A Primate Model of Hyperacute Renal Allograft Rejection

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Hyperacute renal allograft rejection is initiated by primary immune injury to vascular endothelium and is propagated by secondary vasoconstriction, platelet aggregation and intravascular coagulation. Previous dissociation of these primary and secondary events, with graft survival in one human, suggested that the more usual graft failure was due to secondary injury. As a basis for further modification studies, this primate model most closely resembled its counterpart in man, as the onset and intensity of functional, morphologic and biochemical alterations were similar. Unmodified allografts failed within 5 minutes. The earliest and most abnormal finding was marked reduction in renal blood flow affecting all compartments. By 5 minutes, histologic changes of hyperacute rejection as well as antibody and faint C3 deposits were noted, but biopsies suggested that the initial flow reduction was more likely due to vasoconstriction, which was then followed by vascular obstruction. Glomeruli appeared most damaged, but at the highest antibody titer arterial injury was more prominent. Early red cell sequestration and stasis was marked, followed by progressive platelet clumping and neutrophil infiltration. While the decline in renal venous C3 levels was prompt, as in man, early intrarenal activation of the coagulation, fibrinolytic and kinin-forming systems could not be demonstrated, and fibrin formation was sparse by light and fluorescence microscopy. Qualitatively similar histologic and functional alterations were noted in autograft controls. While the initiating event was unclear and may have been accentuated by the arteriovenous shunts utilized, the final mechanism was probably marked vasoconstriction with renal ischemia. Intrarenal C3 consumption was an important finding and was not associated with tissue deposits of antibody or complement; it may provide a parallel with the progressive complement-mediated injury associated with acute myocardial ischemia noted by others. Endothelial injury was not seen in arteries, and all alterations were delayed in onset and progressed more slowly than in allografts. These findings may elucidate the mechanism of early malfunction of most autografts. Treatment of additional autografts with increasing doses of heparin progressively reversed these changes and even prevented the initial reduction in blood flow. Therefore, many alterations consistent with hyperacute rejection which are probably immediately responsible for graft failure can also be initiated by nonspecific, nonimmunologic events and, where injury is less intense, can be prevented pharmacologically. This model provides a means of dissecting the injurious events and subsequent evaluation of the effectiveness and interaction of various agents on the damaging secondary alterations which occur during hyperacute rejection.
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HYPERIMMUNE HUMAN RENAL ALLOGRAFT REJECTION has become an important clinical problem, as transplant centers accumulate increasing numbers of presensitized potential recipients. Such rejection may cause a graft to fail immediately (hyperacute) or more gradually over several days to weeks.[1] Circulating antigraft antibody is formed by certain recipients in response to immunologic challenges such as repeated blood transfusions for treatment of chronic anemia,[2] multiparity, cross-reaction with microbial antigens,[3] and possibly to early, poorly controlled, cellular rejection episodes with release of graft antigens.

The clinical and morphologic patterns of hyperimmune rejection reflect primary and secondary injurious events. Primary injury occurs in renal blood vessels through binding of circulating antibody to foreign histocompatibility antigens present on the endothelium, followed by complement-mediated membrane damage. Secondary injury rapidly ensues due to activation of proteolytic enzymes and probable release of vasoactive substances and includes the triad of platelet aggregation, local intravascular coagulation and vasoconstriction. This results in increased vascular resistance and obstruction, markedly reduced blood flow and progressive ischemic necrosis of the graft.

Abrogation of hyperacute rejection has been the subject of considerable investigation, with limited results. However, successful pharmacologic dissociation of primary and secondary injury with survival of one human graft suggested that in some presensitized humans, graft failure may be prevented.[4,5] This finding encouraged us to pursue a systematic evaluation of the secondary injury and its possible modification in an animal model.

Prior studies in rodents and canines revealed poor reproducibility in the former and variable onset and severity in the latter.[6,7] In contrast, in primates these patterns appeared reproducible and similar to those in man.[1,8]

This report presents the primate model, documents the latter similarities, and dissects the concurrent alterations within the primate kidneys in the vascular, complement, coagulation, fibrinolytic and kallikrein systems. The changes observed in allografts are compared with those in autografts. These results form the basis for other studies which examine the mechanisms whereby anticoagulant, antithrombotic and other agents fail or succeed in modifying the secondary injury.

Materials and Methods

Presensitization, Transplantation and Treatment

Macaca speciosa monkeys of either sex and weighing 6 to 12 kg were paired; when appropriate, donor-specific presensitization was achieved by serial exchange

of full thickness skin grafts. For each exchange, two skin grafts, each measuring approximately 3 cm in diameter, were transferred to the back within each donor recipient pair under Sernylan® (phencyclidine hydrochloride) anesthesia. When skin was rejected as a "white graft," lymphocytotoxic antibody titers (LCT) were performed 1 day prior to kidney transplantation using a standard microdroplet test.[9] Animals were segregated into the following treatment groups. *Group 1—SP,* arteriovenous (A-V) shunt perfusion controls without a kidney in the system and with no treatment. *Group 2:* Autografts (autoperfusion controls) subdivided as follows: *2a-AP,* Autoperfusion. Following excision, the animal's own kidney was anastomosed to an indwelling A-V shunt; no treatment was given. *2b-LDHAP,* Low dose heparin autoperfusion. Same as 2a, but animal pretreated with heparin (2 mg/kg) intravenously prior to anastomosis of kidney to A-V shunt. *2c-HDHAP,* High dose heparin autoperfusion. Same as 2b, but animal also treated with continuous heparin infusion (1 mg/3 kg/hr) into the arterial limb of the A-V shunt, utilizing a peristaltic pump. *Group 3—NTAL.* Nontreated renal allograft. Donor kidney anastomosed to A-V shunt in presensitized recipient. No treatment was given.

At transplantation, paired monkeys were anesthetized with Sernylan. Excised donor kidneys were flushed with Ringer's injection solution containing 25 mg heparin and 5 mg xylocaine per 500 cc (pH adjusted to 7.2 with sodium bicarbonate, at room temperature) until the venous effluent appeared free of red blood cells. Ischemia time for all kidneys was within the range of 11 to 27 minutes.

All kidneys were anastomosed to the common femoral vessels utilizing A-V shunts (Extracorporeal Medical Specialities, Inc) which had been stored overnight in a sterile 1.0% of a 1:1000 heparin solution. All recipients received intravenous normal saline infusion (50 ml/hr) intraoperatively. All experiments were terminated arbitrarily at 180 minutes postanastomosis.

Total Renal Blood Flow

Onset of renal cortical cyanosis and loss of turgor, and cessation of venous blood flow were used as indicators of initial injury to and failure of the kidney, respectively. Total arterial and venous blood flow (in milliliters per minute) were each approximated by timed collection of samples at 2, 6, 12, 25, 40, 60, 120 and 180 minutes after anastomosis, as available, into calibrated test tubes, by controlled interruption of the appropriate vessel tip connectors. Venous samples were collected first.

Differential Renal Blood Flow

Normal distribution of outer and inner cortical, and medullary blood flow was measured in undisturbed donor kidneys (controls). A 16-gauge Intracath (Deseret Pharmaceutical Co) was threaded to a premeasured length via the femoral artery to the midthoracic aorta and 2 to 3 $\times$ 10^5 microspheres (15-μ diameter, 3M Company) labeled with chromium-51 were injected.[10] Differential blood flow was subsequently measured in the kidney grafts utilizing cerium-141- and strontium-85-labeled microspheres (2 to 3 $\times$ 10^4) injected directly into the arterial limb of the shunt at 5 and 30 minutes, respectively, posttransplantation. Following graft excision, multiple, weighed coronal slices of outer and inner cortex and medulla were evaluated for sequestration of microspheres,[11] utilizing a differential gamma cell scintillation counter with pulse height analysis. Isotope data were converted to counts per minute per gram of tissue and then to mean percent radioactivity (distribution) per compartment, assuming approximately equal proportions of renal mass in the outer cortex, inner cortex and medulla.[12,13] Lungs were removed when animals were sacrificed at termination of the experiments and evaluated for radioactivity

(entrapment of microspheres) to assess possible juxtamedullary shunting of blood flow.

