Platelets in Early Hyperacute Allograft Rejection in Kidneys and Their Modification by Sulfinpyrazone (Anturan) Therapy

An Experimental Study

Hari M. Sharma, MD, FRCP(C), S. Moore, MB, BCh, H. W. Merrick, MD, and M. R. Smith, MD

Experiments were carried out on mongrel dogs to study the early ultrastructural changes in hyperacute rejection of kidney. Renal allografts were performed on dogs presensitized by three to five skin grafts. The animals were grouped in pairs, control and treated with sulfinpyrazone, a platelet inhibitor. The electron microscopic studies showed that the earliest change in the kidney was aggregation of platelets mainly in the peritubular capillaries and a few glomeruli. Later, platelets showed degranulation with vascular damage leading to rejection of kidney. Treated dogs showed less tendency for platelets to aggregate, and graft survival was prolonged. The role or platelets in the hyperacute rejection is discussed (Am J Pathol 66:445–460, 1972).

THROMBOSIS OF SMALL CORTICAL VESSELS and cortical necrosis was first reported by Kissmeyer-Neilson *et al*[1] in hyperacute renal allograft rejection. Subsequently, many papers have appeared in the literature implicating intravascular coagulation and platelets in hyperacute rejection.[2–6] However it is not clear whether the platelets play the primary part (initiators) in this early rejection episode or are involved in the rejection episode secondarily. There are also reports of some modification of vascular lesions by antithrombic drugs.[3,7]

Our experiments were designed to study the early phase of hyperacute allograft kidney rejection in dogs and also the effect of sulfinpyrazone (Anturan, Geigy Laboratories Ltd, Toronto, Canada), a platelet inhibitor, on the survival of the graft. The results seem to indicate that platelets play the primary role in the hyperacute rejection and are the initiators of vascular damage. Sulfinpyrazone therapy also could prolong the survival of the graft by inhibiting platelet aggregation.

From the Department of Pathology and Lady Davis Institute of Jewish General Hospital, and the Department of Experimental Surgery, Division of Hematology and the Department of Pathology, McGill University, Montreal, Canada.

Supported in part by grant MT-2168 from the Medical Research Council of Canada, and the Quebec Heart Foundation.

Accepted for publication September 1, 1971.

Address reprint requests to Dr. H. M. Sharma, Department of Pathology, Indiana University Medical Center, 1100 West Michigan Street, Indianapolis, Ind 46202.

Materials and Methods

The experiments were carried out on healthy adult mongrel dogs weighing between 15 and 20 kg. The dogs were presensitized by three to five full-thickness skin grafts applied in succession until the last graft was rejected as a white graft. This was taken as evidence of presensitization. The dogs later underwent cross renal transplantation. The donor kidney after removal was flushed with ice-cold saline and transplanted immediately. The anoxia time in both groups was 20–30 minutes. One of the dogs of the pair acted as control and the other was injected intravenously with sulfinpyrazone, 125 mg/kg body weight, 1 hour before transplantation and maintained on a dose of 200 mg every 6 hours by mouth till anuria occurred. The drug dosage was determined in a separate set of experiments by giving different quantities of sulfinpyrazone to dogs and testing the inhibition of their platelet aggregation by using a turbidimetric method employing collagen, adenosine diphosphate and thrombin. The optimal platelet-inhibiting effect of sulfinpyrazone was found to be 125 mg/kg body weight, which was used in this experiment. Urine flow was used as an index of function of renal allografts. Serial determination of renal artery and vein platelet counts, white blood cell (WBC) and red blood cell (RBC) counts were done up to 2 hours after transplantation. These counts were later done on the peripheral venous blood. Serial biopsies of the kidneys were also taken starting at 1 minute after transplantation and subsequently at 7, 10 and 30 minutes, 1 and 2 hours, and before transplantation for light and electron microscopic and immunofluorescent studies. The details of the experiments, including determination of sulfinpyrazone dosage, results of blood counts, light microscopic and immunofluorescent data are reported elsewhere.[8] Tissues for ultrastructural study were immediately fixed in 5–6% buffered glutaraldehyde, washed in phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide, dehydrated and embedded in Epon.[9] Unstained sections ½–1 μ thick were studied under a phase microscope for selection of areas with blood vessels and glomeruli. Thin sections were cut on Reichert's ultramicrotome OmU_2, dobule-stained with uranyl acetate [10] and lead citrate [11] and examined with Hitachi HS-8-2 and Hitachi-12 electron microscopes.

Results

Since the purpose of this paper is to discuss mainly the electron microscopic findings, the other results will be summarized only briefly here. The 13 control animals had renal transplants that functioned from 5 minutes to 36 hours, with a mean function time of 7.5 hours, whereas in the treated group (13), the mean time of graft function was 33.1 hours. Serial determinations of renal arterial and venous platelet counts showed little difference in the treated group (4.5%), whereas the mean venous count was 29.5% lower than mean arterial platelet count in the control group. No significant differences in WBC or RBC counts were noted in either group.

Electron Microscopy

This will be discussed in three separate groups, before transplantation and after transplantation in the control and treated group.

