proteins. The posttransplantation development of similar lesions in kidneys perfused only with Ringer's-albumin also militates against an immunologic mechanism due to the perfusate. Perfusion and/or prolonged preservation may alter the cell surface so that normally covert antigenic

sites are exposed and react with high frequency antibodies present in the donor. Such antigenic sites have been demonstrated in cells with surfaces damaged by enzyme treatment or prolonged storage.[26] In the 6 patients studied by indirect immunofluorescence, we were unable to demonstrate antibodies against perfused renal tissue. However, further studies are required to evaluate the role of this possible mechanism. Loss of fibrinolytic activity in the perfused kidney is another factor that could explain some of these findings and should be explored.

Intravascular coagulation was evident only in perfused kidneys, and its presence did not correlate with the total preservation or warm ischemia time (Table 6). The perfusion pressures and flow rates were no different in those kidneys which were judged normal at 1 hour after transplant compared with those which developed the intravascular coagulation lesion (Table 3).

Foreign bodies in glomerular capillaries and arterioles were found in approximately 72% of the perfused kidney. Approximately 70% of kidneys containing foreign bodies also developed the intravascular coagulation lesion; conversely, 66% of kidneys with this lesion contained foreign bodies. Despite this apparent relationship, the presence of foreign bodies alone could not explain the morphologic findings. Foreign bodies were scanty and were rarely seen in the "plug" of platelets and fibrin. Kidneys with very severe changes did not always contain foreign bodies and some biopsies with numerous foreign bodies showed minimal focal intravascular coagulation or none at all. These foreign bodies were seen in kidneys perfused with either plasma or Ringer's-albumin. One type of foreign body could be identified as starch, another consisted of small, fiber-like, refractile particles. The latter could have been introduced into the apparatus during handling or could have originated from the apparatus itself.

In summary: Examination of biopsies obtained 1 to 2 hours after transplantation represents a reliable means of evaluating the condition of the renal transplant and, to a certain extent, of predicting its outcome. Kidneys with severe glomerular and vascular lesions do not function well and usually require removal within a month. Mild to moderate lesions focally limited to the glomeruli are compatible with resumption of adequate function, often after initial depression. Measurement of perfusion pressures and flow rates do not represent a reliable means of predicting the occurrence of these lesions. The gross appearance immediately following surgical implantation is helpful only in those kidneys showing very extensive damage.

Intravascular coagulation is the predominant morphologic lesion (18 of

25) observed 1 hour posttransplantation in kidneys preserved with continuous pulsatile perfusion. This phenomenon must be recognized as an entity and differentiated from hyperacute rejection, to which it has a close morphologic resemblance. The initiating lesion could not be consistently demonstrated in the pretransplant biopsies although it is possible that a subtle endothelial injury during perfusion triggers intravascular coagulation when the kidney is revascularized. Once coagulation has been initiated, a vicious cycle results in further ischemic damage and increased resistance to blood flow. Depending upon the severity of the lesions, biopsies obtained at a later time may show resolution with mild residual glomerular changes or progression to cortical necrosis. The vascular alterations observed in the severely affected kidneys closely resemble those of humoral rejection.