Histology and Immunofluorescence

Portions of renal wedge biopsies, obtained just prior to anastomosis (zero time control) and at 10, 30, 60, 120 and 180 minutes posttransplantation or at the time of graft failure, were processed for routine (hematoxylin and eosin and periodic acid-Schiff staining) light microscopy and were quick frozen for direct immunofluorescent staining with goat anti-human IgG, IgM, IgA, C3 (β1C/β1A), fibrinogen and albumin (Hyland Laboratories).[14] Precipitation of the fluorescein-labeled anti-human sera with *Macaca* serum and plasma was initially demonstrated by immunoelectrophoresis and immunodiffusion.

Renal A-V Blood Studies

Serial renal arterial and venous blood samples obtained at zero time and as enumerated above were evaluated for serum complement (C3) levels by radial immunodiffusion utilizing Immuno-plates® (Hyland Laboratories) as well as for hematocrit levels, white blood cell total and differential counts, platelet counts and for coagulation factor II, VIII, X and XII levels by standard one-stage assay as previously described.[15] Plasminogen levels were measured using a caseinolytic technic after streptokinase activation,[16] titers of fibrin degraduation products (FDP) by staphylococcal clumping [17] and prekallikrein and kallikrein inhibitor levels using tosyl arginine methyl ester as a substrate after kaolin activation.[18] Results of all serial determinations were then expressed as percent normal for each monkey, using its zero time arterial levels as the 100% of normal value. Separate evaluation of alterations resulting from A-V shunt insertion was made by comparison of zero time arterial samples with peripheral venous blood obtained prior to any operative manipulation.

Results

Host Response to Operative Procedure and A-V Shunt

Group 1-SC controls reflected host response to the operative procedure and alterations due to the A-V shunts alone. Blood flow through the shunts was maintained at about 361 ml/min throughout the 180-minute period (Table 1).

Renal venous and arterial C3 levels remained normal (Text-figure 2). Venous hematocrit levels gradually declined to 79% by 180 minutes (Text-figure 3A). In contrast, venous WBC levels increased to 259% of normal by 120 minutes (Text-figure 3B). Venous platelet counts fluctuated between 104 and 85% of normal throughout (Text-figure 3C). There were no detectable A-V differences in these formed elements to suggest sequestration within the 6- to 10-cm shunt segments at these flow rates.

Renal venous levels of factors II, X and VIII showed a transient decline to 73, 81 and 67%, respectively, between 6 and 40 minutes, suggesting activation and consumption (Text-figure 4A–C). Plasminogen levels

Table 1—Summary of Cytotoxic Titer, Renal Failure and Total Renal Venous Blood Flow Data*

Group	Animal No.	Cytotoxic titer	Onset (min)		Renal venous blood flow	
			Cortical cyanosis	Loss of turgor	Duration (min)	Rate at 180 min (ml/min)
1-SC	1	—	—	—	180	332
	2	—	—	—	180	365
	3	—	—	—	180	400
	4	—	—	—	180	348
	Mean	—	—	—	180	361
2a-AP	5	—	63	80	80	—
	6	—	19	55	55	—
	7	—	13	34	34	—
	8	—	40	40	90	—
	9	—	35	65	65	—
	Mean		34	55	65	—
2b-LDHAP	10	—	180	45	180	2
	11	—	38	180	180	15
	12	—	180	180	180	44
	Mean		133	135	180	20
2c-HDHAP	13	—	180	180	180	114
	14	—	180	180	180	126
	15	—	180	180	180	13
	Mean		180	180	180	84
3-NTAL	16	1:512	2	1	5	—
	17	1:128	2	2	4	—
	18	1:128	2	3	7	—
	Mean	1:256†	2	2	5	—

* No cortical cyanosis, loss of turgor or cessation of flow was seen by 180 minutes.
† To the nearest tube dilution.

declined to 70% of normal by 6 minutes but promptly returned to and remained normal (Text-figure 5). However, FDP titers were 1:1024 (768 μg/ml) in the zero time blood samples and remained in that range. This represented a change from a normal upper limit of 1:8 (6 μg/ml) in the peripheral premanipulation samples and thus resulted from the operative procedure and shunt insertion. These titers were therefore of no value for serial studies of fibrinolysis. While factor XII declined in both venous and arterial samples to 48% of normal between 6 to 60 minutes, there was no detectable activation of the kinin-forming system, as prekallikrein and kallikrein inhibitor levels remained normal (Text-figure 6A and B).

Preliminary Observations

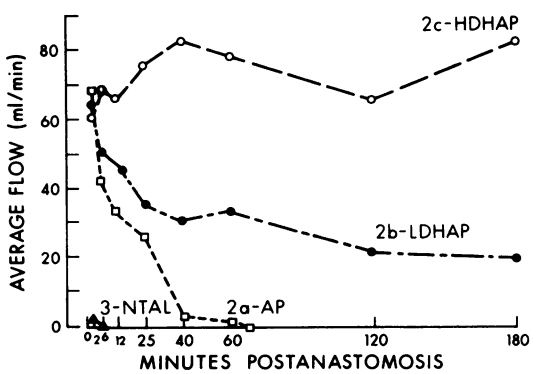
Group 3-NTAL kidneys (mean LCT, 1:256) demonstrated the most marked alterations (Table 1). Progressive deepening of cortical cyanosis and loss of turgor was apparent in all by 2 minutes. Group 2a-AP kidneys had initial improvement in color and cortical perfusion but then all had onset of progressive cyanosis by 34 minutes and loss of turgor by 55 minutes. With heparin therapy (Group 2b-LDHAP), all autografts showed good cortical color and perfusion immediately after anastomosis. Only one kidney showed cortical cyanosis at 38 minutes, while another had loss of turgor at 45 minutes. Group 2c-HDHAP kidneys did not show onset of these changes.

Total Renal Blood Flow

In Group 3-NTAL kidneys, venous flow declined abruptly to 2 ml/min by 2 minutes and ceased at 5 minutes (Table 1, Text-figure 1). In contrast, in Group 2a-AP mean venous flow was 69 ml/min at 2 minutes; this declined to 3 ml/min by 40 minutes and ceased at 65 minutes. With heparin therapy (Group 2b-LDHAP) there was a more gradual decline in venous flow from 65 ml/min at 2 minutes to 20 ml/min by 180 minutes. With intraarterial heparin perfusion (Group 2c-HDHAP), flow was maintained between a mean of 61 and 84 ml/min throughout the 3-hour period.

Differential Renal Blood Flow

Mean compartmental flow values obtained from 9 "normal" animals following midthoracic aortic injection of ^{51}Cr-labeled microspheres, shown in Table 2, are in accord with data from normal canines and rabbits.[11,19] The most marked alterations occurred in Group 3-NTAL by 5 minutes, when a very low counts per minute per gram and low total



TEXT-FIG 1—Mean values of renal venous blood flow.

