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Prolongation of Pig-to-Dog Renal Xenograft Survival by Modification of the Inflammatory Mediator Response

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The pathogenesis of hyperacute renal rejection consists of a nonspecific effector cascade that invokes most of the components of a typical acute inflammatory response. Platelet-activating factor (PAF) represents the most recent and perhaps the most significant mediator and promoting agent of this phenomenon. These studies evaluated SRI 63-441, a novel, synthetic, and the most potent PAF receptor antagonist available, alone and in combination with other prostanoids, for their ability to influence this response and to prolong renal xenograft survival and function in a model of pig-to-dog heterotransplantation. Inhibition of PAF by SRI 63-441 alone, at the dosage and schedule used in these experiments, did not significantly prolong xenograft survival or function. However, the combination of SRI 63-441 with either prostacyclin (PGI₂) or prostaglandin E₁ (PGE₁) infusion demonstrated significant synergism, and resulted in a 6-9-fold increase in kidney survival and a 3-20-fold increase in urine output. Neither PGI₂ nor PGE₁ infusions alone significantly influenced this xenograft model. Electromagnetic flow studies demonstrated significantly delayed diminution in renal artery blood flow in the combination-treated animals. Serial and end-stage histologic examination of kidneys receiving combination therapy demonstrated a delayed onset of the pathologic deterioration and an overall amelioration of the entire process. These studies demonstrate that significant abrogation of a rapid and violent form of hyperacute rejection can be achieved solely by the pharmacologic manipulation of the inflammatory mediator response.

THE PATHOPHYSIOLOGIC EVENTS leading to hyperacute allograft rejection have been relatively well characterized and differ little today from

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the process originally described almost 20 years ago.¹⁻³ The initiating event is clearly a specific immunologic reaction consisting of antibody-mediated injury directed against antigens on the vascular endothelium of the transplanted organ.⁴⁻⁶ This immunologic phenomenon leads to the accumulation of platelet-polymorphonuclear leukocyte-erythrocyte plugs in small arteries and capillaries, followed by intravascular coagulation and ischemic necrosis.^{1,4,5,7}

The sequence of events leading from the initial specific immunologic reaction to the final characteristic pathologic result of hyperacute rejection of a kidney, which can best be described as a failure or breakdown of the microvasculature, represents a nonspecific, intense, and complex inflammatory response. This effector cascade invokes most, if not all, of the classical mediator systems of the acute inflammatory process (*i.e.*, complement, coagulation, fibrinolytic, and kallikrein-kinin systems).^{8,9} During this process, a large number of biologically active molecules are generated that are responsible for the increased vascular permeability, arteriolar vasoconstriction, damage to the cytoskeleton, fibrin deposition, generation of oxygen-free radicals, and the recruitment, activation, and release of various inflammatory cells, which all contribute to the entire pathogenesis of hyperacute kidney rejection.^{8,9}

Many of these chemical mediators of inflammation, including kinins, various chemotactic factors, anaphylatoxins, serotonin, and arachidonic acid metabolites

(prostaglandins, leukotrienes, thromboxanes) have been proposed as central candidates in the overall process of hyperacute rejection, and have served as the targets in attempts to develop therapeutic interventions to regulate organ function. The newest and perhaps most important inflammatory mediator to be nominated as a central candidate in the overall evolution of hyperacute rejection is acetyl glyceryl ether phosphorylcholine (AGEPC).¹⁰ Also known as platelet-activating factor (PAF), as it will be referred to here, because it was initially discovered by its ability to cause potent platelet aggregation and release, it represents the most recently described and novel class of potent lipid autocoids.^{8,11,12}

The implication that PAF plays a central role in the induction of tissue injury is attributed to a spectrum of biological activities that may manifest all of the cardinal signs of acute inflammation.^{8,10-12} PAF is produced by a variety of inflammatory cells including platelets and neutrophils. It is also produced by endothelial cells, which supports its importance as a key regulator of the microvasculature, and more importantly, its disruption under pathophysiologic circumstances. Specific biological activities of PAF that are germane to its role in hyperacute rejection include: platelet and neutrophil release and aggregation, release of thromboxane A₂ and other eicosanoids, increase of vascular permeability, vasoconstriction, increase in hematocrit, systemic hypotension, and generation of oxygen-free radicals.

With the availability of specific receptor antagonists of PAF, further insight into the role of PAF in the pathogenesis of hyperacute rejection is now possible. The potential for a new method of controlling the effector cascade of hyperacute rejection by pharmacologic intervention can now be exploited also. Pig-to-dog renal heterotransplantation results in a rapid and violent form of cortical necrosis and ischemic destruction of the organ, which mimics the situation in humans and is due to preformed humoral antibodies in the recipient.^{4,5,13} It represents an appropriate severe model in which to evaluate new methods of therapeutic intervention. The studies reported evaluated the effect of a novel and potent synthetic PAF receptor antagonist, alone and in combination with other prostanoids, on this renal xenograft model.

Materials and Methods

Animals

Female, white outbred SPF pigs that weighed 15–20 kg were purchased from Shadyside Farms, Ohio and served as donors. Male beagles that were bred for research and weighed 13–16 kg were purchased from Laboratory Research Enterprise, Kalamazoo, Michigan and served as recipients. All animals were housed in a central

animal facility and were allowed to acclimatize for at least 1 week before experimentation. They received standard animal chow and water *ad libitum* and were fasted for 24 hours before surgery.

Surgical Procedures

All animals for both the donor and recipient procedures were anesthetized with intravenous sodium pentobarbital (30 mg/kg). Recipient dogs were maintained with 30% oxygen through an endotracheal tube. During the experimental procedure, dogs were monitored for depth of anesthesia by EKG and arterial pressure. Additional anesthetic (pentobarbital, 3 mg/kg IV) was given as required.

Donor pigs received intravenous Ringer's lactate (1000 mL), mannitol (12.5 g), and Thorazine® (50 mg). After systemic heparinization (5000 units) and *in situ* cooling with cold (4 C) Ringer's lactate through an aortic cannula, the kidneys were removed *en bloc*. The kidneys were then separated and transplanted simultaneously into two recipient dogs, always with less than 1 hour of cold ischemia time. A paired design was therefore performed for each experiment, by assigning one kidney as the control and the other as the test (treated) kidney.

All pig-to-dog kidney heterotransplantations were performed by a standard technique. The porcine renal artery was anastomosed end to end to the recipient right common iliac artery, and the renal vein was anastomosed end to side to the right common iliac vein. The anastomosis time was always less than 30 minutes. The dogs were kept anesthetized and the kidney was left external to the dog for observation. The ureter was cannulated with polyethylene tubing and urine was collected throughout the transplant period. For intra-arterial administration of prostaglandins, an 18-gauge polyethylene catheter was inserted through the middle sacral artery and threaded towards the orifice of the right common iliac artery. To ensure that solution infused into the terminal aorta would exclusively run into the graft renal artery, the left common iliac, middle sacral, and other smaller arteries originating at or near the aortic bifurcation were ligated near their origin. For those experiments where renal vein and artery platelet counts were measured, 18-gauge polyethylene catheters were introduced into the right femoral vein and its tip positioned at the venous anastomosis, and into the left common iliac artery and its tip positioned at the orifice of the right common iliac artery.

Each dog underwent native nephrectomy after the porcine kidney had been revascularized. A change in color or motling of the transplanted pig kidney was considered evidence of the onset of rejection. However, the

end point of the experiment was signaled by a cessation of urine flow or by the onset of frank hematuria.

Drug Administration

Prostaglandins. Naturally occurring prostacyclin (PGI₂) and prostaglandin E₁ (PGE₁) or Prostin-VR were supplied by the Upjohn Company, Kalamazoo, Michigan (M. J. Ruwart). PGI₂ was reconstituted and infused in a 0.1M glycine buffer, adjusted to a pH of 10.5 with 2M Tris solution to enhance its stability. PGE₁ was reconstituted in a stock solution of 1 mg in 100 μL of ethanol. This solution was then diluted to the appropriate concentration in 0.9% sodium chloride for infusion.

All prostaglandins, freshly reconstituted for each experiment, were administered by constant infusion through the catheters placed into the aorta, and the dosage was titrated according to blood pressure tolerance. Control animals for the prostaglandin experiments received infusions of the particular prostaglandin vehicles.