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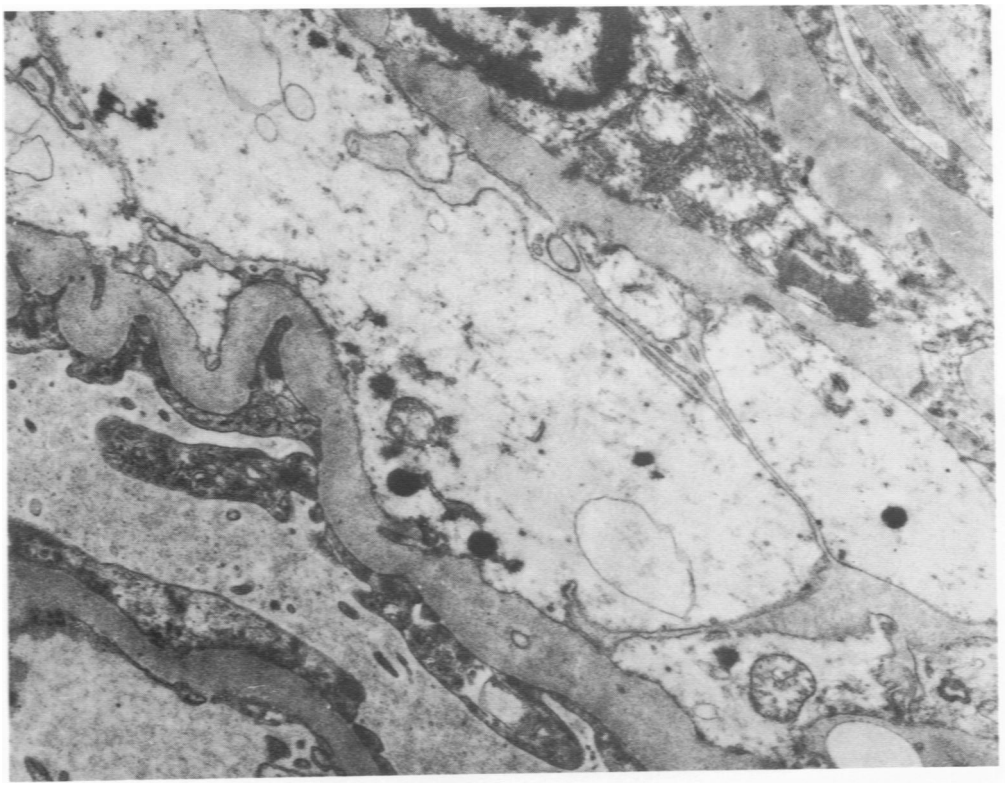


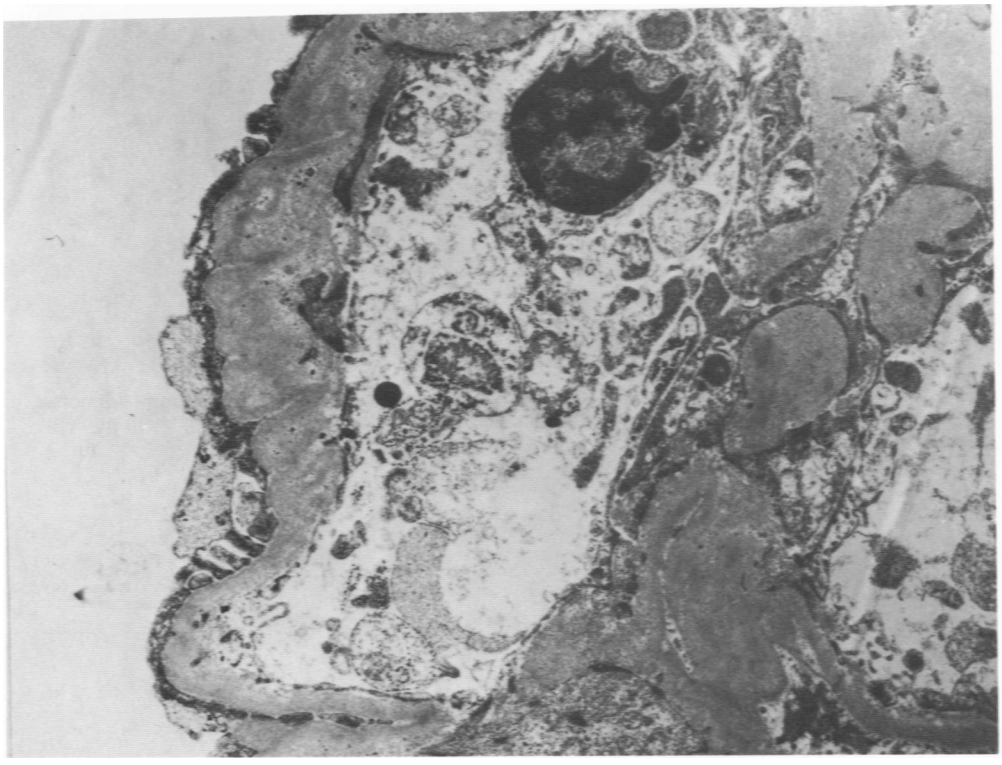


Figure 1—Pretransplant biopsy from a kidney preserved by simple hypothermia. The lumen of a glomerular capillary is compromised by the markedly edematous endothelium. The foot processes of podocytes are fused. (× 9052) **Figure 2**—Pretransplant biopsy from a kidney preserved by simple hypothermia. The endothelium of a glomerular capillary shows nuclear pyknosis and disrupted cytoplasm. The basement membrane is corrugated. (× 5496)