Table 2—Differential Renal Blood Flow (Percent Counts per Minute per Gram per Compartment)

Normal controls (aortic injection)				Experimental animals								
					Ani-	5 minutes			30 minutes			
Control No.	OC	IC	M	Group	mal No.	OC	IC	M	OC	IC	M
1	56	35	9	2a-AP	7	40	28	32	37	37	26
2	56	35	9		8	27	49	24	37	44	19
3	34	61	5		9	50	38	12	37	43	19
4	42	45	13		Mean	39	38	23	37	42	21
5	65	31	4	2b-LDHAP	10	53	33	14	49	37	14
6	58	36	6		11	32	43	25	59	29	12
7	45	50	5		12	55	26	19	41	36	23
8	61	32	7		Mean	47	34	19	50	34	16
9	51	36	13	2c-HDHAP	13	32	45	23	45	40	14
Mean ± SD	52 ± 9	40 ± 9	8 ± 3		14	46	36	18	50	36	14
					15	36	46	18	56	40	4
					Mean	38	42	20	51	39	10
				3-NTAL	16	0	100	0	—	—	—
					17	0	0	0	—	—	—
					18	54	32	14	—	—	—
					Mean	18	44	5			

OC = outer cortex, IC = inner cortex, M = medulla.

flow affected all compartments. Flow distribution in kidneys of Group 2a-AP animals was also persistently abnormal. Outer cortical flow was reduced, and medullary distribution was increased. This altered medullary flow, when compared to the normal controls, was significant ($P < 0.01$) by the Wilcoxon rank sum test. The use of low dose heparin therapy (Group 2b-LDHAP) was associated with a more normal pattern of compartmental flow. The 30-minute outer cortical flow was significantly improved from the corresponding Group 2a-AP value ($P < 0.05$). At 5 minutes, compartmental flow in Group 2c-HDHAP kidneys was similar to that in Group 2a-AP. However, by 30 minutes, distribution was improved in all and was similar to that in aortic injection controls. The improved outer cortical and medullary flow were each significant ($P < 0.05$) when compared to the 30-minute 2a-AP values.

Lungs from 11 animals were counted for both ^{141}Ce and ^{85}Sr. In no instance were these counts significantly above background, thus eliminating juxtamedullary shunting as a possible cause of renal cortical underperfusion.

Light Microscopy

All zero time control biopsies showed only occasional red cell and small platelet aggregates within capillaries following perfusion with Ringer's solution. The tubular epithelium showed very early cytoplasmic degenerative changes in most kidneys.

Group 3-NTAL kidneys showed extensive alterations. By 10 minutes, 90% of the glomeruli and 20% of the peritubular capillaries showed minimal to marked aggregation (predominantly of red cells, but also of platelets), with variable capillary obstruction. Increased numbers of neutrophils within lumina and minimal to moderate endothelial cell damage were also noted (Figure 1). Cortical arteries and arterioles appeared contracted. Forty percent of the arteries contained thrombi with variable obstruction and multifocal endothelial nuclear pyknosis. These changes were most marked in the graft against the highest LCT (1:512), where multifocal cytoplasmic swelling, vacuolization and loss of endothelium were also seen. Neutrophils were marginated along endothelial cells (Figure 3) and segments of denuded vascular basement membrane and in foci showing digestion of internal elastic lamina and adjacent smooth muscle. All of these changes showed progression in nephrectomy specimens obtained at 15 to 30 minutes.

However, Group 2a-AP kidneys also showed alterations. By 10 minutes, 80% of the glomeruli and 30% of the peritubular capillaries contained mild aggregates (predominantly of red cells, but also of platelets within collapsed capillary loops), increased numbers of neutrophils within multifocal thrombi and scattered pyknotic endothelial cells. Early changes in arteries were limited to focal red cell stasis and platelet aggregation. Thirty- to 120-minute biopsies showed little progression of the changes in glomeruli, but those in peritubular capillaries increased in distribution (50%) while 75% of cortical arteries in all grafts contained mural thrombi, some of which appeared occlusive.

With heparin therapy, kidneys of monkeys in Groups 2b-LDHAP and 2c-HDHAP showed minimal changes by 10 minutes. Occasional glomerular and peritubular capillaries contained slightly increased numbers of red cells, neutrophils and scattered platelet aggregates. There were no changes in arteries. However, by 1 to 3 hours, alterations in kidneys of Group 2b-LDHAP animals resembled those in Group 2a-AP and involved 80% of the glomeruli and 40% of the peritubular capillaries. Many glomeruli showed capillary collapse and focal loss of endothelium, more prominently in the inner cortex. While some arteries contained nonocclusive red cell and platelet clumps without evident injury to endothelium or media, most showed the same degree of contraction seen in

Groups 2a-AP and 3-NTAL (Figure 4). In contrast, serial biopsies from Group 2c-HDHAP kidneys did not show progressive alterations and, at 180 minutes, more closely resembled the zero time controls (Figure 5). Cortical arteries in this group did not appear as contracted as those in the other groups.

All kidneys showed progressive tubular epithelial degenerative changes which ranged from fine cytoplasmic vacuolization to pyknosis of up to 20% of the nuclei. These alterations were least prominent in Group 2c-HDHAP.

Immunofluorescence

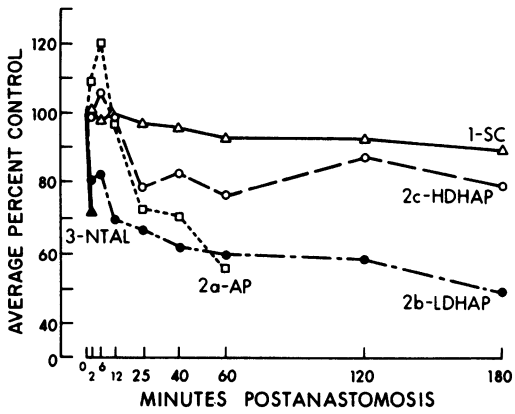
All zero time control and serial biopsies from Groups 2a-AP, 2b-LDHAP and 2c-HDHAP showed only rare, small fibrinogen deposits in capillaries and in arterioles. These deposits often appeared very finely granular and suggested staining of fibrinogen in platelets. IgG, IgM, C3 and albumin stains were consistently negative.

However, Group 3-NTAL grafts showed IgG, IgM, C3 and fibrinogen deposits by 5 minutes after revascularization (Figure 2). The initial mixed linear and finely granular immune deposits in most glomeruli were generally weak and focal, but by 30 minutes were more moderate in intensity and predominantly finely granular in pattern. The latter finding coincided with the progressive glomerular necrosis observed by light microscopy. IgM predominated in one kidney, IgG in another and in the third they were of equal intensity. Glomerular C3 deposits were more focal and of faint intensity. Mural peritubular capillary deposits paralleled those seen in glomeruli but were focal, less intense and more linear in character. In cortical arteries, initial IgG, IgM and C3 deposits were weak, focal, inconstant and limited to the endothelium, whereas in the nephrectomy specimens they were more prominent along the endothelium, and were also seen in the media. Mural fibrin deposits were weak and focal in glomeruli, were less frequently observed in peritubular capillaries, and were not seen in arteries.

Initial immune deposits in glomeruli and arteries were most prominent in the 5-minute nephrectomy specimen from the recipient with the highest LCT (1:512), but by 30 minutes, deposits in a graft removed from a recipient with a titer of 1:128 were of equal intensity.

Serum Complement Levels

Group 3-NTAL kidneys showed prompt reduction in venous C3 levels to 71% by 2 minutes, while arterial levels remained normal (Text-figure 2). In Group 2a-AP grafts, venous C3 declined after the initial 6 min-



TEXT-FIG 2—Mean values of renal venous complement (C3) levels; the normal range is 100 ± 20%. Each animal's zero time sample served as its own 100% of control (normal).