PAF antagonist. SRI 63-441 was provided by the Sandoz Research Institute, Sandoz, Inc. (East Hanover, NJ). This synthetic compound represents the most potent receptor antagonist of PAF responses known to date. SRI 63-441 (molecular weight 662) is a substituted [tetrahydrofuran-yl-(methoxyphosphinyloxy)ethyl] quinolinium compound¹⁴ and is structurally similar to PAF. Structural analyses and purity (greater than 98%) were determined by NMR spectroscopy and thin-layer chromatography.¹⁴ SRI 63-441 is readily soluble in water or sodium acetate buffer at concentrations as high as 5% and can only be given parenterally. In four species tested (rats, guinea pigs, dogs, and primates), SRI 63-441 exhibited dose-dependent inhibition of all major physiologic responses to PAF (*i.e.*, hypotension, hemoconcentration, bronchoconstriction, and increased vascular permeability).¹⁴ For all studies reported here SRI 63-441 was reconstituted as a 10 mg/mL solution in 0.68% sodium acetate and 0.9% sodium chloride (pH 5.3). Before its administration, this solution was placed in a warm water bath (24–25 C) for 5 minutes and then administered as a bolus intravenous injection. Control dogs received the sodium acetate buffer intravenously.

Experimental Design

Four experimental groups are reported: Group I: the administration of SRI 63-441 alone was compared with controls; Group II: the combination of SRI 63-441 and PGI₂ was compared with controls; Group III: the combination of SRI 63-441 and PGE₁ was compared with controls; Group IV: the combination of SRI 63-441 and PGE₁ was compared with PGE₁ alone.

Both prostaglandins (PGI₂, PGE₁) were administered at a dose of 0.5–1.0 μg/kg/min (depending on systemic blood pressure) by continuous intra-arterial infusion commencing at 30 minutes before revascularization. SRI 63-441 was administered at 5 mg/kg/dose by intravenous bolus beginning at 5 minutes before and then 5 minutes after, and then every 15 minutes after revascularization. For each study, experiments were performed on control and test animals simultaneously.

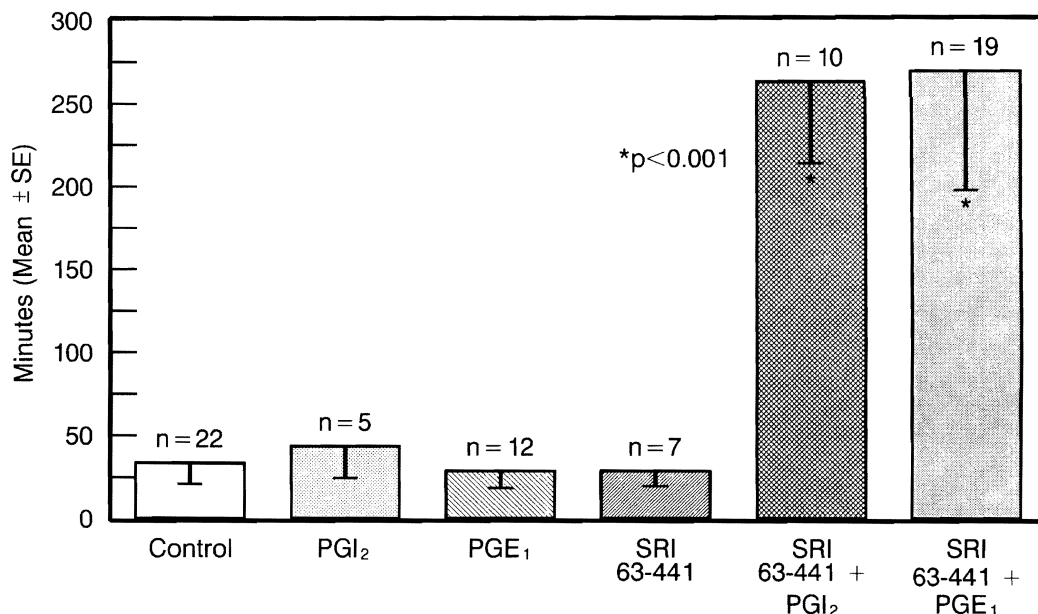
Kidney survival was assessed for each experiment as described above. Urine was collected for the entire transplant period, and the total volume was used as an indicator of renal function. For six experiments in Group II and for three experiments in Group IV, platelet counts in the renal artery, renal vein, and peripherally were determined serially (every 10 minutes for the first hour and then every 30 minutes for up to 4 hours in the treated groups). Renal arterial blood flow in the transplanted kidneys was monitored electromagnetically using a Cliniflow II, Model FM701D Electromagnetic Flowmeter (Carolina Medical Electronics Inc., NC). Flows (in milliliters per minute) were determined every 10–15 minutes in six separate experiments in Group IV (controls received PGE₁ infusion). Serial kidney biopsies (every 15 minutes) were performed in an extra group of animals that are not included in survival statistics. Biopsies were also done at the end point of the experiment, (using the criteria outlined above) in all groups studied.

Pathologic Studies

Tissue preparation. Histologic evaluation of serial biopsies from one experiment and of sections of kidneys taken at the end stage of additional experiments are presented. In the serial studies, wedge biopsies were obtained at baseline (back table), and at 5, 15, 30, 45, 60, 75, and 90 minutes after revascularization. Tissues were formalin-fixed, paraffin-embedded, cut at 3 μm, and stained with hematoxylin and eosin.

Histologic parameters. One microscopic section from each biopsy was selected. The slides were randomized and evaluated in blind fashion. The percentage of glomeruli with segmental or global thrombosis was determined. The glomeruli with necrosis or PMNs were also counted. Acute tubular necrosis (ATN), characterized by disintegration and sloughing of epithelial cells, was graded as mild (rare foci), moderate (numerous foci), or severe (confluent). Blood in the tubular lumens and mitoses were noted. The interstitium was rated as normal, congested, or hemorrhagic; the latter was graded as mild (few foci), moderate (numerous foci), or severe (confluent). Arteries and arterioles containing thrombi were noted.

FIG. 1. Survival of renal xenografts after pig-to-dog kidney transplantation assessing the effect of single and combination treatment with a PAF receptor antagonist (SRI 63-441) and two prostanoids (PGI_2 and PGE_1). Results are expressed as total survival time (mean minutes \pm SE).



Statistical Evaluation

All statistical evaluations were performed on an IBM PC-AT microcomputer using statistical analysis software (SPSS/PC+, SPSS Inc., Chicago, IL and BMDP/PC, BMDP Statistical Software, Los Angeles, CA). The mean and standard errors for urine volume and kidney survival for control and treated groups were compared using a nonparametric statistical test (McNemar) for the comparison of mean differences between two groups. The mean and standard errors for platelet counts were compared using a repeated measure design analysis. For all tests, a p value of less than 0.05 was considered statistically significant.

Results

Renal Xenograft Survival

Figure 1 depicts the survival for transplanted porcine kidneys in the four groups studied. The control dogs in all experiments consistently demonstrated violent rejection of the pig kidney within a mean time of approximately 30 minutes. The administration of SRI 63-441 as a single agent at the dosage and schedule described here did not result in a significant increase in graft survival. The addition of either prostanoid (PGI_2 or PGE_1) to SRI 63-441 treatment resulted in a marked synergism and a significant increase in kidney survival. SRI 63-441 and PGI_2 resulted in a greater than sixfold (217.1 ± 39.4 vs. 37.4 ± 11.1 minutes, $p < 0.001$) and SRI 63-441 and PGE_1 in a greater than sevenfold (268.6 ± 27.7 vs. 37.2 ± 5.6 minutes, $p < 0.001$) increase in renal xenograft survival when compared with their respective controls. PGI_2 or PGE_1 infused as single agents had no significant

effect on kidney survival. In fact, in Group IV, the combination of SRI 63-441 and PGE_1 resulted in a greater than ninefold increase in kidney survival when compared with PGE_1 infusion alone (280.4 ± 31.8 vs. 31.0 ± 11.1 minutes, $p < 0.001$). PGE_1 in this experiment behaved no differently than the control vehicles in the other experiments.

Renal Xenograft Function

Renal function as determined by total urine output for each transplant is depicted in Figure 2. The results parallel those observed for kidney survival already presented above. SRI 63-441 alone did not significantly improve renal function. The combination of SRI 63-441 and PGI_2 resulted in a greater than threefold (99.5 ± 30.4 vs. 29.5 ± 13.7 mL, $p < 0.05$) and of SRI 63-441 and PGE_1 in a greater than 20-fold (436.2 ± 134.8 vs. 21.2 ± 7.3 mL, $p < 0.02$) increase in total urine output. Neither prostanoid alone resulted in a significant increase in urine output. The combination of SRI 63-441 and PGE_1 resulted in a 12-fold increase in total urine output (370.9 ± 78.9 vs. 32.7 ± 6.4 mL, $p < 0.003$) when compared with PGE_1 alone, which did not differ significantly from vehicle controls.