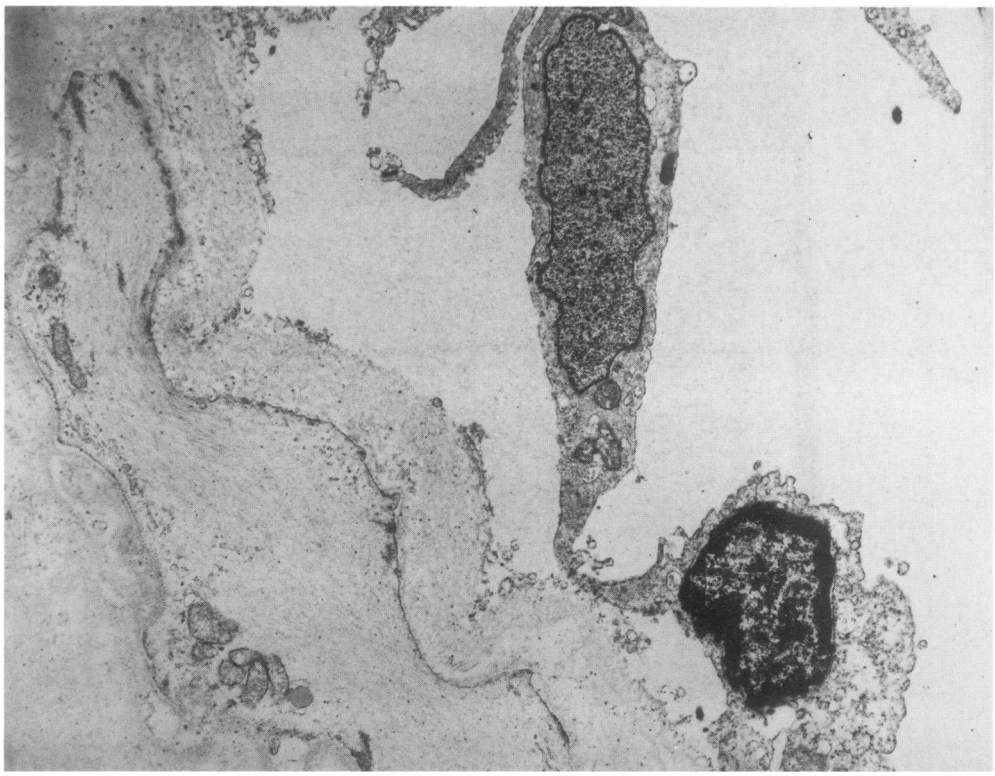
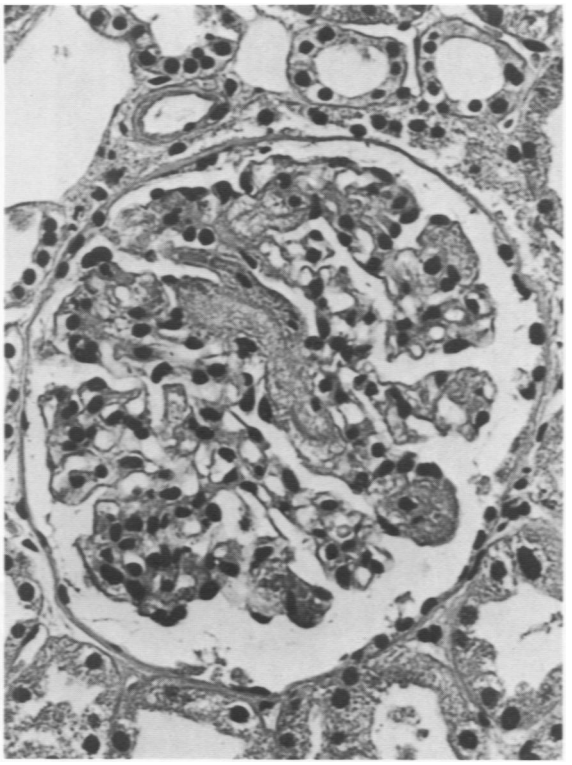
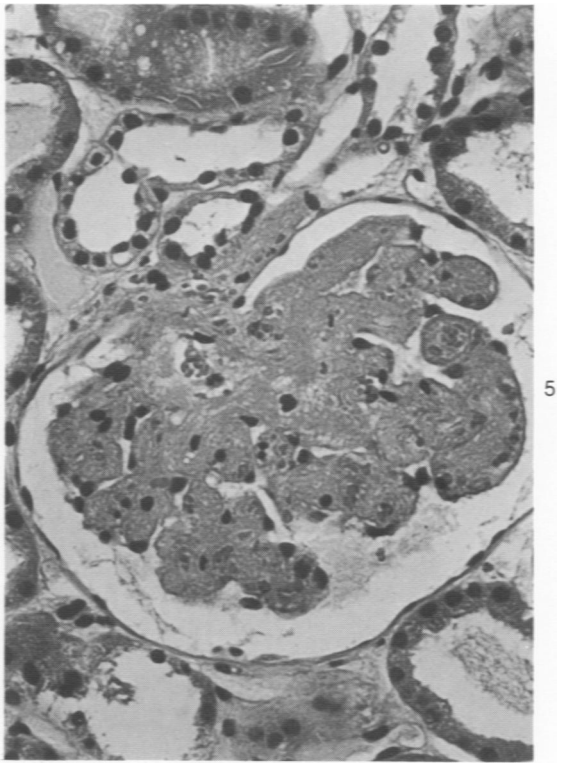