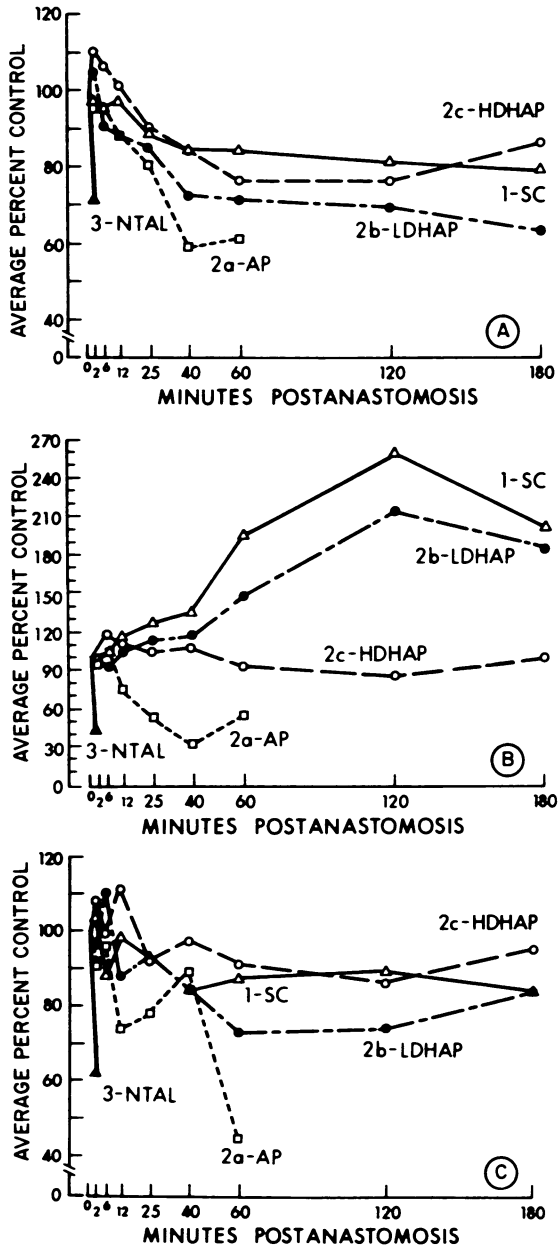
utes to 55% by 60 minutes, while arterial levels remained normal. With low dose heparin therapy (Group 2b-LDHAP), venous C3 was reduced to 69% within 12 minutes, and then more gradually to 48% by 180 minutes. After 6 minutes, arterial C3 levels gradually declined with a progressive 9 to 22% A-V difference. With higher doses of heparin (Group 2c-HDHAP), the venous C3 level was minimally depressed by 25 minutes with a maximum 12% A-V difference at that time.

Formed Blood Elements

Kidney of animals in Group 3-NTAL showed immediate and marked alterations in venous levels of formed blood elements (Text-figure 3A–C). By 2 minutes after revascularization, the mean venous hematocrit was 71% of normal (89% arterial level), the WBC count was 41% (77% arterial level), and the platelet count was 63% with a normal arterial level. No venous samples were obtainable thereafter.

In contrast, in Group 2a-AP grafts, the venous hematocrit was 95% at 2 minutes but then declined progressively to 59% with a 79% arterial level by 40 minutes. Similarly, the renal venous WBC count declined, with a progressively widening A-V difference, from 97% at 2 minutes to 33%, with a 70% renal arterial level by 40 minutes. Renal venous platelet counts averaged 91% of normal at 2 minutes but declined to 46% by 60 minutes, while arterial levels remained normal throughout.

In Group 2b-LDHAP, these changes were less pronounced. The normal 2-minute venous hematocrit declined gradually to 63% by 180 minutes. A 13% A-V difference noted at 12 minutes gradually narrowed. In contrast, the venous WBC levels were similar to those in Group 1-SC and increased progressively to 213% of normal by 120 minutes. Arterial levels were parallel and 10 to 20% higher after the initial 12 minutes.



TEXT-FIG 3—Mean renal venous values. Each animal's zero time served as its own 100% of control (normal). A—Hematocrit. B—White blood cell counts. C—Platelet counts.

Venous platelet levels gradually decreased to 74% of normal by 60 minutes but then returned to more normal levels, while arterial counts remained normal.

In Group 2c-HDHAP the renal venous hematocrit was 110% at 2 min-

utes, declined to 76% by 60 minutes, but returned to 86% by 180 minutes. There were no significant A-V differences. In contrast to all other groups, the renal venous WBC and platelet levels remained normal throughout the 180-minute period.

Renal arterial and venous differential white blood cell counts revealed that sequestration of white blood cells within the kidneys almost exclusively involved neutrophils (92%). In those animals with initially elevated peripheral eosinophil counts, these cells were also sequestered.

Coagulation Factor II

Group 3-NTAL grafts showed no alterations in renal venous levels by 2 minutes (Text-figure 4A). In Group 2a-AP, venous levels declined to 52% by 40 minutes. Both heparin-treated groups showed a more gradual decline, with 40-minute values of 72% for 2b-LDHAP and 78% for 2c-HDHAP, but by 60 minutes no difference was observed between these two groups. Renal arterial factor II levels remained normal in all groups.

Coagulation Factor X

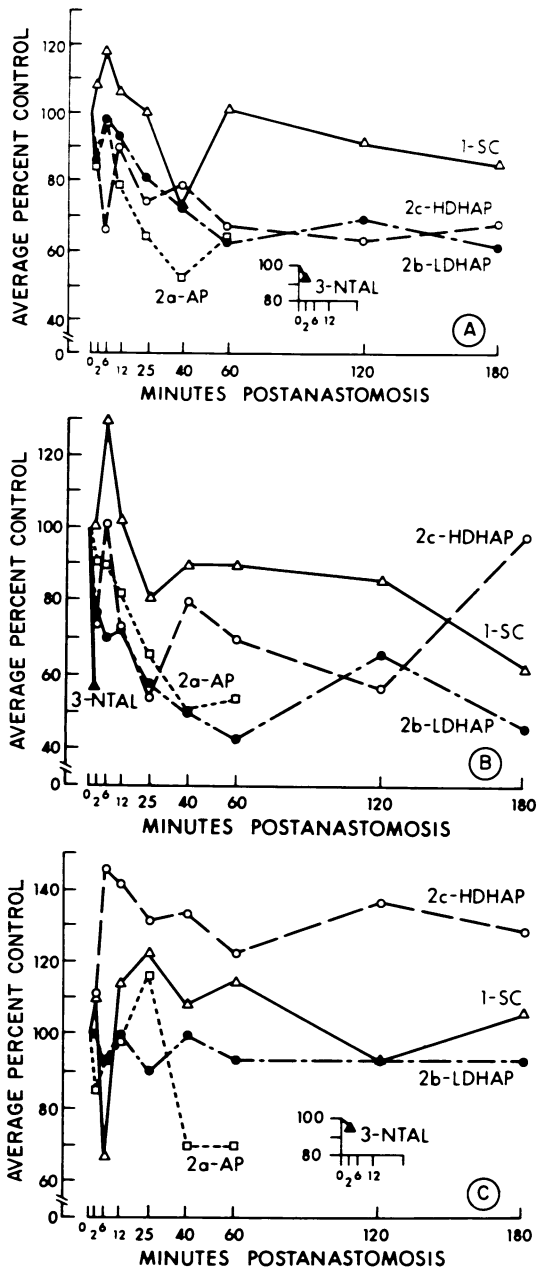
Group 3-NTAL grafts showed a decline in renal venous levels to 57% of normal (79% arterial level), by 2 minutes (Text-figure 4B). In Group 2a-AP, venous levels declined to 51% by 40 minutes, at which time the arterial level was 90%. Heparin therapy did not alter this decline in Groups 2b-LDHAP and 2c-HDHAP. However, only when higher dose heparin therapy was utilized was there a return toward normal venous factor X levels by 60 to 180 minutes.

Coagulation Factor VIII

Group 3-NTAL showed no change in venous levels at 2 minutes (Text-figure 4C). After the initial 25 minutes, Group 2a-AP showed consumption with venous and arterial levels of 70 and 160%, respectively, by 40 minutes. Group 2b-LDHAP did not show any deviations from normal levels and Group 2c-HDHAP showed a mild increase in factor VIII which was equal in both arterial and venous samples.