Platelet and Flow Studies

The serial platelet counts for six experiments in Group II (SRI 63-441 + PGI_2 vs. controls) and three experiments in Group IV (SRI 63-441 + PGE_1 vs. PGE_1) are depicted in Figures 3 and 4, respectively. There was a significant difference between the renal artery and renal vein platelet counts in Group II for both

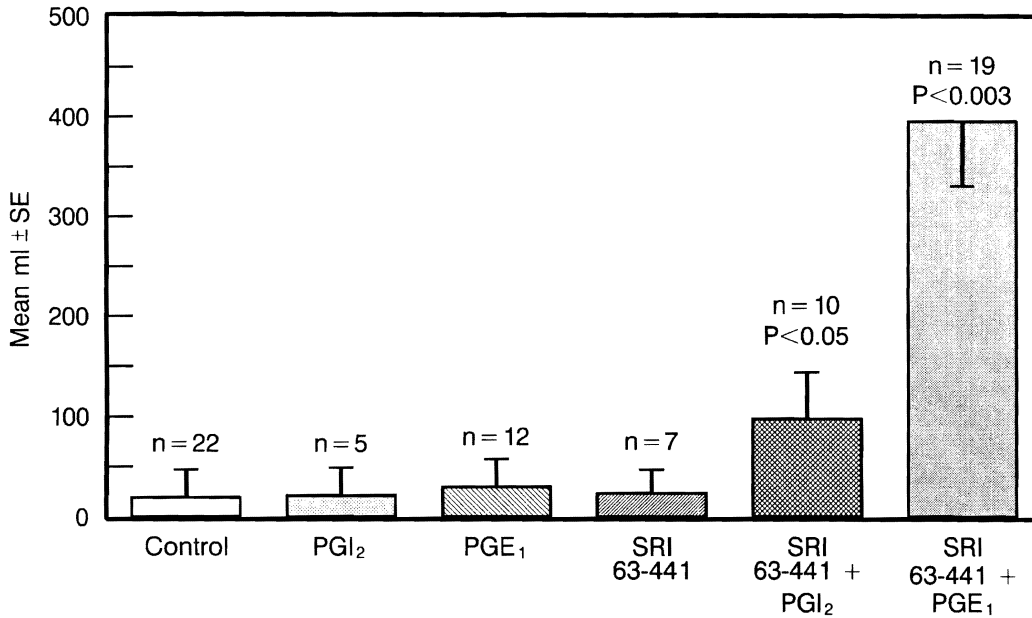


FIG. 2. Function of renal xenografts after pig-to-dog kidney transplantation from experiments described in Figure 1. Results are expressed as total urine output for the entire transplant period (mean milliliters ± SE).

control and treated animals, with a more pronounced gradient evident in the control group. No significant gradient in platelet count between renal artery and vein was observed in Group IV dogs.

The flow studies from six experiments in Group IV (SRI 63-441 + PGE₁ vs. PGE₁) are depicted in Figure 5. With the drug combination treatment group, as already evident in renal xenograft survival and function, blood flow to the xenografted kidneys was consistently maintained at significantly higher levels than in the control group (in this case already receiving a vasoactive agent, PGE₁). This is depicted up to 100 minutes after revascularization in Figure 5, since before that time, the PGE₁ alone group had already demonstrated severe diminu-

tion of graft flow. However, the combination treatment group maintained a degree of significant flow for most of the duration of the experiment.

Pathologic Evaluation

Microscopically, in the serial experiment, baseline biopsies from both control and treated animals contained normal glomeruli, with normal cellularity, and patent, empty capillary loops (Fig. 6). After reperfusion, both kidneys exhibited progressive obliteration of capillary loops by eosinophilic granular material mixed with red blood cells (Fig. 7). Similar thrombi were found in arteries and arterioles as early as 15 minutes. Tubules

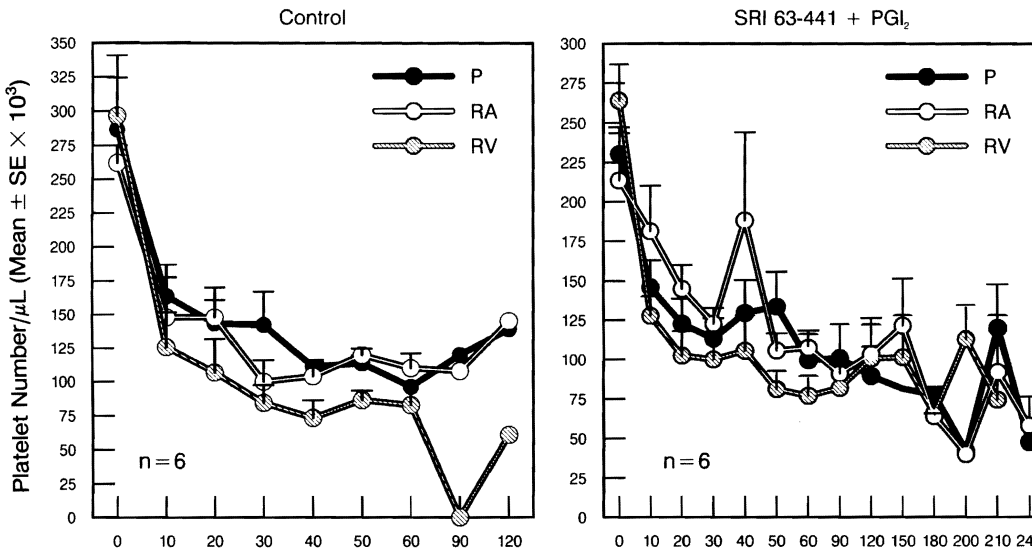


FIG. 3. Platelet counts (mean ± SE) determined for the renal artery (RA), renal vein (RV), and periphery (P) for six sets of experiments in Group II (SRI 63-441 + PGI₂ vs. control).

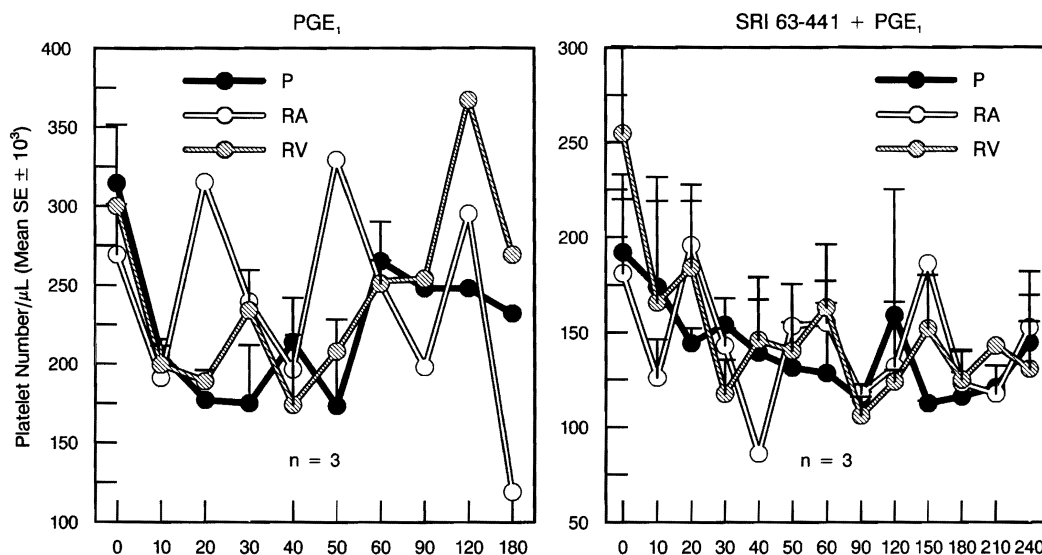


FIG. 4. Platelet counts (mean ± SE) determined for the renal artery (RA), renal vein (RV), and peripherally (P) for three sets of experiments in Group IV (SRI 63-441 + PGE₁ vs. PGE₁).

remained well preserved until 1 hour. There was progressive edema, then congestion, then hemorrhage in the interstitium. At the end stage, when renal function ceased, all specimens showed diffuse thrombosis of glomeruli, arteries, and vasa rectae. There were interstitial hemorrhages and early changes of ATN.

Sequential biopsies. Biopsy specimens at 5, 45, and 90 minutes are shown in Figures 8–10. Qualitatively, the glomerular changes were similar in the control and treated groups. However, the development of glomeru-

lar thrombosis proceeded at a slower rate in the treated kidneys. Table 1 shows the results from one experiment. The control animal reached end stage with thrombosis in 94% of the glomeruli at 45 minutes, whereas the process reached completion in the treated kidney at 90 minutes. At end stage, red blood cell thrombi were more prominent in the treated kidney (Fig. 10).