Before Transplantation

The ultrastructural study of kidney biopsies taken before unclamping of the renal vessels showed the normal structure of the glomerulus and the peritubular capillaries. Occasionally, in a few glomerular capillary loops, a completely degranulated degenerating platelet was seen (Fig 1). This degranulation of platelets resulted possibly from the saline perfusion of the kidney before transplantation.

After Transplantation

Since the results were more or less similar in all the kidneys examined in the control group and the same sequence of events occurred in all, they will be discussed together.

Control Group. The earliest finding, which occurred at 1 minute after transplantation (release of clamps), was the aggregation of platelets in arterioles (Fig 2). Subsequently, the majority of the platelet aggregates were noted in the peritubular capillaries and few in the glomerular capillaries. The aggregated platelets were noted first before any evidence of injury to the vascular endothelium could be detected (Fig 2). Subsequent biopsies that were removed up to 15 minutes after transplantation demonstrated many tight aggregates of platelets, again mainly in the peritubular capillaries (Fig 3). In most of the capillaries, these aggregates almost completely filled the lumen. The platelets in the center of the aggregates still had some of their granules intact, whereas those at the periphery and near the vascular endothelium showed partial to complete degranulation (Fig 3 and 5). Pseudopod formation was also a prominent feature (Fig 3 and 4). The vascular endothelium showed swelling and disruption of the cytoplasm, with lifting of the cell surface from the basement membrane. At places, the vascular endothelium and basement membrane were completely disrupted, with platelets lying in direct contact with collagen fibrils of the interstitium of the kidney. The platelet pseudopods were seen touching and/or extending through the vascular endothelium and at a few places, were lying between the endothelial cytoplasm and the vascular basement membrane (Fig 3 and 4). In other places, the pseudopods were extending through both the endothelium and the basement membrane (Fig 4). Platelet granules were seen lying free in the lumen of the peritubular capillaries and in the interstitial space of the kidney (Fig 3). Detached fragments of platelets were also present in the interstitial space near the area of vascular damage (Fig 4). Furthermore, in a few instances, red blood cells were seen lying in the interstitial space (Fig 5). There was marked edema in the interstitial space

manifested by marked separation of collagen fibrils (Fig 3 and 4). There was no morphologic difference in the biopsies taken from 15 minutes to 2 hours after transplantation. The important negative finding at this early phase of rejection was the absence of fibrin and leukocytes in all the specimens examined up to 2 hours after transplantation.

Treated Group. We were impressed by the absence of platelet aggregation and vascular injury in the majority of the kidneys of this group examined up to 2 hours after transplantation. However, in a few instances, in those animals in which the graft was rejected earlier, platelet collections could be found in a few of the peritubular capillary networks. These platelet aggregates were loose, with less tendency to pseudopod formation and degranulation than in the control group (Fig 6). Also, there was very little edema in the interstitial tissue. Rarely, an area of vascular damage was seen where platelets were degranulated and pseudopods extended through the vascular endothelium. Here again, the number of platelets was markedly less than in the control group.

Discussion

Our experimental data show that in the presensitized dogs the earliest change noticed in the rejecting transplanted kidney is the aggregation of platelets before any vascular injury is demonstrable. These platelet aggregates later show degranulation, release of their contents and vascular damage leading to ischemia and graft rejection. Sulfinpyrazone therapy largely prevented the platelet aggregation and their degranulation, thus preventing vascular damage and prolonging the graft survival.

Prior sensitization of the recipient by repeated skin grafting in order to study the early phase of allograft rejection has been done before.[5] The concept that immunoglobulin response is also involved in homograft rejection has frequently been proposed after the publications of Gorer and O'Gorman [12] and Stetson.[13] The presence of high concentrations of circulating antibody as measured by lymphocytotoxicity has been seen in hyperacute rejection in recipient dogs deliberately or inadvertently sensitized to prior exposure to donor antigen.[7,15,16] Passive transfer of donor-specific hyperimmune serum in animal models results in intense vascular damage and rapid destruction of the graft.[17–19] In clinical transplantation, graft rejection can also occur within minutes or hours and is characterized by the presence of preexisting antibodies which produce this hyperacute rejection. Previous dialysis, pregnancy or even bacterial exposure may have produced this presensitization.[1,20,21]

Hyperacute rejection of a renal allograft can also occur in patients presensitized by multiple blood transfusions [22] and previous kidney transplants [23,24] in the presence [25] or absence [1,24,26,27] of ABO-incompatible donors and recipients. Our experimental model of hyperacute rejection in the dog is analogous to clinical transplantation in presensitized recipient patients in which performed antibodies to the donor tisssue are present.