Figure 3—Pretransplant biopsy from a kidney preserved by simple hypothermia. The endothelium of a small artery appears detached; one of the endothelial cells (lower) shows degeneration with nuclear pyknosis and ill-defined organelles. (× 4900)





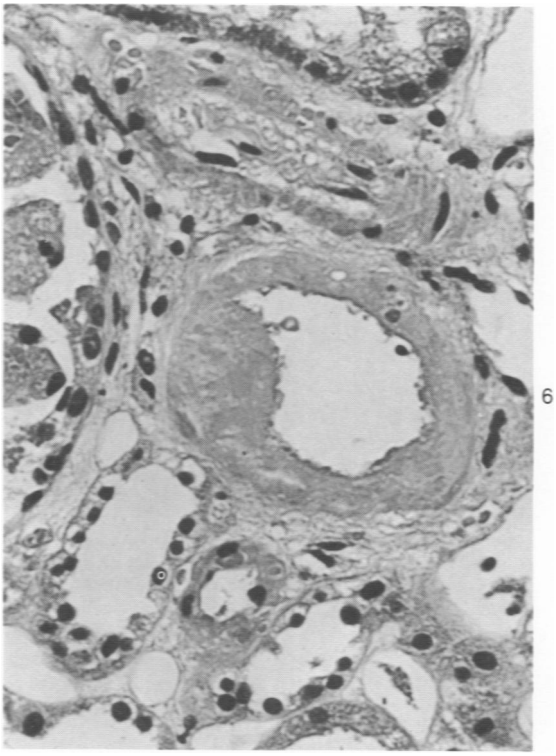


Figure 4—One-hour posttransplant biopsy from a perfused kidney; a glomerulus with mild IVC lesion (loop in center of glomerulus) (H&E, × 325). **Figure 5**—One-hour posttransplant biopsy from a perfused kidney; a glomerulus with severe IVC lesion (H&E, × 325). **Figure 6**—One-hour posttransplant biopsy from a perfused kidney with severe IVC lesions; an arteriole shows a homogeneous, structureless wall and loss of endothelium (H&E, × 365).