Necrosis of tubular epithelial cells was noted in the control kidney with blood in the lumens at 60 minutes. These changes persisted until the end of the experiment.

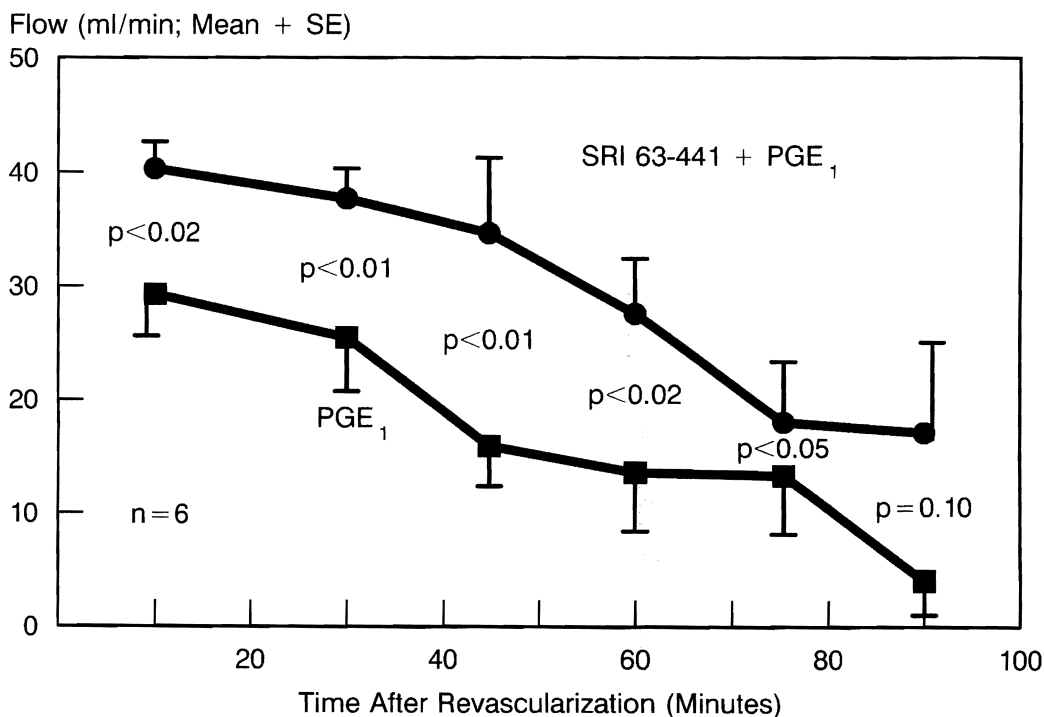


FIG. 5. Electromagnetic flow studies (mean mL/min ± SE) for six sets of experiments in Group IV (SRI 63-441 + PGE₁ vs. PGE₁).

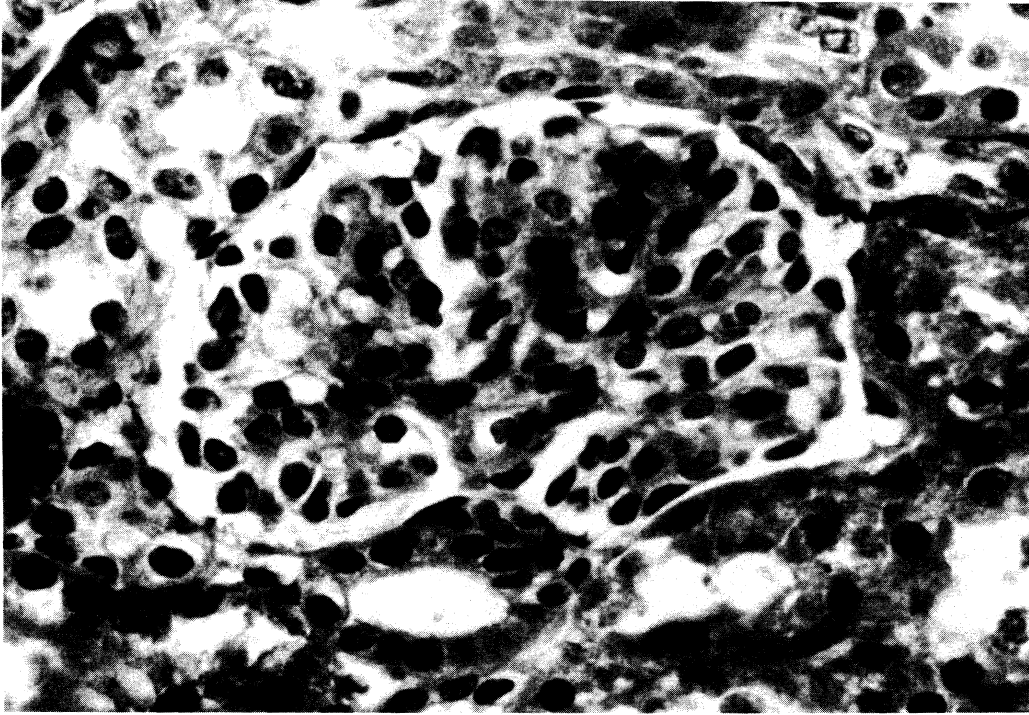


FIG. 6. Baseline biopsy specimen showing normal, empty glomerulus (hematoxylin and eosin, original magnification $\times 960$).

It is important to note that significant necrosis was not noted in the treated kidney.

There was a difference between the two kidneys in the amount and onset of interstitial hemorrhage. Focal hemorrhages were noted in the control kidney at 15

minutes; this progressed to diffuse hemorrhage by 45 minutes and persisted (Fig. 9). In contrast, the treated kidney showed only focal hemorrhages at 30 minutes, and again at 90 minutes. There were no significant differences between the control and treated kidneys with