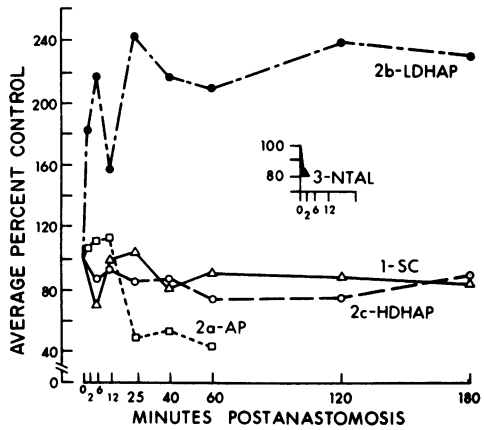
Fibrinolytic System

Only Group 2a-AP showed a marked decrease to 49% of normal in venous plasminogen levels by 25 minutes with an A-V difference of 114%, suggesting significant fibrinolysis (Text-figure 5). The remaining groups showed either no change from the range of normal, or an elevation of plasminogen activity over control values (Group 2b-LDHAP).



TEXT-FIG 4—Mean renal venous levels. Each animal's zero time served as its own 100% of control (normal). **A**—Factor II. **B**—Factor X. **C**—Factor VIII.

FDP levels were consistently elevated to 1:1024 (768 μg/ml) in the zero time control arterial blood samples in all groups and remained in that range.



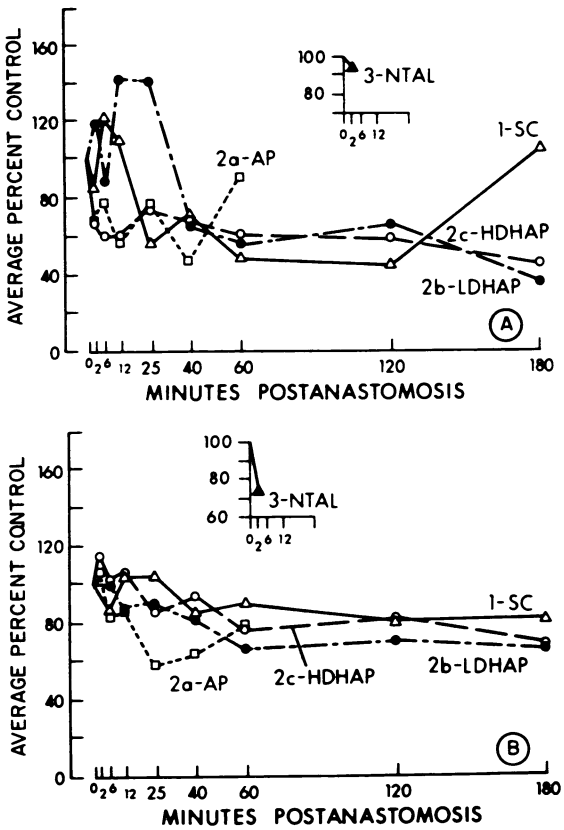
TEXT-FIG 5—Mean renal venous plasminogen levels; normal range is 100 ± 20%. Each animal's zero time sample served as its own 100% of control (normal).

Kallikrein System

Group 2a-AP showed the most marked alterations with a gradual reduction of venous levels of factor XII to 47%, prekallikrein to 58% and kallikrein inhibitor to 82% of normal by 40 minutes (Text-figure 6A–B). Arterial levels remained normal, suggesting progressive kallikrein formation within the kidneys. After the initial 25 minutes, Group 2b-LDHAP grafts showed a marked decline in venous factor XII levels to 66% by 40 minutes (49% A-V difference) but a more gradual decrease in venous prekallikrein to 67% by 60 minutes, with a 74% A-V difference in kallikrein inhibitor levels which also suggested intrarenal kallikrein formation. In Group 2c-HDHAP kidneys, factor XII levels declined to 60% by 6 minutes, but a reduction in prekallikrein and kallikrein inhibitor levels did not occur until 120 to 180 minutes. Group 3-NTAL did not show significant intrarenal kallikrein formation by 2 minutes.

Discussion

The onset, pattern and intensity of hyperacute renal allograft rejection in this primate model are reproducible and similar to those observed clinically in man.[1,8] Within 5 minutes, morphologic changes predominated in glomeruli where prominent red cell aggregation and stasis was followed by progressive platelet aggregation, neutrophil margination and infiltration, destruction of vascular endothelium and usually by mural or intraluminal thrombus formation. At this time, immunoglobulin and C3 deposits also predominated in glomeruli where an early, mixed focal-linear and finely granular pattern became more intense and finely granular on serial biopsies as endothelial necrosis progressed and time before nephrectomy increased.



TEXT-FIG 6—Mean values in the kinin-forming system. **A**—Renal venous factor XII levels. **B**—Renal venous prekallikrein levels. Normal range is 100 ± 20%; each animal's zero time served as its own 100% of control (normal).

Initial arterial lesions were minimal. Intraluminal thrombus formation, endothelial injury and immunoglobulin and C3 deposits were focal. Invasion of endothelium by neutrophils and focal necrosis with immune deposits in the media were generally later events. Deposits of IgG, IgM and C3 in the media may represent protein transudation into areas of injury, but the absence of IgA and albumin in these and most human grafts suggests immunologic specificity. Local release of lysosomal enzymes by platelets and neutrophils with subsequent disruption of vascular basement membrane [20] may permit access of specific antibody to histocompatibility antigens present on sarcolemmal plasma membranes.

The intensity of initial tissue injury and immune deposits in this and other studies [21,22] appears related to the lymphocytotoxic antibody titer. Our own observations suggest that at the highest titers, early injury appears more prominent in cortical arteries where prompt and marked vasospasm may reduce capillary flow, thus affording relative protection to the distal microcirculation. At lower titers (about 1:128 and below)

initial injury predominates in glomeruli and at times in peritubular capillaries. Here, the reduced linear velocity of flow may allow antibody greater access to the larger capillary endothelial surface area, while the smaller surface, higher velocity and laminar flow through larger cortical arteries may afford relative protection to the latter. Either IgG or IgM may initiate rejection and be deposited in the kidney. The immunoglobulin class involved may merely reflect the duration of presensitization.

While A-V differences in lymphocytotoxic titers were not measured, earlier studies [8] showed only marginal differences at comparable times in the same primate model, and suggested that relatively small amounts of antibody may initiate the process. It is unlikely that ABO incompatibility was related to the changes observed, as this species of monkey has only blood group B.[23] The markedly reduced 2-minute renal venous C3 levels associated with only faint C3 tissue deposits suggested concurrent, nonspecific, fluid-phase activation.

These studies confirmed immediate renal injury in a hyperimmune, unmodified allogeneic host with the initial flow of blood. The earliest and most abnormal finding was a profound reduction in total blood flow which affected all compartments. The absence of radioactivity in lungs excluded significant renal juxtamedullary shunting, although increased numbers of microspheres reached the medulla in some instances. Thus, entry of arterial blood into the kidney is rapidly and progressively reduced.

In sharp contrast to the other groups, these allografts sequestered a mean of 1,073,000 red cells, 64,489 platelets and 16,022 white blood cells per milliliter of blood within the initial 2 minutes. This preponderance of red cells and platelets correlated with their relative histologic prominence. Sequestration of this extensive red cell mass has received little attention and must contribute significantly to further vascular stasis, and by release of ADP, to further platelet aggregation. Although present in smaller numbers, neutrophils and platelets have a potentially more damaging role due to their local release of proteolytic enzymes, vasoactive and procoagulant factors. However, despite the A-V differences, initial histologic changes in these allografts, in earlier primate studies [8] and in human grafts usually do not show entrapment of formed elements of sufficient magnitude and distribution to account for the immediate, marked reduction in blood flow by obstruction alone, as has been suggested.[25] While endothelial cell swelling may also be contributory, it was not a prominent, early finding. These observations and the early contracted appearance of cortical arteries strongly suggest that

intense vasoconstriction may be the initially most important determinant of graft failure.