We speculate that circulating antibodies first bind to the endothelial plasma membrane HL-A antigens.[28–31] These antigen-antibody complexes lead to platelet aggregation. *In vitro* studies with platelet-rich plasma or platelet suspensions from humans,[32–34] guinea pigs,[35,36] rabbits [37] and pigs [32] demonstrated that adding antigen-antibody complexes induced platelet aggregation. Movat *et al* [38] further suggested that aggregation of human and pig platelets by antigen-antibody complexes is induced by release of adenosine diphosphate (ADP) during phagocytosis of the complexes. This aggregation *in vitro* is associated with release of histamine, serotonin and ADP. In addition to these, the constituents that may be released include epinephrine, potassium, free amino acids, small amounts of lipoproteins and proteins, mucopolysaccharides, enzymes such as β-glucuronidase and acid phosphatase, and platelet factors 3 and 4.[39–45] Extracts of human platelets have also been shown to contain elastinolytic protease which damages elastic tissue.[46] A factor that increases the permeability of the vessels and causes disruption of endothelium in the microcirculation has also been demonstrated when platelets are exposed to antigen-antibody complexes.[47] The aggregation of platelets by antigen-antibody complexes with loss of their internal structure and their disruption as seen in the experiments of Packham *et al* [47] corresponds to the situation in our experimental model. The effect of platelet aggregation on organ structure and function has been reported.[47] ADP-induced platelet aggregation in the myocardial [48] or renal circulation [47] causes tissue injury and organ dysfunction. Platelet-aggregate embolism arising from a source of emboli placed in the thoracic aorta has also been shown to cause endothelial damage.[49] These data support our findings that intravascular aggregation of platelets with subsequent degranulation is followed by vascular damage manifested by disruption of vessel wall and subsequent hemorrhage.

Antithrombotic and anticoagulant drugs have been shown to modify the vascular lesions of allograft rejection.[3] In the experiments of MacDonald *et al* [7] using presensitized dogs, aspirin treatment delayed the vascular damage when compared to the azathioprine-treated and un-

treated controls. Systemic heparin therapy used in cadaveric renal transplantation resulted in improvement in transplant function.[50] McMillan[50] proposed that dispersion of platelet aggregation in glomerular capillaries by heparin therapy resulted in improved function of the graft. Sulfinpyrazone is a pyrazole derivative (1,2-diphenyl-4-[2-(phenylsulfinyl)ethyl]-3,5-pyrazolidinedione). It is a uricosuric agent and is used clinically in the chronic phases of gout. The side effects are rare and mild.[51] Sulfinipyrazone prolongs platelet turnover[52–54] and also decreases platelet adhesiveness.[54] In experimental studies using rabbits, it was shown that sulfinipyrazone and phenylbutazone inhibited platelet aggregation induced by gamma-globulin coated surfaces, collagen and antigen-antibody complexes.[55] Packham *et al*[55] suggested that this inhibition of surface-induced platelet aggregation resulted from a diminished release of platelet constituents. Because of these platelet-inhibitory activities, and because it is a nontoxic drug, sulfinpyrazone was chosen in our experimental model. Our results clearly show that sulfinpyrazone inhibited platelet aggregation, thus prolonging graft function.

Our experimental data show that platelets play a primary part in the initiation of graft rejection. The evidence for this is twofold. Firstly, the appearance of platelets was noticed before any other blood element or vascular injury. Secondly, in the treated group, in areas devoid of platelet aggregates, there was no evidence of vascular injury up to 2 hours after transplantation. To our knowledge, sulfinpyrazone has not been found to have any effect on antigen-antibody complexes; thus prevention of platelet aggregation in the treated group should have resulted in vascular damage if this is caused by antigen-antibody complexes on the vascular endothelium as proposed by Busch *et al*.[56] Because of these observations, we propose that as a first event, platelets aggregate in response to antigen-antibody complexes formed on the vascular endothelium. Then, secondarily, they cause vascular damage which results in further platelet aggregation and the process goes on. We propose that polymorphonuclear leukocytes are only secondarily involved in this process as is fibrin formation.

In conclusion, our experimental data have shown that in presensitized dogs, hyperacute rejection is triggered by platelet aggregation caused probably by antigen-antibody complexes on the vascular endothelium of the grafted kidney.

References

1. Kissmeyer-Neilsen F, Olsen S, Petersen VP, Fjeldborg O: Hyperacute rejection of kidney allografts associated with preexisting humoral antibodies against donor cells. Lancet 2:662–665, 1966