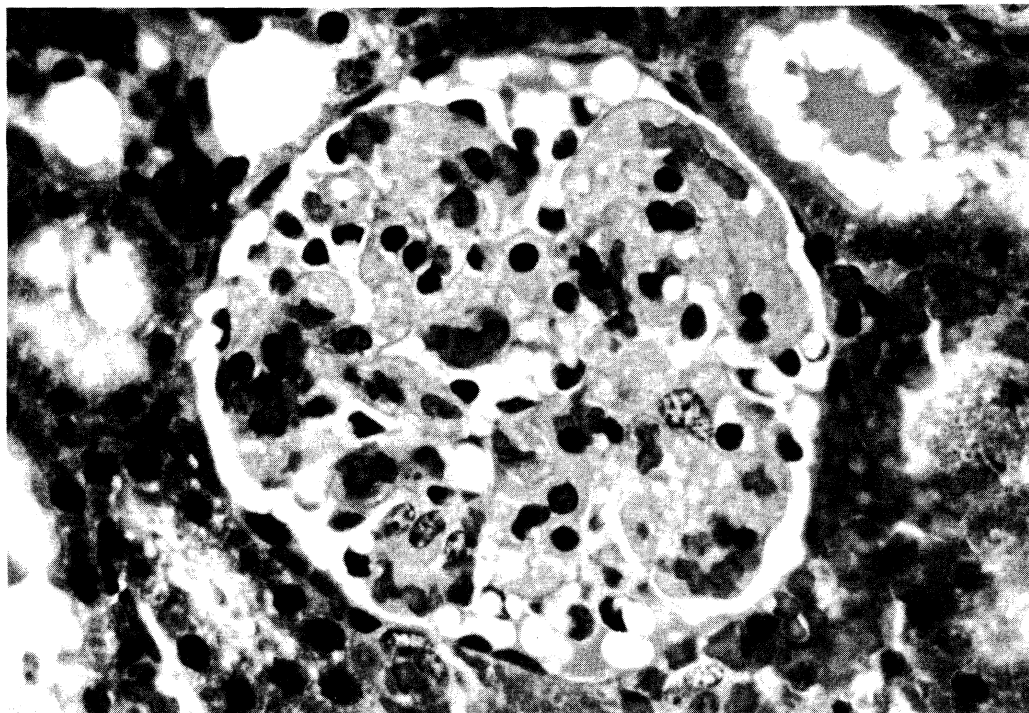
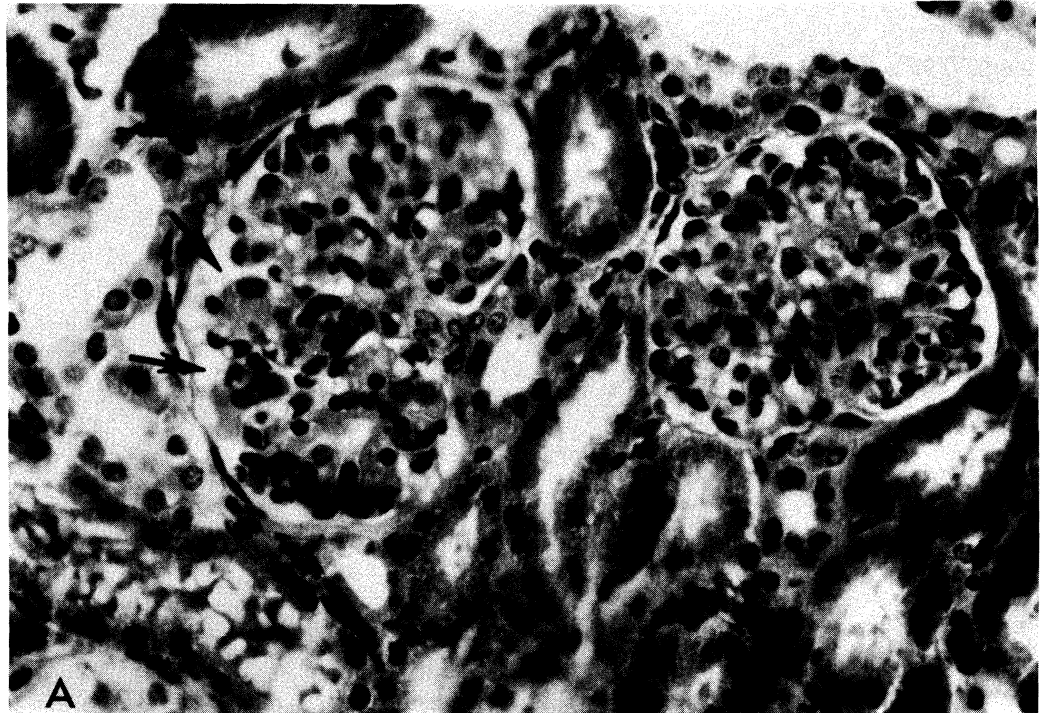
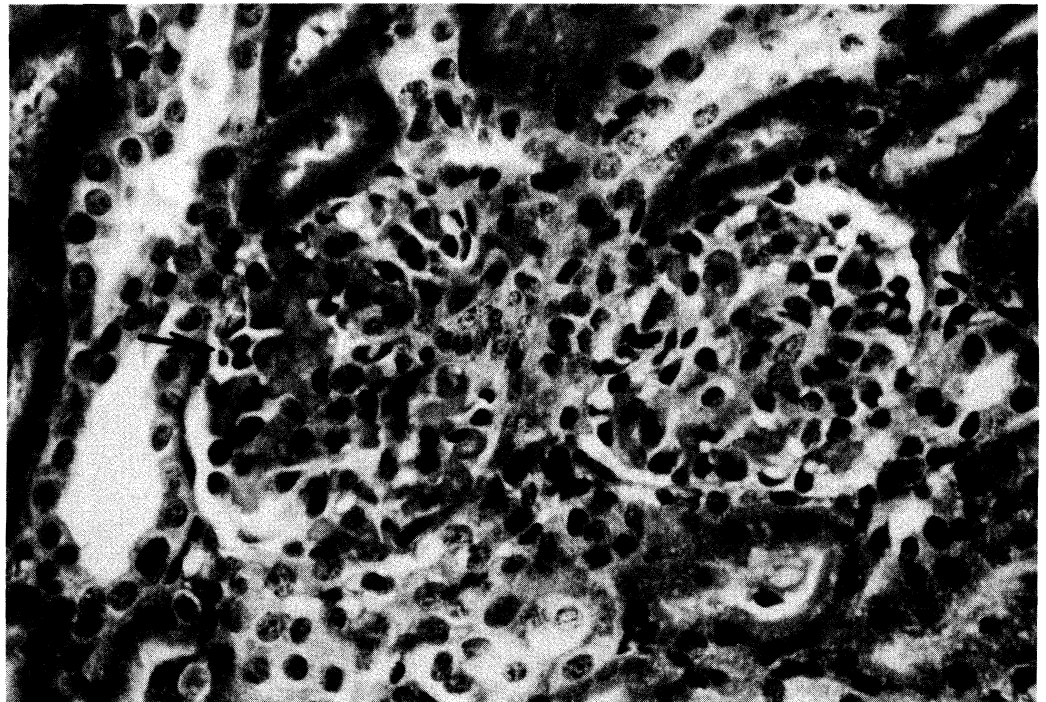


FIG. 7. Glomerulus filled with thrombi composed of RBCs in a pale granular matrix (hematoxylin and eosin, original magnification $\times 960$).



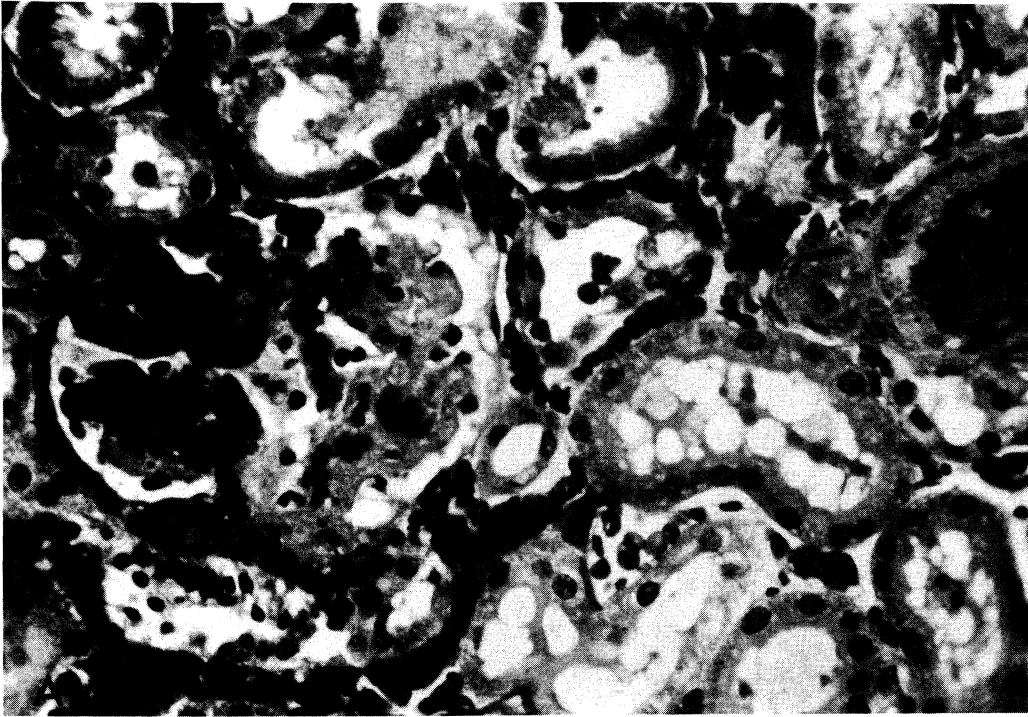
FIGS. 8A and B. Serial study, biopsy specimens at 5 minutes: *A*. control; *B*. treated. Note thrombi in some glomerular capillaries (arrows), and relatively normal tubules and interstitium (hematoxylin and eosin, original magnification $\times 625$).



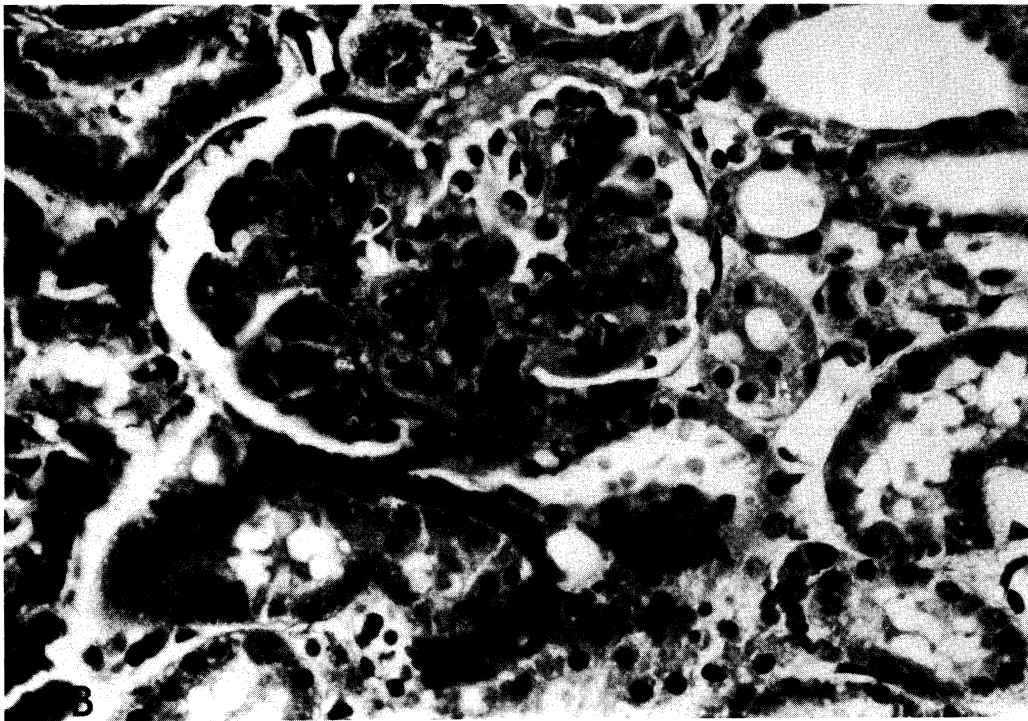
regard to the presence of thrombi in arteries and arterioles.