By 2 minutes, only factor X appeared activated within the grafts, whereas the remaining coagulation, fibrinolytic and kallikrein parameters appeared unaltered. These findings and the sparse fibrin formation observed by light and fluorescence microscopy suggest that activation of these systems occurs later, or that the rapidly reduced flow through injured areas precludes evidence of such activation in the venous effluent. However, the early A-V differences in venous formed elements and in C3 levels mitigates against the latter hypothesis.

These observations are all consistent with our previously reported [1,4,15,24] and subsequent studies of hyperacute rejection in man.

It is artificial to isolate the multiple mediators of graft injury. However, our observations suggest five phases of injury. Antibody-induced, complement-mediated vascular endothelial injury occurs with the initial passage of blood. Vasoconstriction is immediate and progressive, possibly due to locally released vasoactive substances. This may be maximal in some renal segments, would most effectively impair flow at the arteriolar level and markedly reduces flow to all compartments. Concomitant sequestration of red cells, platelets and neutrophils due initially to immune adherence and specific aggregation and later to stasis and nonspecific entrapment initiates progressive vascular obstruction and renal ischemia. This is compounded by further platelet aggregation due to altered red cell and platelet release of ADP. Thereafter, chemotactic factors derived from both specific and fluid-phase complement activation gradually recruit increasing numbers of neutrophils. In the unmodified primate, detectable activation of the coagulation, fibrinolytic and kinin-forming systems would appear to be the final events.

In sharp contrast, the gradual decline in hematocrit to 79% of normal in the shunt controls probably reflected serial blood sampling and intravenous fluid administration. Normal host response to the shunt was consumption of coagulation factors II, X, VIII and XII, a brief period of fibrinolysis and progressive systemic leukocytosis, all of which could be detected only after the allografts had already failed. The shunts per se had no demonstrable effect on the hematocrit, leukocyte and platelet counts or on activation of the complement or kinin systems.

However, in the untreated autografts, many morphologic changes, the reduction in venous blood flow and complement levels, as well as the sequestration of formed elements, mimicked qualitatively those observed in the hyperacuately rejected allografts. Alterations in the coagulation, fibrinolytic and kinin systems were more marked than in shunt

controls. Most importantly, they differed from the allografts in that there was no evidence for immunologic injury (IgG, IgM and C3 tissue deposits). The histologic alterations were of a milder appearance, and changes in cortical arteries other than those suggesting intense vascular contraction were absent. Although the earliest change noted was a reduction in total blood flow, progression to the low levels seen in allografts was delayed. All renal compartments continued to be perfused although distribution was altered. The reduced renal venous complement levels without tissue deposits of antibody or complement again suggested fluid-phase activation. The latter requires factor XII [26] which was activated. Sequestration of formed elements was delayed and more gradual, as was consumption of factors II, X and VIII and plasminogen. While factor XII was decreased by 2 minutes, mild kinin activation occurred later. Thus, the entire sequence of renal failure occurred more slowly.

It is well recognized that early function of most autografts is not normal although the reasons for such malfunction have not been elucidated. The autografts in this series were considered to have failed when venous flow ceased. When kidney grafts were implanted in this primate model via direct vascular anastomosis, similar early gross and functional abnormalities were noted, but venous flow was not measured and the autografts recovered, whereas allografts in presensitized recipients failed in the same manner noted here.[27] The earliest changes in the shunt controls which might have contributed to the alterations in the current autograft studies were mild, transient consumption of factor VIII and plasminogen, neither of which is known to trigger vasoconstriction. While malfunction may have been accentuated by the A-V shunts, the initiating event was not clear. Intraoperative ischemia time was comparable for all groups. However, the only possible final event was marked renal ischemia which progressed to secondary changes and organ failure which in many respects appeared similar to that observed in the hyperacutely rejected kidneys. These findings may provide some insight into the poor, early function of most autografts. A most important finding was the intrarenal consumption of C3, as similar nonspecific, but injurious, complement activation with subsequent chemotaxis and progressive tissue injury has been observed in a model of acute cardiac ischemia.[28] Perhaps complement has a similar role in certain forms of potentially reversible acute renal failure.

Thus, Group 3-NTAL, represents initial immunologic vascular injury with specific as well as nonspecific activation of complement and other mediators of tissue damage, while Group 2a-AP represents a model of

marked, acute renal ischemia, with nonimmunologic activation of the same secondary mediators. Specific histologic alterations during the early stages of hyperacute rejection are subtle and demand confirmation of immune vascular injury by fluorescence. The more readily evident intraluminal findings may in large measure reflect the effects of very rapidly superimposed profound ischemia.

An allograft undergoing primary immunologic injury may survive.[4,5] Successful pharmacologic modification of hyperacute rejection may require prevention of the early, intense vasoconstriction and renal ischemia prior to onset of vascular obstruction.

Heparin was used in an attempt to prevent the changes seen in nonmodified autografts. All animals had clotting times of infinity. Increasing doses progressively reversed the functional and morphologic abnormalities, and at the highest dose even the early decreased total blood flow was prevented. Only the decline in factor XII remained unaltered. The action of heparin in this setting is not clear. This agent may have exerted some effect(s) other than its known action on the coagulation system. Most importantly, these experiments show that secondary injury, which is qualitatively similar to that seen during hyperacute rejection and is probably immediately responsible for graft failure, can be abrogated pharmacologically. The intrarenal effects of heparin during hyperacute rejection, where injury is more immediate and intense, will be reported separately.

The incidence of hyperacute rejection in humans is minimized by nonselection of known presensitized individuals. Transplantation in these patients will require therapy which reliably prevents the immunologic injury or significantly modifies its effect on the target organ. Enhancement, either active or passive (*eg*, use of $F(ab')_2$ fragments), which alters the immunologic injury, would be the preferable approach but, to date, has achieved only limited success in animal models.[8,29] Current efforts may therefore require combined biologic and pharmacologic modification. This primate model, which most closely resembles its clinical counterpart in man, provides a system for dissecting the events which occur during hyperacute renal allograft rejection and a basis for evaluating pharmacologic agents for their effective action and varied interactions on the damaging secondary alterations.

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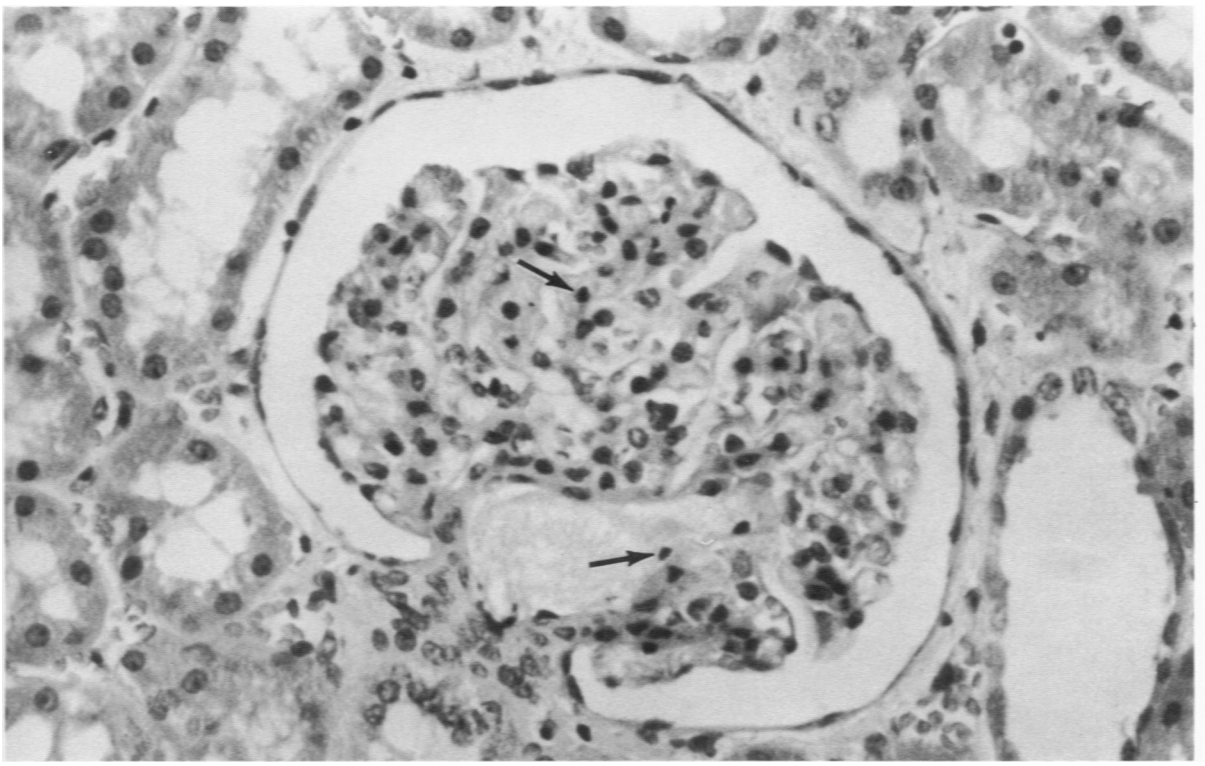
Legends for Figures