2. Busch GJ, Braun WE, Carpenter CB, Corson JM, Galvanek ER, Reynolds ES, Merril JP, Dammin GJ: Intravascular coagulation (IVC) in human renal allograft rejection. Transplantation 1:267–270, 1969
3. Kincaid-Smith P: The pathogenesis of the vascular and glomerular lesions of rejection in renal allografts and their modification by antithrombotic and anticoagulant drugs. Australas Ann Med 3:201–214, 1970
4. Kincaid-Smith P, Mckenzie IFC, Morris PJ, Marshall VC: Biopsy features of early acute rejection in cadaveric renal grafts. Transplantation 1:287–289, 1969
5. Lowenhaupt R, Nathan P: Platelet accumulation observed by electron microscopy in the early phase of renal allotransplant rejection. Nature 220: 822–825, 1968
6. Simpson KM, Bunch DL, Amemiya H, Boehmig HJ, Wilson CB, Dison FJ, Coburg AJ, Hathaway WE, Giles GR, Starzl TE: Humeral antibodies and coagulation mechanisms in the accelerated or hyperacute rejection of renal homografts in sensitized canine recipients. Surgery 68:77–85, 1970
7. Macdonald A, Busch GJ, Alexander JL, Pheteplace EA, Menzoian J, Murray JE: Heparin and aspirin in the treatment of hyperacute rejection of renal allografts in presensitized dogs. Transplantation 9:1–7, 1970
8. Merrick HW, Smith MR, Sharma H: Modification of hyperacute renal allograft rejection by platelet inhibition. Fed Proc 30:455, 1971, abstr
9. Luft JH: Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol 9:409–414, 1961
10. Huxley HE, Zubay G: Preferential staining of nucleic acid containing structures for electron microscopy. J Biophys Biochem Cytol 11:273–296, 1961
11. Reynolds ES: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17::208–212, 1963
12. Gorer PA, O'Gorman P: Cytotoxic activity of isoantibodies in mice. Transplantation 3:142–143, 1956
13. Stetson CA: The role of humoral antibody in the homograft rejection. Adv Immunol 3:97–130, 1970
14. Yamada T, Key JH: Kidney homotransplantation with special reference to cytotoxic antibody response. Surgery 63:637–645, 1968
15. Holl-Allen RT, Scharli A, Rippin A, Busch GJ, Simonian SJ, Wilson RE: Cytotoxic antibody after antigen pretreatment: enhancement of renal allografts. Surg Forum 20:276–278, 1969
16. Wilson RE, Rippin A, Dagher RK, Kinneart P, Busch GJ: Prolonged canine renal allograft survival after pretreatment with solubilized antigen. Transplantation 7:360–371, 1969
17. Altman B: Tissue transplantation, circulating antibody in the homotransplantation of kidney and skin. Ann R Coll Surg Engl 33:79–104, 1963
18. Dubernard JM, Carpenter CB, Busch GJ, Diethelm AG, Murray JE: Rejection of canine renal allograft by passive transfer of sensitized serum. Surgery 64:752–760, 1968
19. Najarian JS, Perper RJ: Participation of humoral antibody in allogeneic organ transplantation rejection. Surgery 62:213–220, 1967
20. Williams GM, Lee HM, Weymouth RR, Harlen WR, Stanely CM, Willington CA, Hume DM: Studies in hyperacute and chronic renal homograft rejection in man. Surgery 62:204–211, 1967

21. Terasaki PI, Thrasher DL, Hauber TH: Serotyping for homotransplantation. XIII. Immediate kidney transplant rejection and associated preformed antibodies, Advances in Transplantation. Baltimore, The Williams & Wilkins Co, 1968, pp 225–229
22. Carpenter GB, Winn HJ: Hyperacute rejection. N Engl J Med 280:47–48, 1969
23. Patel R, Terasaki PI: Significance of the positive cross-match test in kidney transplantation. N Engl J Med 280:735–739, 1969
24. Williams GM, Hume DM, Hudson RP, Morris PJ, Kano K, Milgram F: Hyperacute renal homograft rejection in man. N Engl J Med 279:611–618, 1968
25. Starzl TE, Marchioro TL, Holmes JH, Hermann G, Brittain RS, Stonington OH, Talmage DW, Waddell WR: Renal homografts in patients with major donor-recipient blood group incompatibilities. Surgery 55:195–200, 1964
26. Busch GJ, Braun WE, Carpenter CB, Corson JM, Galvanek ER, Reynolds ES, Merrill JP, Dammin GJ: Intravascular coagulation (IVC) in human renal allograft rejection. Transplantation 1:267–270, 1969
27. Starzl TE, Lerner RA, Dixon FJ, Broth CG, Brettschneider L, Terasaki PI: Shwartzman reaction after human renal homotransplantation. N Engl J Med 278:642–648, 1968
28. Braun WE, Murphy JJ: HL-A antigens of the human renal glomerulus, Histocompatibility Testing. Edited by PI Terasaki. Copenhagen, Munkogaard, 1970, p 323
29. Kahan BD, Reisfeld RA, Pellegrino MA, Curtoni ES, Mattiuz PL, Cepellini R: Water soluble human transplantation antigen. Proc Natl Acad Sci USA 61:897–904, 1968
30. Mann DL, Rogentine GN, Fahey JL, Nathensen S: Human lymphocyte membrane (HL-A) alloantigens, isolation, purification and properties. J Immunol 103:282–292, 1969
31. Rapaport FT, Dausset J, Converse JM, Lawrence HS: Biological and ultrastructural studies of leucocyte fractions as transplantation antigens in man. Transplantation 3:490–500, 1965
32. Movat HZ, Mustard JF, Taichman NS, Urinhara T: Platelet aggregation and release of ADP, serotonin and histamine associated with phagocytois of antigen–antibody complexes. Proc Soc Exp Biol Med 120:232–237, 1965
33. Bettex-Galland M, Luscher EF: Untersuchungen über die auslösung der viskösen metamorphose der menschlichen blutplättchen durch immunokomplexe. Pathol Microbiol (Basel) 27:533–541, 1964
34. Mustard JF: Antigen–antibody complexes and platelet aggregation. Fed Proc 23:548, 1964, abstr
35. Siqueira M, Nelson RA Jr: Platelet agglutination by immune complexes and its possible role in hypersensitivity. J Immunol 86:516–526, 1961
36. Ishizaka T, Ishizaka K: Biological activities of aggregated γ-globulin. V. Agglutination of erythrocytes and platelets. J Immunol 89:709–716, 1962
37. Humphery JH, Jaques R: The release of histamine and 5–hydroxytryptamine (serotonin) from platelets by antigen-antibody reaction (in-vitro). J Physiol (Lond) 128:9–27, 1955
38. Movat HZ, Mustard JF, Taichman NS, Urinhara T: Platelet aggregation and release of ADP, serotonin and histamine associated with phagocytosis of antigen–antibody complexes. Proc Soc Exp Biol Med 120:232–237, 1965