End-stage biopsies. The end-stage biopsies obtained after implantation represented a later stage than the sequential biopsies. In these sections, RBCs were a promi-

nent component of the glomerular thrombi in the treated kidneys, whereas in the control groups the thrombi were composed of mostly granular eosinophilic material consistent with platelet-fibrin thrombi, similar to the changes noted in the sequential biopsies. There



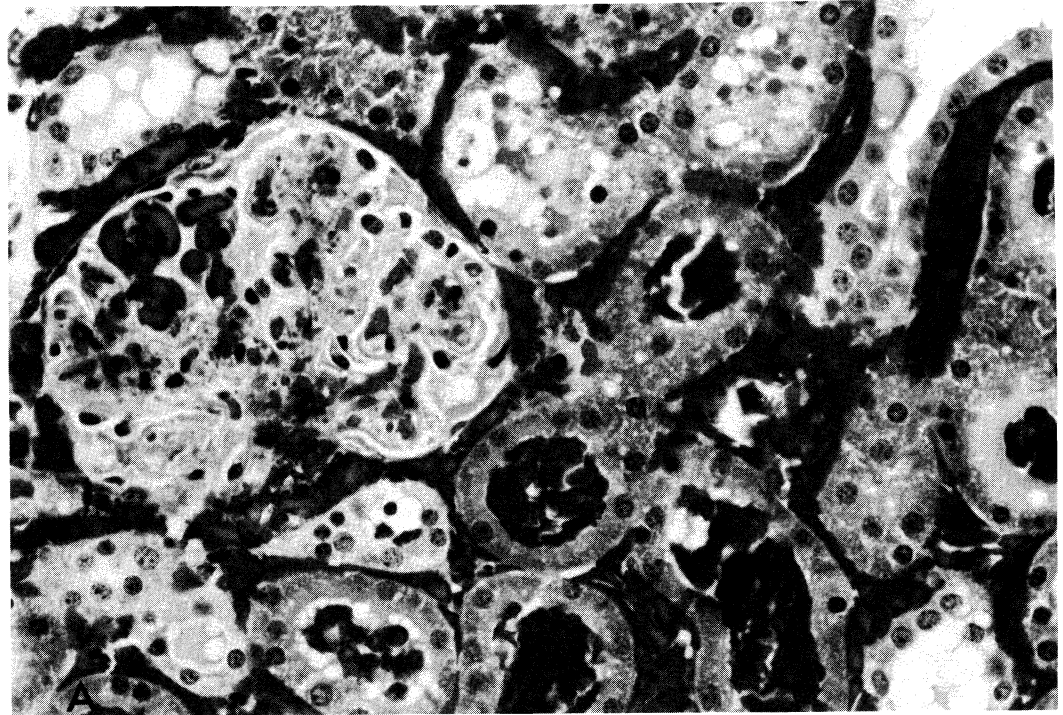
FIGS. 9A and B. Serial study, biopsy specimens at 45 minutes: *A.* control; *B.* treated. Note hemorrhages in the interstitium in the control kidney. Both glomeruli contain thrombi (hematoxylin and eosin, original magnification $\times 625$).



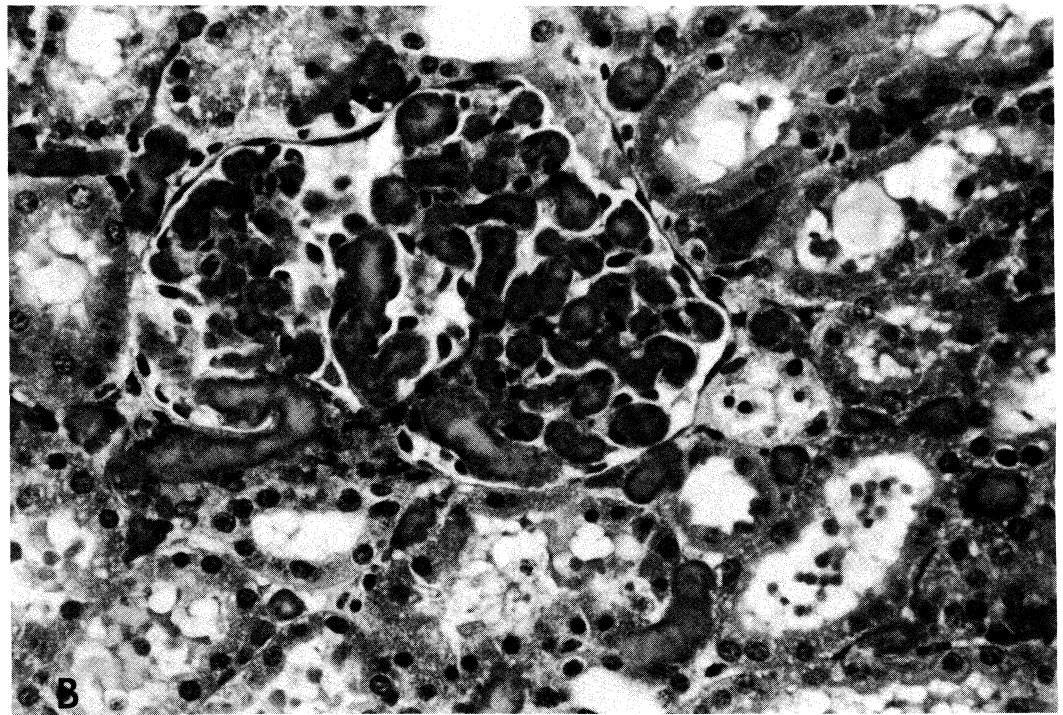
was more interstitial hemorrhage in the treated dogs than in the controls in five cases, and less hemorrhage in four cases. Three of the treated kidneys and none of the control kidneys exhibited up to 20% glomeruli free of thrombi.

Discussion

The candidacy of platelet-activating factor (PAF) as a pivotal biologic mediator in the pathogenesis of antibody-mediated hyperacute allograft rejection has been



FIGS. 10A and B. Serial study, biopsy specimens at 90 minutes: *A*. control; *B*. treated. Note interstitial hemorrhages and blood in the tubules in the control kidney (hematoxylin and eosin, original magnification $\times 625$).



clearly established. *In vitro* studies have demonstrated that PAF is released when endothelial cells are incubated with antibody to cell surface angiotensin converting enzyme.¹⁵ This has important implications for hyperacute rejection, in which the specific initiating event

is a transplantation antigen-antibody interaction on the endothelial surface. In a model of hyperacute renal allograft rejection in sensitized rabbits, PAF was identified in the blood effluent from the transplanted kidney, following antibody and complement fixation to the endo-

TABLE 1. Progression of Histologic Findings in Sequential Biopsies of Control and Treated Kidneys

Time (Min)	Control			Treated		
	Glomerular Thrombosis (%)	ATN	Interstitial Hemorrhage	Glomerular Thrombosis (%)	ATN	Interstitial Hemorrhage
5	26	0	0	13	0	0
15	69	0	Focal	46	0	0
30	83	0	Focal	60	0	Focal
45	94	0	Severe	73	0	0
60	91	Focal	Severe	80	0	0
75	88	Focal	Moderate	77	0	0
90	78	Focal	Severe	97	Focal	Moderate

thelium of glomerular and peritubular capillaries.¹⁰ No PAF could be detected in rabbits without clinical or histologic signs of rejection. The PAF release was followed by the recruitment of platelets, endothelial sticking, and obliteration of capillaries by platelet-PMN plugs and evidence of cellular degranulation.¹⁰

The importance of PAF, even in the absence of inflammatory cells, was demonstrated in a model of *ex vivo* perfusion of the rabbit heart with transplantation alloantibodies.¹⁶ The release of PAF followed bradycardia, conduction arrhythmias, decreased coronary flow, and cessation of heart action. These alterations in cardiac function and, moreover, the release of PAF, were prevented by complement inactivation. PAF itself induced a negative inotropic effect and reduction of coronary flow in isolated guinea pig heart muscle by a direct action on the Ca⁺⁺ slow channel. Moreover, the injection of synthetic, purified PAF into the renal artery of rabbits reproduced a pathologic picture identical to that seen in a Shwartzman reaction of the kidney.¹⁷ The pathophysiology of hyperacute renal rejection in humans was originally described as a Shwartzman reaction.¹

The nonspecific effector inflammatory cascade that ensues once an antigen-antibody reaction has initiated hyperacute rejection has served as the target for therapeutic intervention by many different approaches. Classically, the platelet-coagulation system has been considered to be the most important and has received the greatest attention. These therapeutic modalities have included heparin, aspirin, dextran, citrate, defibrinating agents, cobra venom factor, induction of thrombocytopenia by antibodies etc., and various prostaglandins.¹⁸⁻²¹ All these approaches have been either too toxic or the results too inconclusive to be accepted for generalized use.