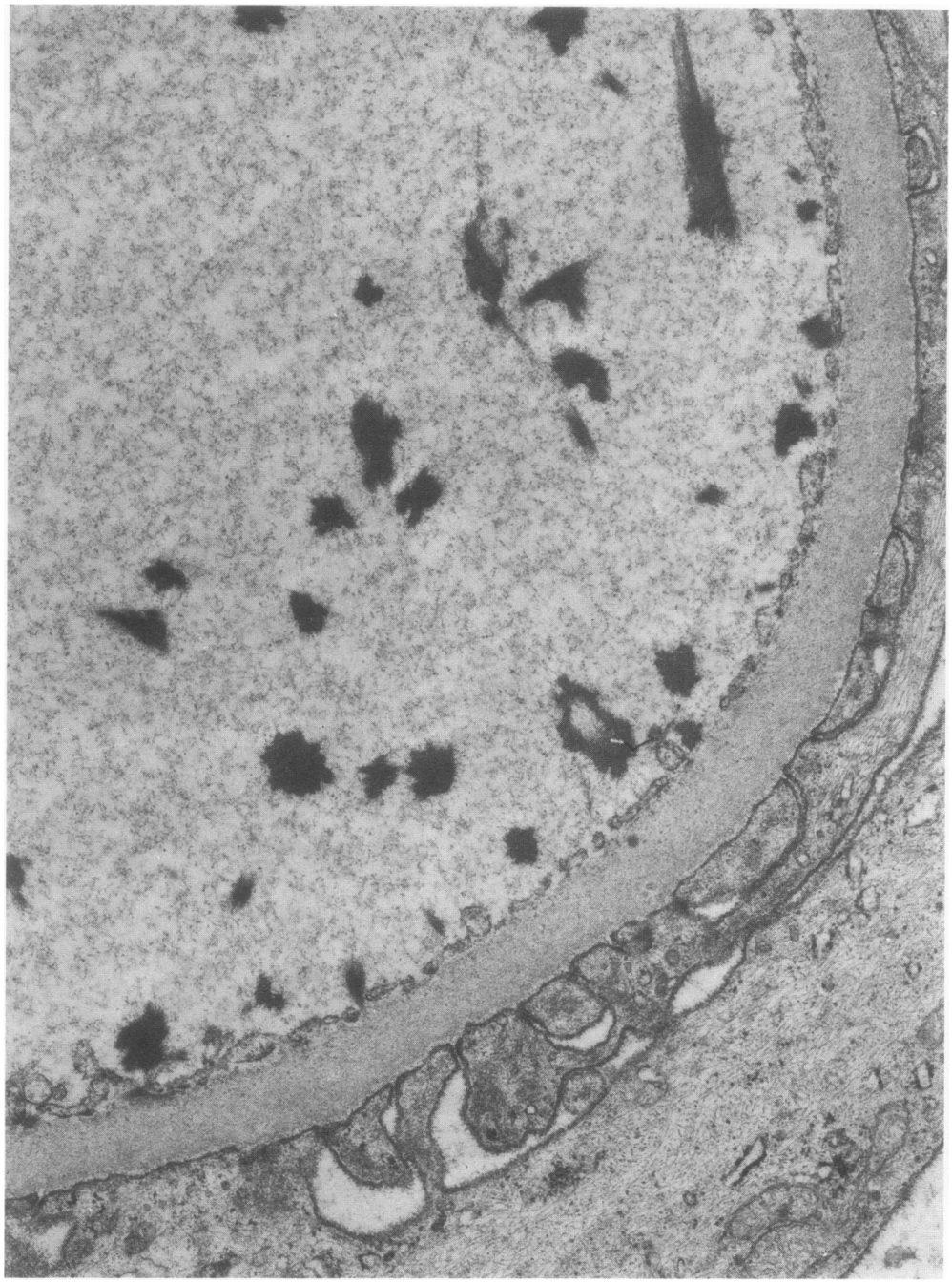


Figure 7—Thirty-minute posttransplant biopsy from a perfused kidney with IVC. A glomerular loop contains fine, granular material and tactoids with periodicity characteristic of fibrin. Note the tactoids apposed to the basement membrane through the pores of endothelium, which is still present. (× 21,552)