39. Holmsen H, Day HJ, Stormorken H: The blood platelet release reaction. Scand J Haematol: suppl 8:1–26, 1969
40. Buckingham S, Maynert EW: The release of 5-hydroxytryptamine, potassium and amino acids from platelets. J Pharmacol Exp Ther 143:332–339, 1964
41. Hinterberger H, Anthony M, Vagholkar MK: Platelet 5-hydroxytryptamine and adenine nucleotides, serum arginylesterase and plasma 11-hydroxycorticosteroids in migraine. Clin Sci 34:271–276, 1968
42. Mills DCB, Robb IA, Roberts GCK: The release of nucleotides, 5-hydroxytryptamine and enzymes from human blood platelets during aggregation. J Physiol (Lond) 195:715–729, 1968
43. Niewiarowski S, Poplawski A, Lipinski B, Farbiszewski R: The release of platelet clotting factors during aggregation and viscous metamorphosis in platelets in hemostasis. Exp Biol Med 3:121–128, 1968
44. Pachter MR, Johnson SA, Neblett TR, Truant JP: Bleeding, platelets and macroglobulinemia. Am J Clin Pathol 31:467–482, 1959
45. Zieve PD, Gamble JL, Jackson DP: Effects of thrombin on the potassium and ATP content of platelets. J Clin Invest 43:2063–2069, 1964
46. Legrand Y, Robert B, Szigeti M, Pignaud G, Robert JCL: Études sur une protéase élastinolytique des plaquettes sanguines humaines. Atherosclerosis 12:451–465, 1970
47. Packham MA, Nishizawa EE, Mustard JR: Response of platelets to tissue injury. Biol Pharmacol: Suppl:171–184, 1968
48. Jorgensen L, Rowsell HC, Hovig T, Glynn MF, Mustard JF: Adenosine diphosphate-induced platelet aggregation and myocardial infarction in swine. Lab Invest 17:616–644, 1967
49. Moore S, Lough J: Lipid accumulation in renal arterioles due to platelet aggregate embolism. Am J Pathol 58:283–293, 1970
50. McMillan R: Heparin in delayed transplant function. Lancet 1:1178–1180, 1968
51. Anturan, Vademecum. International edition (English). Eighteenth edition. Montreal, J Morgan Jones Publications Ltd, 1971, p 42
52. Mustard JF, Murphy EA, Robinson GA, Rowsell HC, Ozge A, Crookston JH: Blood platelet survival. Thromb Diath Haemorrh 13:245–275, 1964
53. Mustard JF, Rowsell HC, Murphy EA: Platelet economy (platelet survival & turnover). Br J Haematol 12:1–24, 1966
54. Smythe HA, Ogryzlo MA, Murphy EA, Mustard JF: The effect of sulfinpyrazone (anturan) on platelet economy and blood coagulation in man. Can Med Assoc J 92:818–821, 1965
55. Packham MA, Warrior ES, Glynn MF, Senyi AS, Mustard JF: Alteration of the response of platelets to surface stimuli by pyrazole compounds. J Exp Med 126:171–188, 1967
56. Busch GJ, Reynolds ES, Galvanek EG, Braun WE, Dammin GJ: Human renal allografts—the role of vascular injury in early graft failure. Medicine 50:29–83, 1971

Miss Ina Ifrim provided valuable technical assistance.
Presented in part at the Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill, 1971.

[*Illustrations follow*]

All electron micrographs were taken from sections stained with uranyl acetate and lead citrate.