The difficulty in overcoming the inflammatory process in hyperacute rejection and in explaining the discrepancies in therapeutic results rest not only in the complexity of the biologic reactions, but also in their

resiliency and redundancy. That is, there are numerous alternative mediator pathways that can backup the same set of functions. Thus, the inhibition of one arm of an inflammatory reaction can easily be replaced by the release of other inflammatory autocoids with complete mediation of a disease process. PAF may represent somewhat of an exception to this hypothesis, because of its potency and its unique potential for mediating almost every aspect of acute inflammatory reactions. Nonetheless, the argument for polypharmaceutical therapy of hyperacute rejection is clear.

The current study represents the first known report describing the therapeutic efficacy of a receptor antagonist of PAF in any *in vivo* model of hyperacute rejection. These studies not only lend further support to the prominent role of PAF in the regulation of the inflammatory response leading to hyperacute rejection, but also introduce a new and potentially significant therapeutic approach to this entity. The agent used in these studies, SRI 63-441, is currently the most potent and specific PAF receptor antagonist available. It represents a family of compounds that can be synthesized and modified to meet desired specifications, *i.e.*, an oral compound recently has been produced.

At the single dosage and schedule of SRI 63-441 evaluated in these studies, inhibition of PAF alone was not sufficient to improve renal xenograft survival or function, although a trend did exist. From studies in rats and other recent studies in dogs, we have determined that the critical consideration for the efficacy of SRI 63-441 is to administer it at a much higher dose and before revascularization (unpublished data). However, the addition of either one of two prostanoids, PGI₂ or PGE₁, resulted in a synergistic response and a manyfold increase in renal xenograft survival and function. Although a higher dosage and more appropriate scheduling of SRI 63-441 may improve the effectiveness of single drug therapy, the need for multiple drug treatment eluded to above may be necessary to adequately modulate this violent inflammatory process.

There are many reasons for choosing PGI₂ and PGE₁ as complimentary agents to SRI 63-441. They both inhibit platelet function by stimulation of receptor-coupled adenylate cyclase and increase intracellular cAMP, a receptor mechanism different from that required for SRI 63-441 inhibition.²² To varying degrees, they both exhibit other properties that establish them as excellent therapeutic candidates for such inflammatory processes. These biological activities include vasodilatation, fibrinolytic action, inhibition of the generation of certain deleterious metabolites of the arachidonic acid cascade, inhibition of oxygen-free radical generation, and the property known as cytoprotection.²² Moreover, both these prostaglandins have been evaluated in other models of hyperacute rejection. Treatment with PGE₁ prolonged hamster-to-rat cardiac xenograft survival.²³ PGI₂ administration significantly prolonged kidney survival in both a model of hyperacute renal allograft rejection in presensitized dogs and in cat-to-dog xenografts,^{21,23} but had no effect on guinea pig-to-rat cardiac xenograft survival.²³ Neither PGI₂ nor PGE₁, when administered alone as a constant infusion without combination SRI 63-441 treatment, had a significant effect on pig-to-dog renal xenograft survival or function as described in our studies. Furthermore, the therapeutic effectiveness of these prostaglandins in combination with SRI 63-441 was evident only when they were infused intra-arterially; peripheral administration of prostaglandins did not exhibit synergism with SRI 63-441 (unpublished results). This is most likely due to inactivation of prostaglandins as they pass through the lungs.²²

The pig-to-dog renal xenograft model evaluated in these studies probably represents the most severe animal model of hyperacute rejection known. Rapid and violent kidney rejection is the rule and there have been only a few reports of successful abrogation of this process. Shons and Najarian reported a significant increase in pig renal xenograft survival from a mean of 5 minutes in controls up to a mean of 45 minutes in dogs treated with a variety of antiplatelet agents.¹⁹ Merkel et al. reported renal xenograft survival times of 120, 90, and 180 minutes in three dogs that had preoperative plasmapheresis.²⁴ Kux et al. reported a mean rejection time of 85 minutes in 15 kidneys treated with intra-arterial citrate infusion; these experiments were limited by significant toxicity.²⁰ The control xenografts for the latter two sets of experiments rejected in less than 10 minutes.^{20,24}

Consistent and reproducible results were achieved in the same model in the current studies by combining SRI 63-441 with either PGI₂ or PGE₁. Pig kidneys surviving for 3–4 hours were consistently achieved with maintenance of good urine output in all combination treatment groups. There were three kidneys in this series of experiments that functioned satisfactorily for 6–9 hours.

These results compare most favorably with any previous reports of this particular model.

Although the differences in the pathologic evaluation of treated and control animals were subtle, there is no doubt that on serial histologic examination, treated animals demonstrated a delay in the evolution of the final morphologic deterioration. Furthermore, the end-stage histologic examination in treated kidneys demonstrated an overall amelioration, albeit minor, of the entire pathologic process. The histologic findings correlated with the clinical course. The significant improvements in renal xenograft survival and function were also paralleled by a significantly delayed diminution in renal artery blood flow and a significantly improved gradient of the platelet count between renal artery and vein in combination treated animals.

Emphasis on platelet counts and gradients may not be as important as once believed. It is clear that although PAF is a potent modulator of platelet aggregation and release, it can promote the pathophysiologic process of hyperacute rejection by various other pathways.^{8–12} Furthermore, rats with a defect in platelet aggregation exhibited the same time course and morphologic features of hyperacute cardiac rejection as in normal rats,⁶ and platelet aggregation was demonstrated not to be essential in a model of renal xenograft rejection.²⁵

In summary, these studies further confirm the central role of platelet-activating factor in the inflammatory cascade that destroys kidneys undergoing hyperacute rejection. Furthermore, this report introduces a novel, reproducible, and potentially effective therapeutic approach to this difficult problem, which relies on a better understanding of the basic pathophysiologic process of inflammation and which utilizes as its basis, a synthetic receptor antagonist that specifically inhibits the potent and unusually diverse spectrum of biologic activities of one of the key mediators of classical inflammation.

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DISCUSSION

DR. EDWIN W. SALZMAN (Boston, Massachusetts): This is a very interesting paper, but there are several concerns I have about some of the details of the use of the inhibitor of platelet activating factor (PAF).

One of the most striking things about PAF is its species specificity. There is evidence, for example, in humans, although perhaps not in rabbits or in dogs, that the action of this agent is mediated through cyclo-oxygenation of arachidonic acid, and ultimately through the generation of prostaglandin endoperoxides and thromboxanes. These processes are totally blocked by aspirin, and I wonder if the observations made on these animals would be relevant in humans. Might one not expect that in humans the effect of aspirin would be as great as the effect of the specific inhibitor of PAF?

The second question relates to the dose of prostacyclin that was used, which is about 40 times the maximum tolerable dose in humans. Is there reason to believe that the findings with such a large dose of this prostaglandin would be relevant to the human situation?

Finally, we have seen that the histology after the graft finally failed was different in the animals treated with the antagonist to PAF and with prostacyclin. This is a situation in which there is virtually total paralysis of platelet function, and I wonder if the author would comment further on the presumed difference in mechanism of graft failure in the animals receiving the PAF antagonist.

DR. P. O'LEARY (Dallas, Texas): I enjoyed your paper very much. I have one question.

It looked to me as though the platelet count did not fall as far in the treated animals, but the extraction arterial to venous was as great. I wonder if you attempted to quantify the extraction of platelets as they passed through the kidney.

DR. M. WAYNE FLYE (St. Louis, Missouri): You state that there is abrogation of the hyperacute rejection, but in fact, the graft still fails at about 400 minutes. Your histology shows some clearing; however, it does not show the expected deposition of fibrin and platelets onto the

endothelium. Would you comment on why the graft fails even though there is some prolongation of survival?