Fig 1—Biopsy from a Group 3-NTAL allograft (recipient LCT = 1:512) at the time of failure (5 minutes). Although many glomeruli showed less or more severe injury, most exhibited multifocal capillary red cell stasis, some increase in intraluminal neutrophils and platelet aggregates as shown here. Scattered endothelial cells have pyknotic nuclei (*arrows*). Most glomerular capillaries are not occluded. Platelet occlusion of the hilus, illustrated here, was not widespread, and peritubular capillaries were patent after venous blood flow had ceased (H&E, × 160).

Fig 2—Immunoflourescent stain of the same biopsy as in Figure 1 illustrates IgG deposited in most glomeruli in this graft by 5 minutes; IgM and C3 deposits had a similar pattern but C3 was less intense. In this graft, the initial pattern along glomerular capillary walls was predominantly finely granular, while in the remaining allografts, against lower antibody titers, it was mixed focal-linear and finely granular, and similar intensities were not observed until 30 minutes. Such staining was not observed in the autoperfusion controls (autografts) (Goat antihuman IgG, × 200).



1

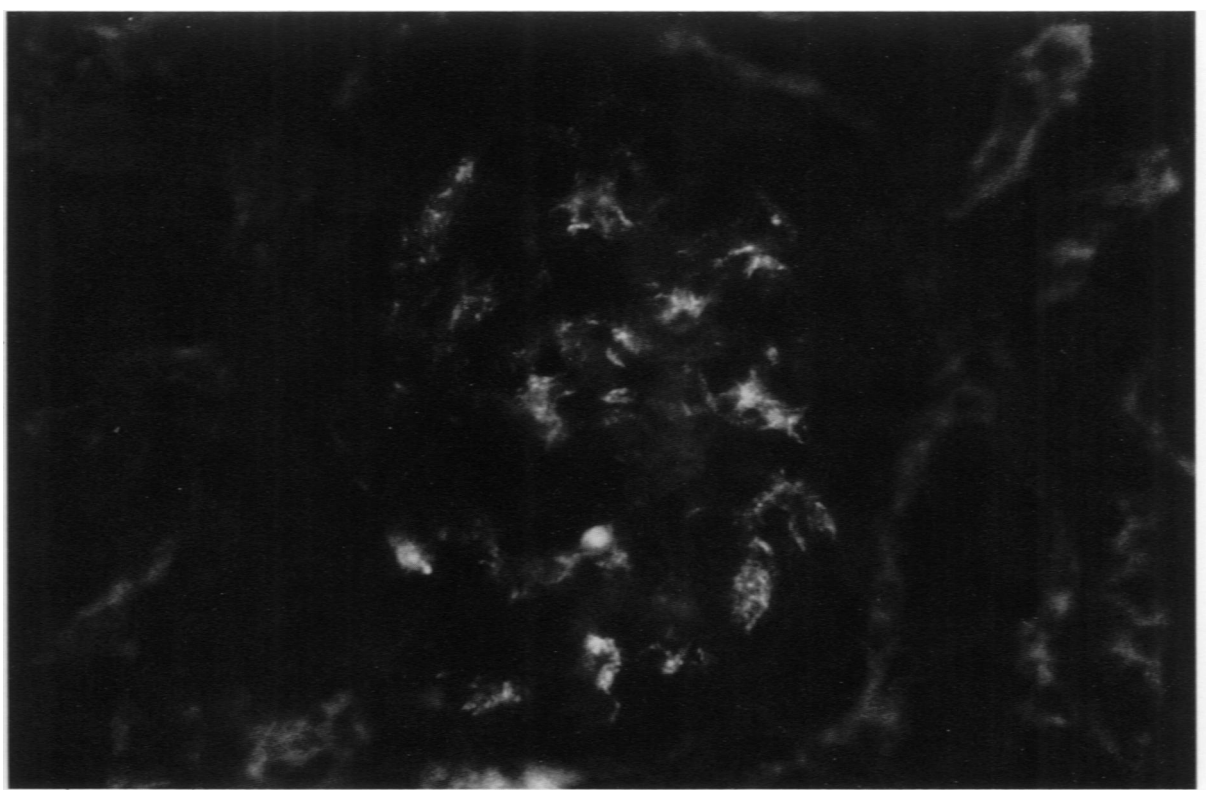


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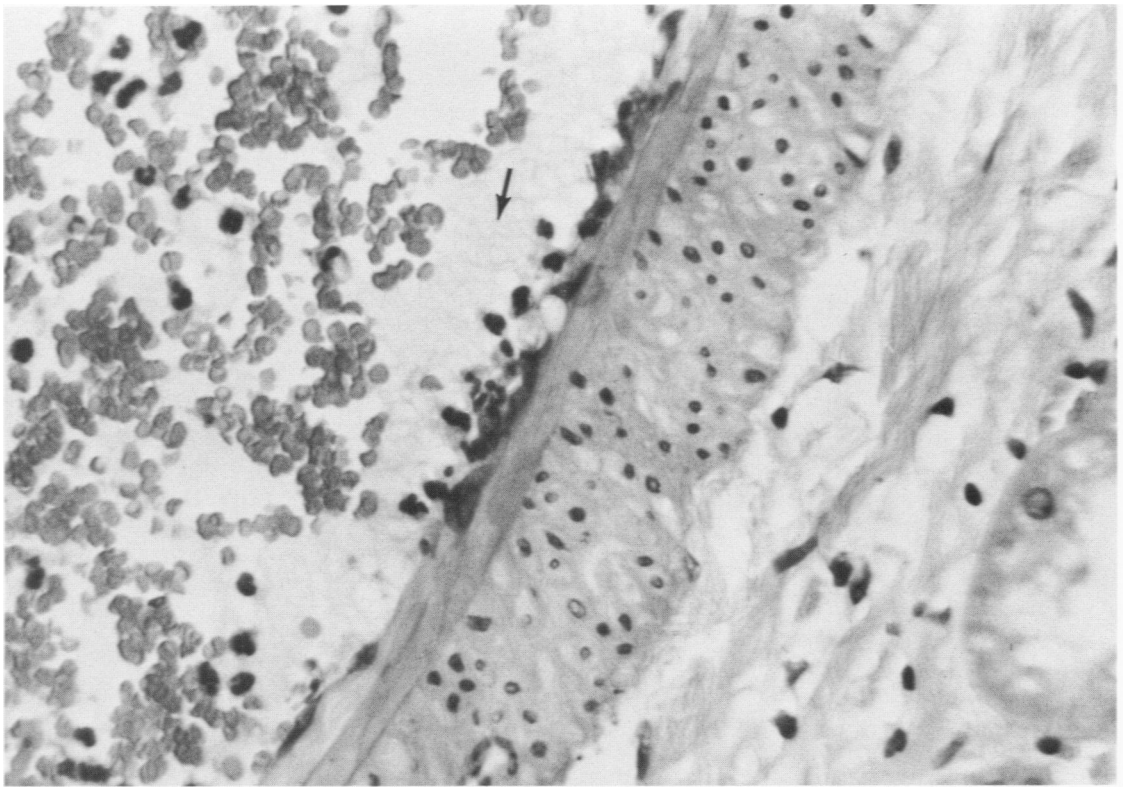
Fig 3—The proximal portion of an interlobular artery from the same biopsy illustrated in Figures 1 and 2, shows neutrophil margination along and early infiltration between vacuolated and pyknotic endothelial cells. The vacuolated structures on the luminal aspect of the endothelium (*arrow*) may represent swollen and degranulated platelets. The internal elastica and media appeared intact at this time (H&E, × 200).