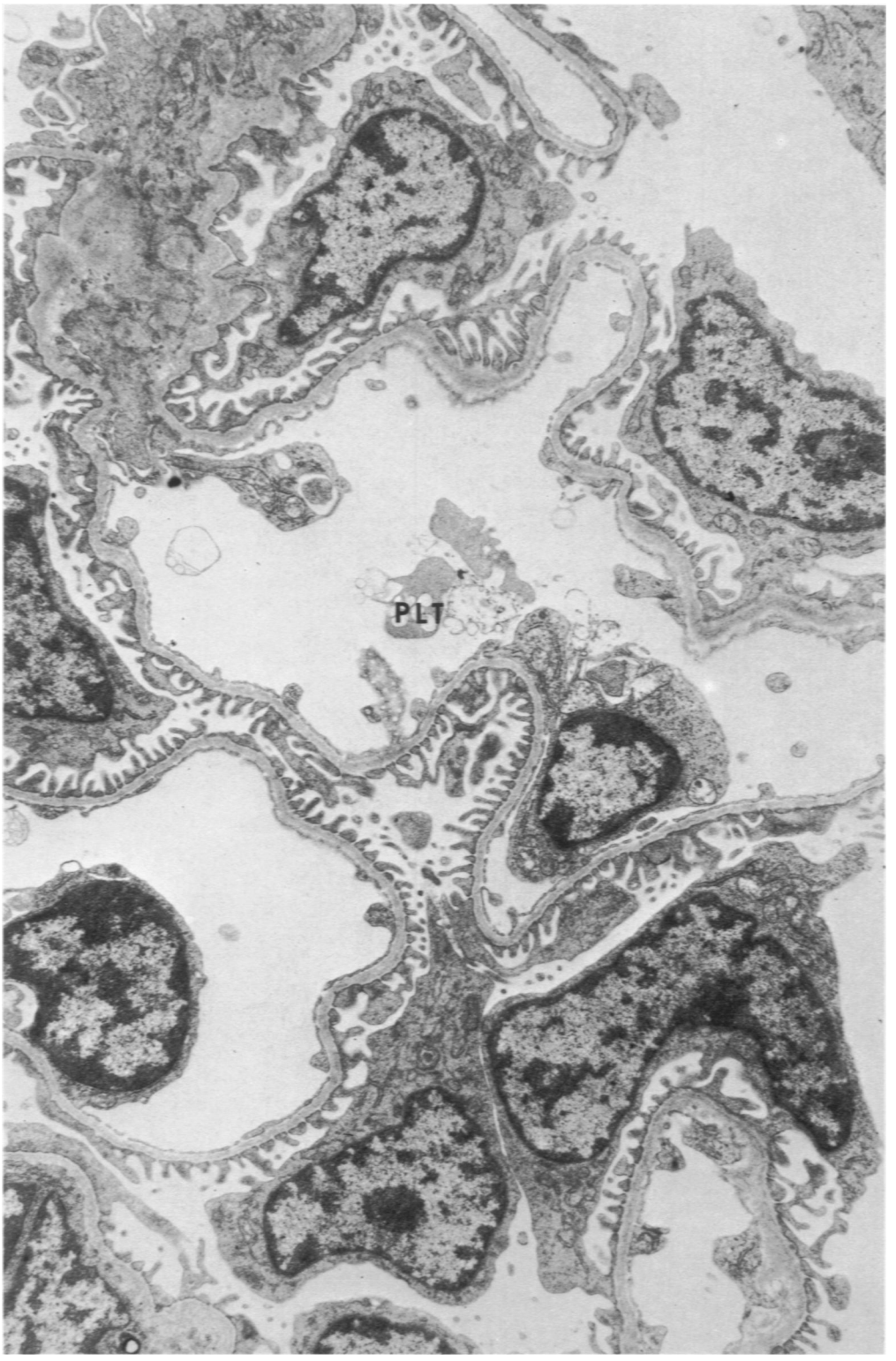

Fig 1—Glomerulus before unclamping of renal vessels, showing a few completely degranulated and degenerated platelets (*PLT*) (× 12,926).