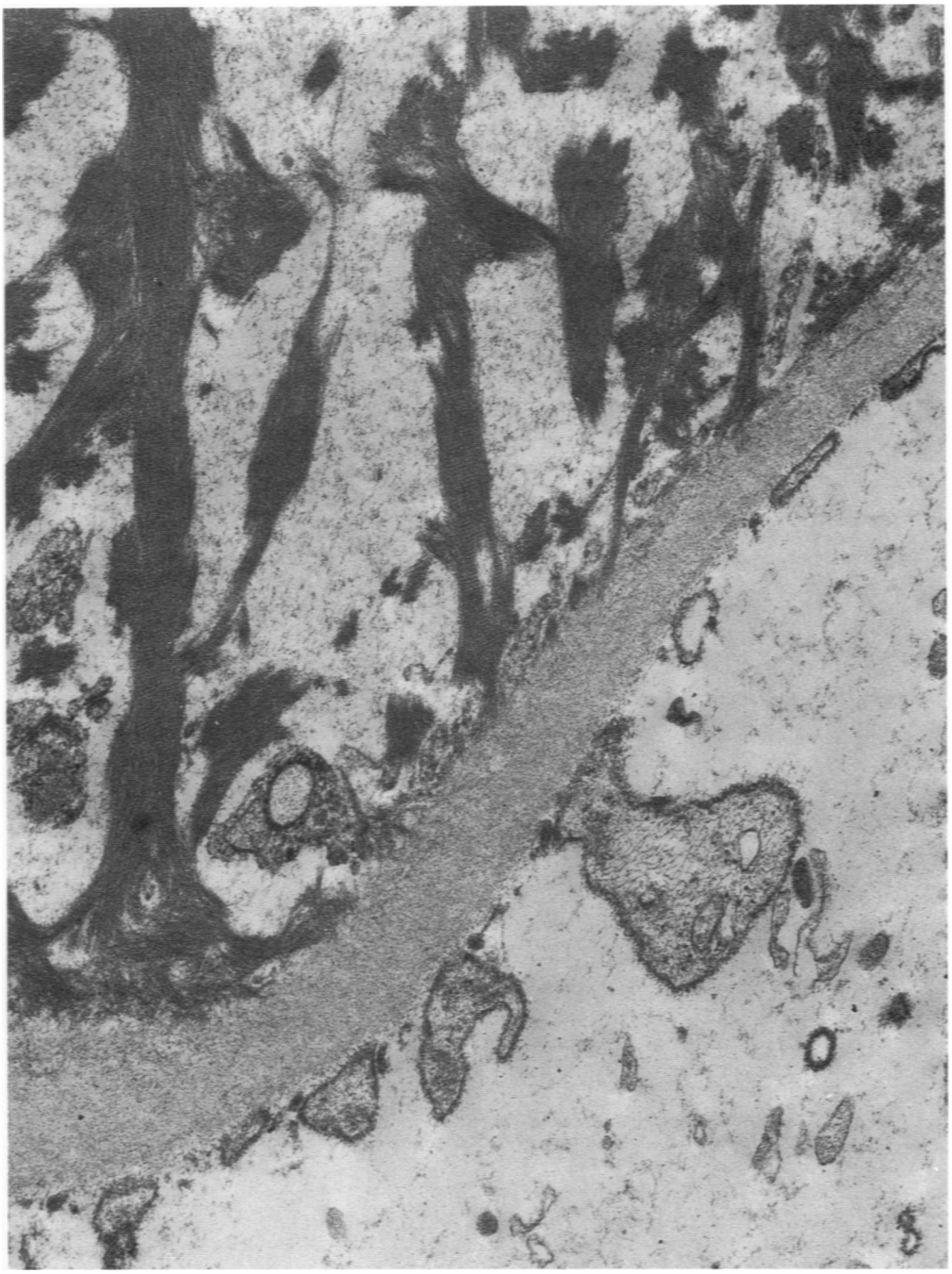
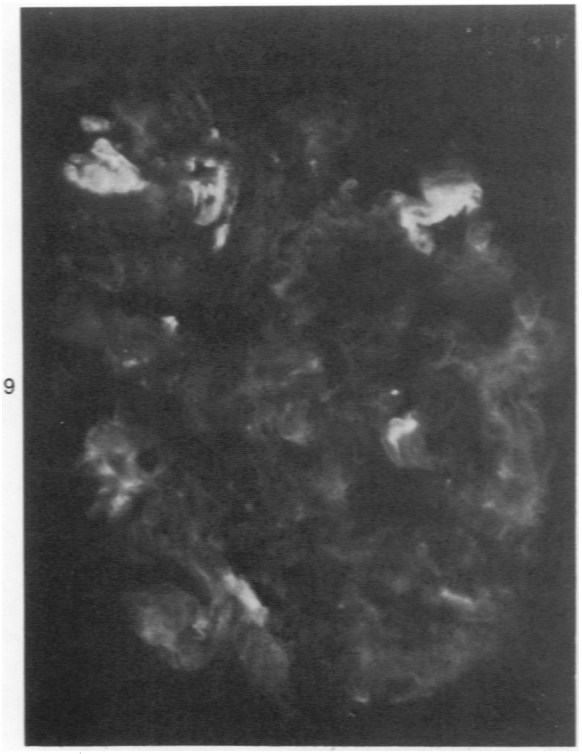


Figure 8—One-hour posttransplant biopsy from a perfused kidney with IVC. Glomerular loop containing fibrin; the endothelium is mostly detached and fibrin is apposed to the basement membrane. (× 30,980)