Fig 4—A 180-minute biopsy from a Group 2b-LDHAP autograft shows marked changes. A representative inner cortical glomerulus shows extensive capillary occlusion by red cells and platelets. Much of the endothelium is not recognizable. Neutrophiles and nuclear debris are present. Adjacent peritubular capillaries show similar changes, but many are patent. An intralobular artery (*lower right*) shows marked folding of internal elastica and smooth muscle cell nuclei along with intraluminal projection of endothelium, all suggestive of intense vascular contraction. There is no evidence for direct injury to endothelium. There is focal tubular necrosis (*lower left*) (PAS, × 160).



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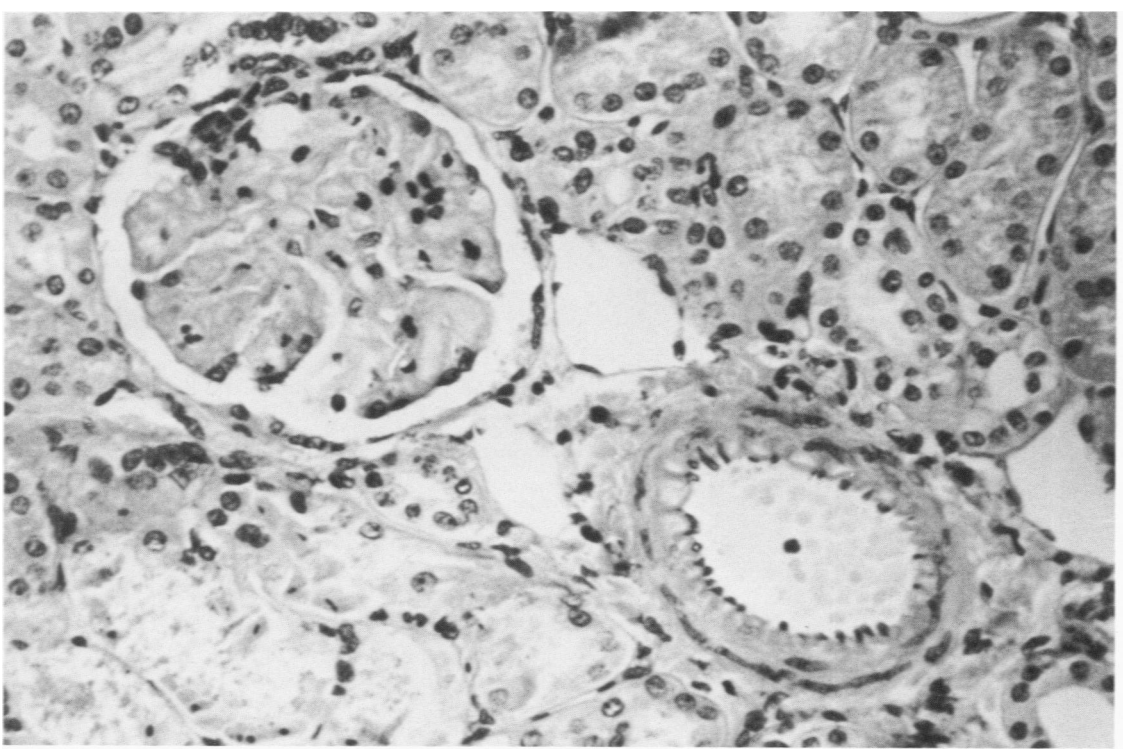

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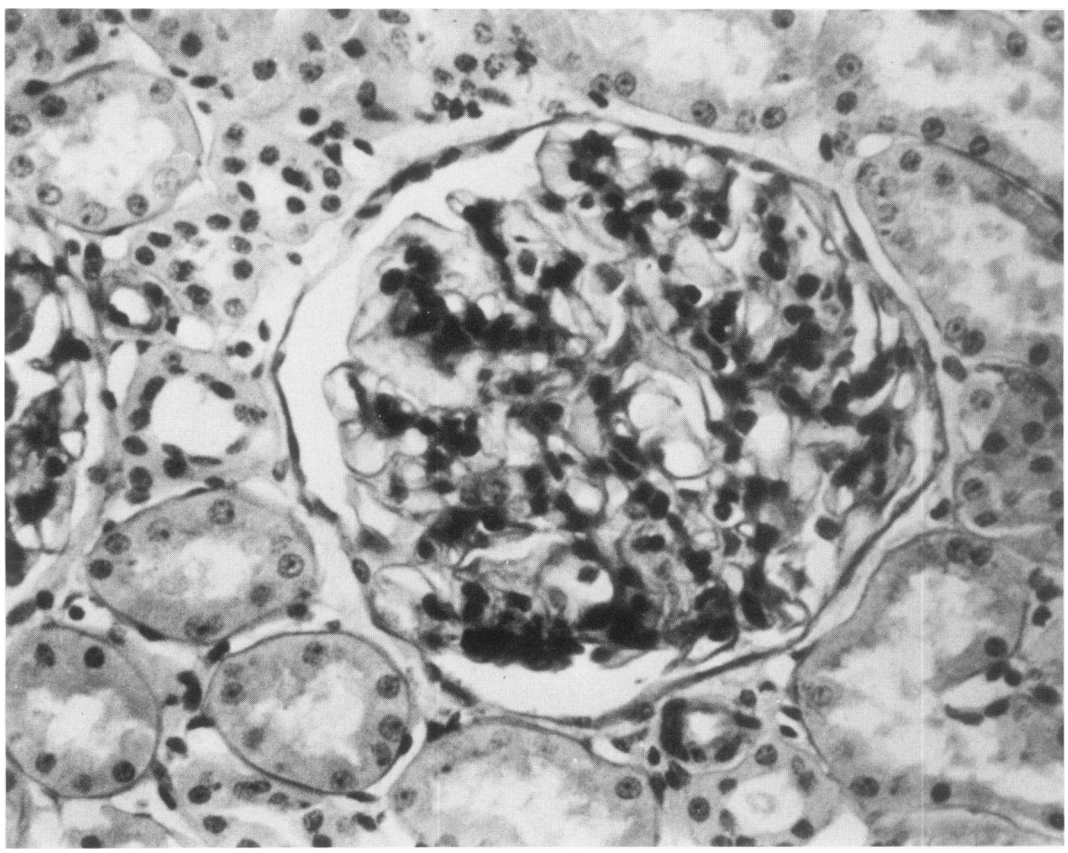

Fig 5—A thick section of a more severely involved glomerulus from a Group 2c-HDHAP graft at 180 minutes is in sharp contrast to that seen in Figure 4. Occasional capillary loops contain red cells, small platelet aggregates and increased numbers of neutrophils. Only occasional endothelial cells appear pyknotic. Most glomerular and peritubular capillary lumina appear patent (PAS, × 200).