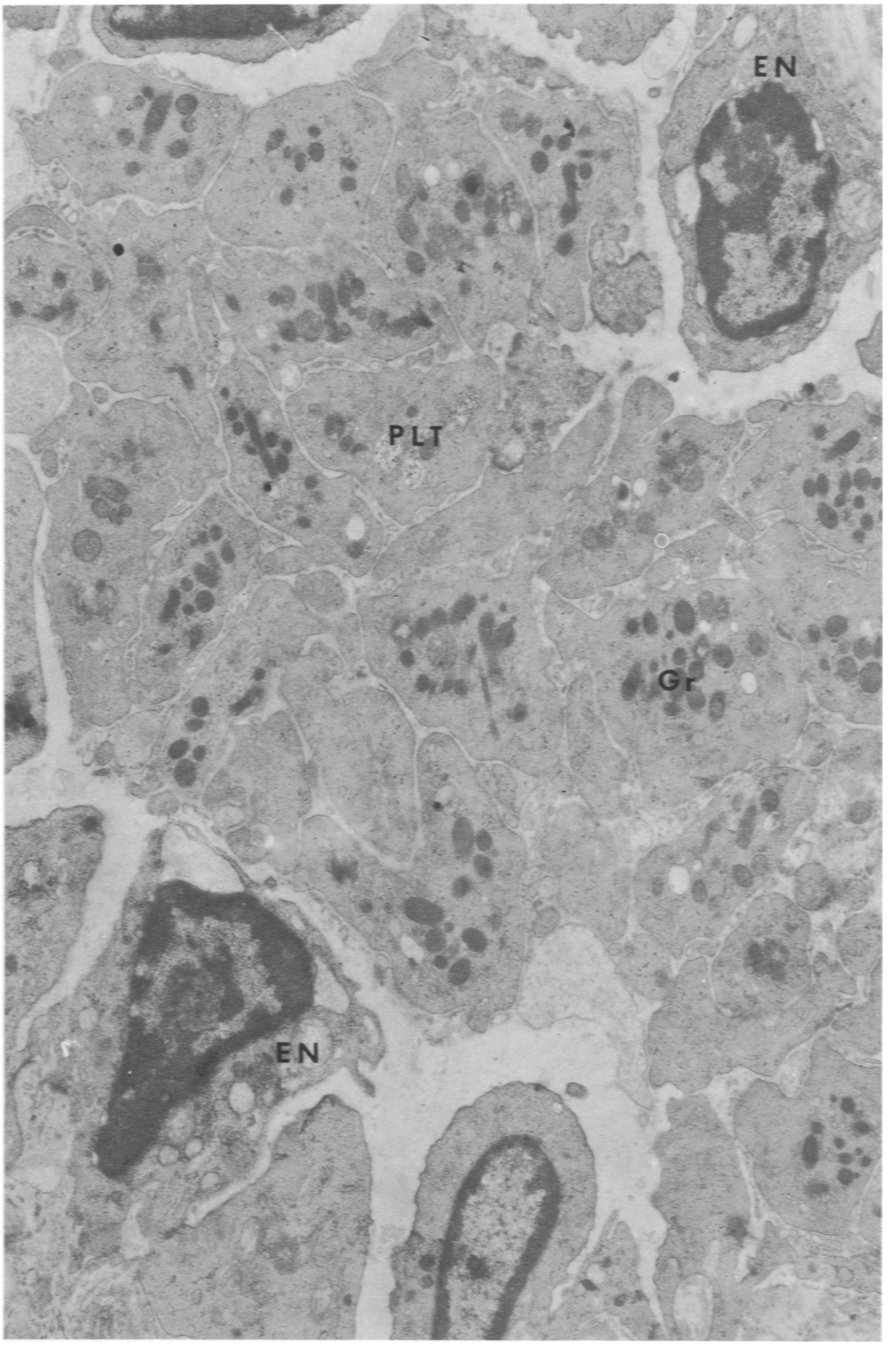

Fig 2—Arteriole in control kidney, 1 minute after unclamping of vessels (transplantation), shows aggregate of platelets (*PLT*). Most of the platelets have their granules (*Gr*) intact. *EN* indicates endothelium (× 28,700).