Figure 9—One-hour posttransplant biopsy from a perfused kidney with mild IVC lesion. A glomerulus reacting focally with the antifibrinogen serum. (H&E, × 400)

Figure 10—One-hour posttransplant biopsy from a perfused kidney with severe IVC. A glomerulus showing positive reaction along the capillary loops and in their lumina, with the antifibrinogen serum. (× 325)

Figure 11—Five-day posttransplantation biopsy from a perfused kidney with severe IVC. Several glomerular loops are denuded of endothelium. (× 10,259)



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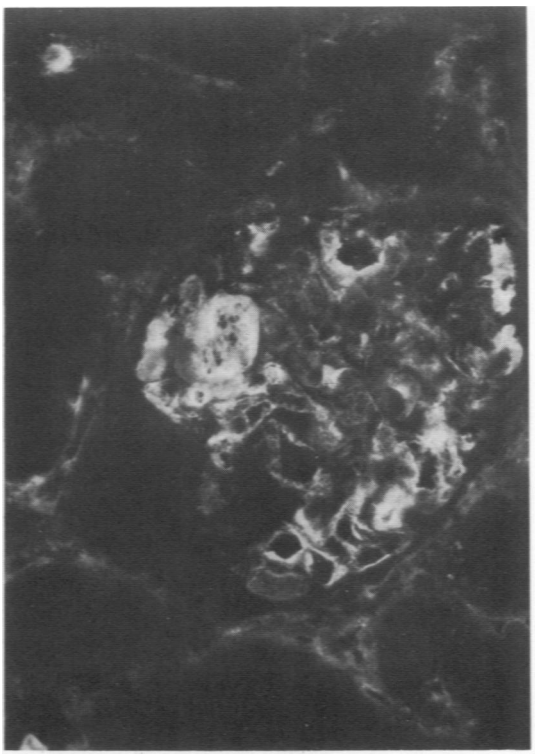

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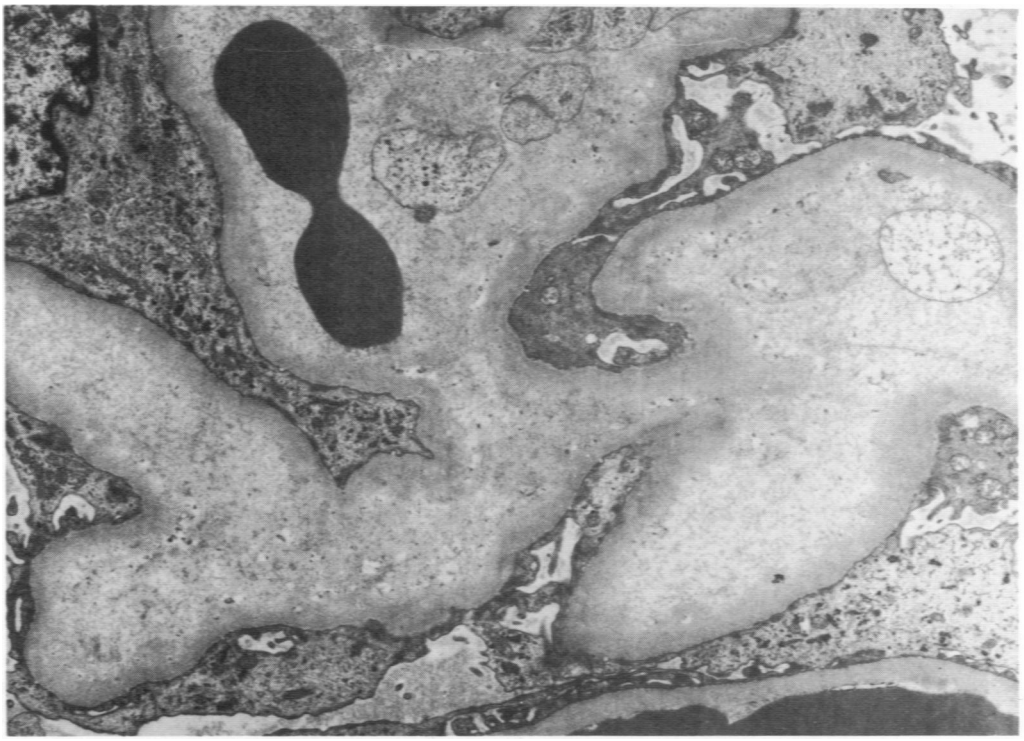