DR. COURTNEY M. TOWNSEND, JR. (Galveston, Texas): That was a very nice study. There was a lot of work in it. It was very well presented. I wondered why you had to give the prostaglandins in addition to the PAF antagonist in the pig-to-dog model and did not apparently do that in the rat model.

DR. WILLIAM E. NEVILLE (Newark, New Jersey): This was a beautifully presented paper and their results were stimulating.

As I look back at what our group did in the 1960s and early 1970s on heterologous cardiac transplants in animals, I realize now that I should have continued with this work. However, we did not have the drugs that today seem to prevent early rejection between certain heterologous animals. We initially inserted the hearts of calves, sheep, goats, and pigs orthotopically into dogs on cardiopulmonary bypass using only Ringer's lactate to prime the pump oxygenator. The hearts were initially pink and contracting but deteriorated rapidly. Histologic and electromicroscopic studies showed microcirculatory red cell agglutination without fibrin despite platelet aggregation and inflammatory reaction. We then reversed the experiments and placed dog hearts into calves, sheep, goats, and dogs using the same technique. All of the transplanted hearts were easily defibrillated and adequately maintained the circulation until the dogs were killed hours later. Microscopic examination of the dog hearts showed few ultrastructural changes. The capillaries were invariably patent with no significant alterations of the endothelial cells. The myocardium only showed some patchy interstitial edema and slight mitochondrial swelling and glycogen depletion. The reason for this striking contrast in the two groups was not clear; but we reasoned and published that it might have something to do with the disproportion between the red blood cells and capillaries, thus disregarding an antigen-antibody reaction.

I would like to see the group from Pittsburgh use this relatively new drug alone or in combination with other antiplatelet drugs to see

whether the Schwartzman reaction could be prevented in xenograft hearts inserted into dogs on cardiopulmonary bypass. We definitely need heterologous organs for transplantation but we do have much to learn regarding the use of these organs in humans.

DR. NICHOLAS L. TILNEY (Boston, Massachusetts): I also enjoyed the paper very much. We have been looking at a slightly different model in a rat heart graft system, so-called accelerated rejection that occurs about 36 hours after transplantation instead of in a few minutes as occurs in a presensitized rat.

In the beginning of the process, we found more or less what these authors have found, a very accelerated rejection that is primarily a humoral event. There are a few infiltrating cells; however, most of which are activated cells bearing interleukin II receptors on their surfaces.

We can abrogate this early rejection and get grafts out to about 3 or 4 weeks using either cyclosporine in various doses or monoclonal antibodies directed against the interleukin 2 receptors. Regardless of treatment, the grafts ultimately reject at about 3 weeks, but the primarily humoral rejection has been converted to a primarily cellular one, an extremely interesting observation.

I would like to ask the authors whether they had a chance to look at the infiltrating cell populations during the time their grafts are still viable, because I believe they will find these are a very interesting cell subgroup and mostly interleukin 2 receptor positive.

DR. LEONARD MAKOWA (Closing discussion): I would like to thank all the speakers for their helpful comments and interesting questions.

Many of your questions have concentrated on the importance of platelets in the overall process of hyperacute rejection and, therefore, I will try to cover these together. There is no doubt that platelets play a role in this process. However, over the last 20 years, since this entity was described, there has been a hangup on the importance of platelets and the major role that they play in the overall pathophysiology of this process. Many investigators had demonstrated platelet consumption by kidneys that were undergoing hyperacute rejection by studying platelet gradients between the renal artery and vein of these kidneys. Histologically, platelet accumulation and aggregation was a major feature of the final pathologic process. Nonetheless, it is now clear that the role and the importance of platelets in this process is just one small component of a much bigger picture, and that the entire evolution of hyperacute rejection must be redefined.

There is considerable evidence in the literature that indicates platelets are not the sole or major contributor to the process of hyperacute rejection. These experiments have demonstrated that the identical clinical and histopathologic entity of hyperacute rejection can occur in the face of platelet depletion, or in animals with a hereditary deficiency of platelet function. In fact, it now appears that other cellular components, especially the neutrophil, may play an equal or even more important role.

Currently, hyperacute rejection must be considered the end product of a set of complex nonspecific inflammatory reactions, which are initiated by a specific antigen-antibody reaction. I tried to demonstrate some of these contributory inflammatory arms in the first slide, and to emphasize their resiliency and redundancy. Thus, many alternative backup inflammatory systems can result in the same set of functions and final pathologic process. The relative importance of each of these inflammatory pathways is as yet unknown, and furthermore, the relative importance of blocking or inhibiting the biologic activity of any one specific mediator remains to be fully defined.

I must re-emphasize that the designation of the mediator that we are discussing today, as platelet activating factor, is a misnomer. PAF plays a role in most of the classical mediator systems of the acute inflammatory process, in the generation of arachidonic acid metabolites, and in the generation of oxygen free radicals, etc. Although PAF is one of the most potent inducers of platelet aggregation and release, and thus by

this mechanism plays a contributory role in hyperacute rejection, there is now clear evidence that establishes PAF as a central candidate in the overall evolution of this inflammatory process and as a key biological mediator in the pathogenesis of antibody-mediated hyperacute allograft rejection. This led to our hypothesis that antagonism of PAF-induced reactions offered a potential therapeutic approach to this entity by inhibiting many of the key inflammatory pathways.

It is true that one of the actions of the PAF antagonist is to inhibit the cyclo-oxygenase pathway, thereby inhibiting the formation of various biologically active autocooids of the arachidonic acid cascade, as well as interfering with platelet function. Aspirin exhibits these same specific properties, but does not influence the numerous other inflammatory pathways as the PAF antagonist can. Dr. Najarian's group described a set of elegant experiments in Transplantation Proceedings in 1974, where the effect of aspirin was evaluated in this same model of pig-to-dog renal xenografts. They found that the mean graft survival time of 5 minutes in control animals was increased to 21 minutes by aspirin treatment. In fact, the addition of dextran to aspirin treatment further increased the mean graft survival time to 45 minutes. At that time, these were considered significant results. Obviously, the numerous backup pathways could overcome this single-pronged approach in such a severe xenograft model. That is why we have initially taken the approach of multipharmaceutical therapy, and demonstrated a synergistic effect by combining a prostaglandin with the PAF antagonist.

In our studies, we consistently achieved renal xenograft survival of 3 to 4 hours and in a few cases achieved 9-hour survival. These results significantly surpassed previously published reports using this same pig-to-dog model, which we believe is probably the most severe experimental model of hyperacute rejection known.

I agree that the dose of prostacyclin that we have reported in these particular studies today was too high. We did observe some hypotension in these animals. Subsequently, we have repeated these studies using a much lower dose of prostacyclin and have achieved the same efficacy. Similarly, for prostaglandin E₁, we have used dosages as low as 0.01 $\mu\text{g}/\text{kg}/\text{min}$, which is well tolerated in humans.

One of the problems with the studies that I have described to you today, is that based on our subsequent rat studies, we probably were not using enough of the PAF antagonist. In these studies, we used 5 mg/kg. From subsequent studies of hyperacute rejection using a model of heterotopic cardiac transplantation in hypersensitized rats, we have learned two critical points. A much higher dose of the drug can and should be given, and the dose given before revascularization is the key for efficacy. In fact, we have demonstrated significant prolongation of cardiac allograft survival in this model using 15 or 20 mg/kg given 4 minutes before revascularization.

It appears that it is much easier to overcome this process in the rat heart model using the PAF antagonist alone. As I already mentioned, this may be due to a dosage and scheduling inefficiency in the xenograft model reported today. However, in the xenograft model we are probably dealing with a much more potent initiating immunologic reaction. Therefore, the trigger for this overall process may be different, and may require a modified therapeutic approach, *i.e.*, that of utilizing polypharmaceutical therapy. Alternatively, we must appreciate the contributions of the different microvasculatures of the kidney and the heart.

I agree completely with Dr. Tilney, that the importance of the rat model should be emphasized. Unfortunately, these initial studies were presented in the closing two slides, but it is apparent that in the rat model, we have converted the process of hyperacute rejection into one of classical cell-mediated rejection. We have not as yet had the opportunity to characterize the infiltrating cells in the hearts that underwent classical rejection. We are currently in the process of combining the PAF antagonist with standard immunosuppression in this model. We certainly agree that the combination of the ability to overcome the inflammatory process resulting from the initial antibody insult and of controlling cell-mediated rejection with appropriate immunosuppression, at least in the homograft rat heart model, may allow us to overcome the entire process of hyperacute rejection.