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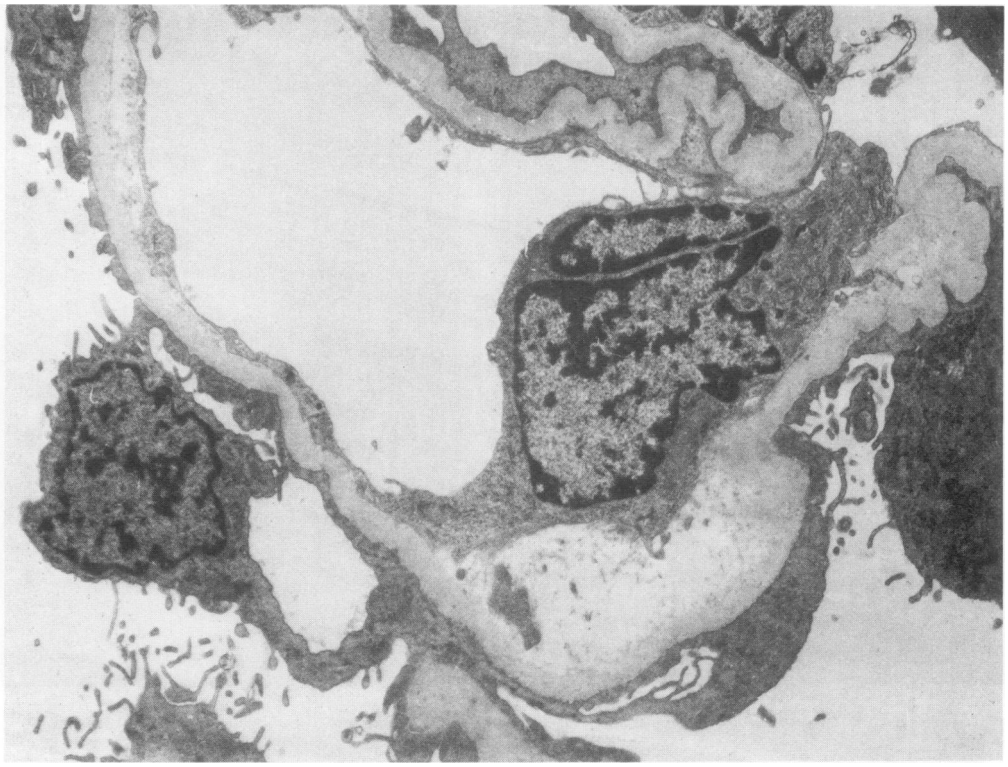

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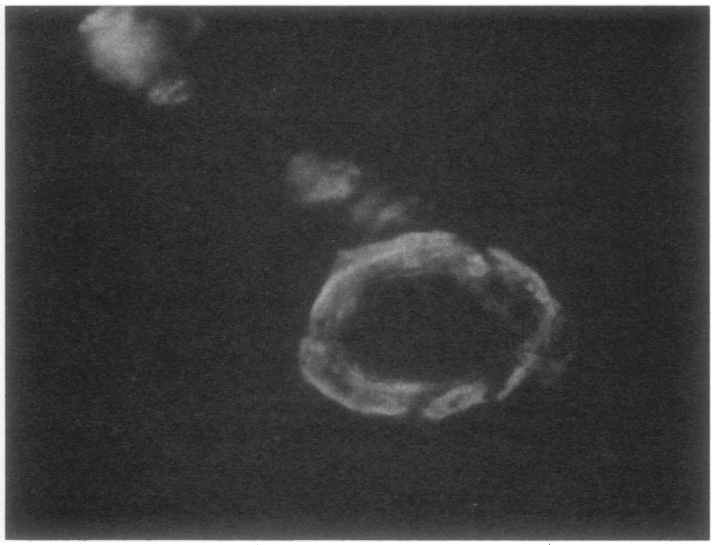

Figure 12—Five-day posttransplantation biopsy from a perfused kidney with severe IVC. A glomerular capillary with large subendothelial electron-lucent space. Foot-process fusion is also present. (×6400) **Figure 13**—Five-day posttransplantation biopsy from a perfused kidney with severe IVC. Small vessels showing strongly positive staining of their walls with the anti-IgM serum. (× 400)