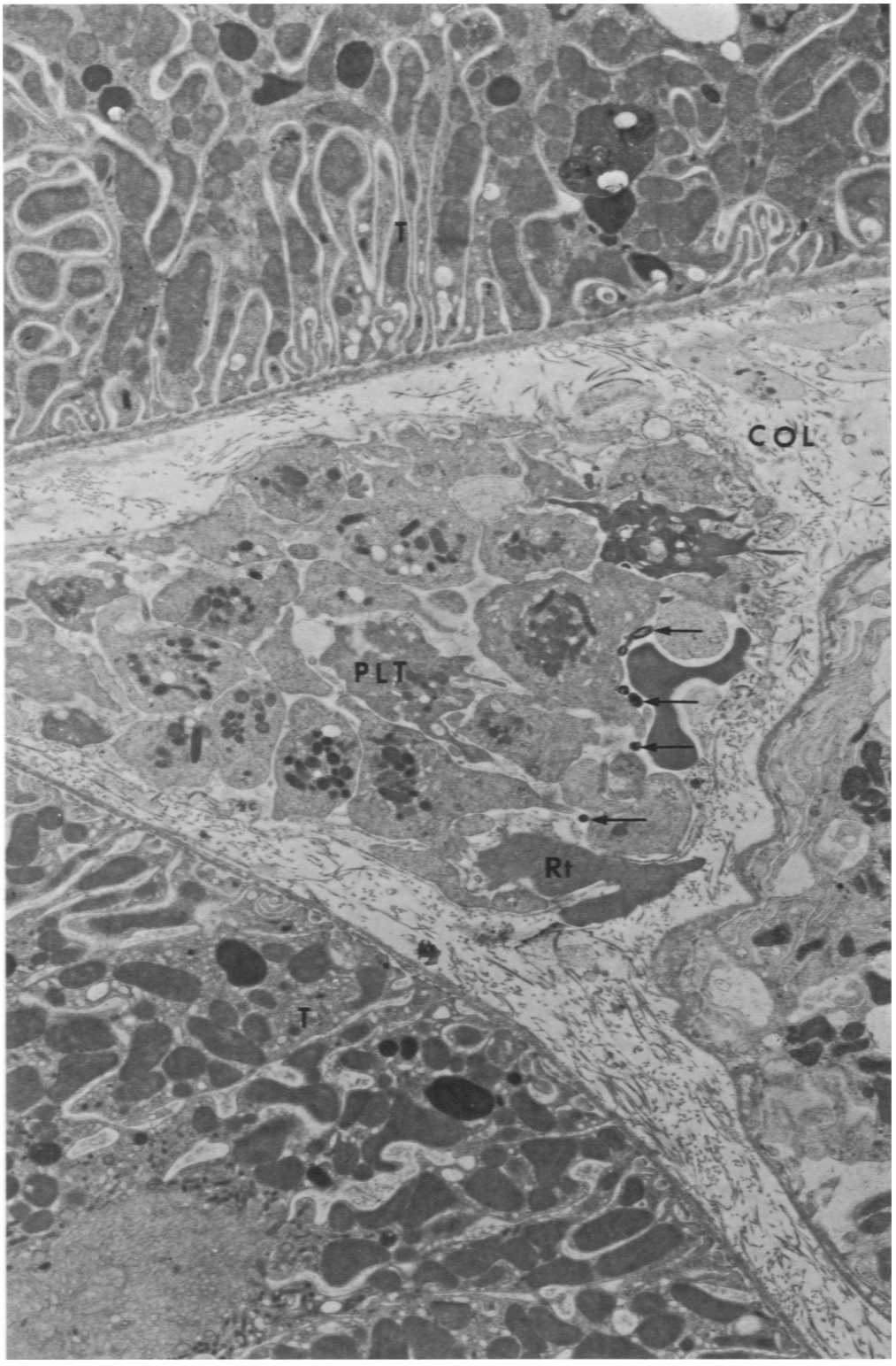

Fig 3—Peritubular capillary in control kidney 15 minutes after transplantation. The lumen is obliterated by platelets (*PLT*) which show active pseudopod formation and degranulation. Some of the granules are lying freely in the lumen (*arrows*). Note the area of vascular endothelial damage with a reticulocyte (*Rt*) extending out of the vessel. Collagen fibres (*COL*) in the interstitial tissue are widely separated by edema. *T* indicates tubule (× 9300).

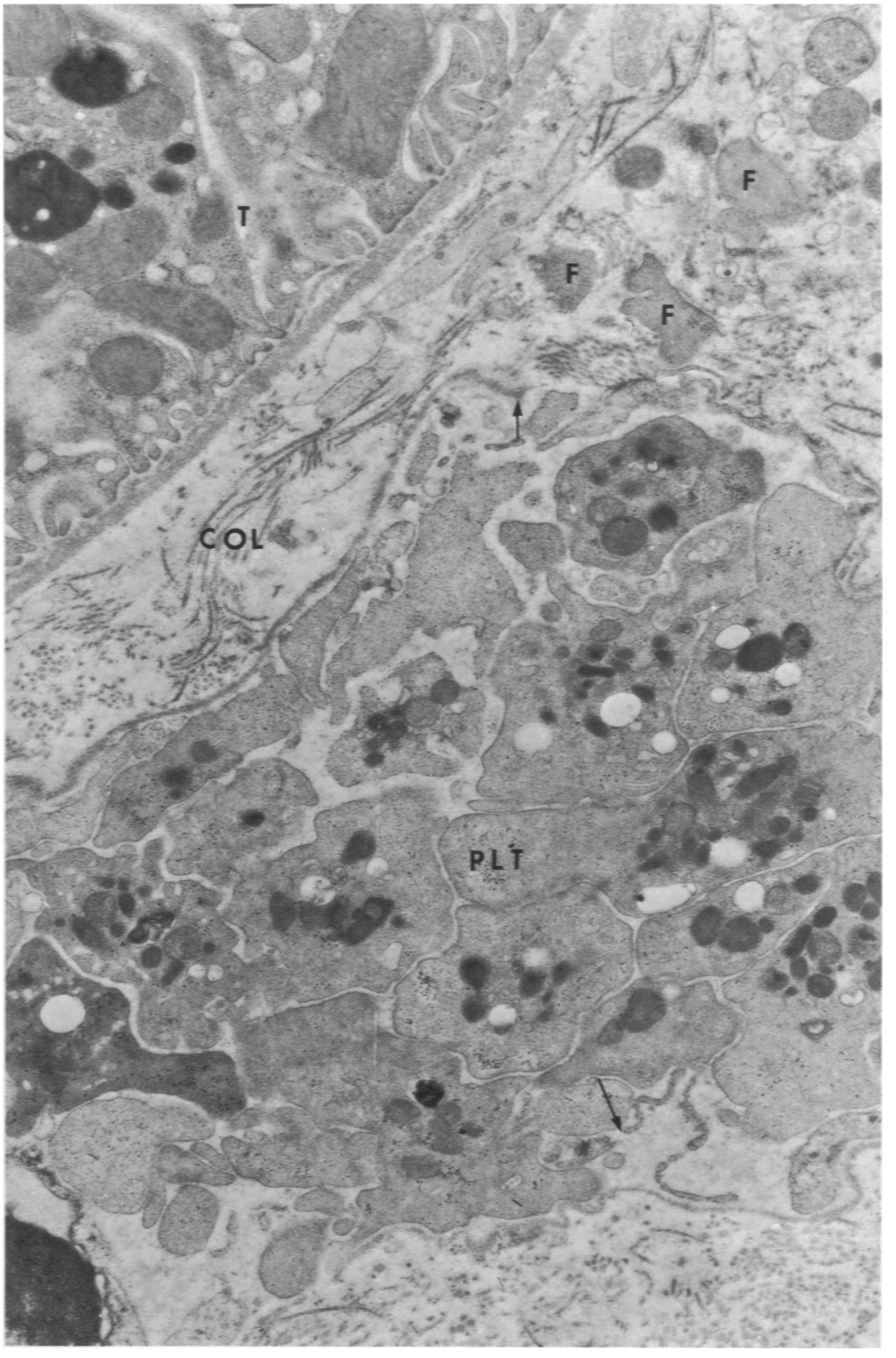

Fig 4—Peritubular capillary in control kidney 15 minutes after transplantation, capillary lumen is obliterated by platelet (*PLT*) aggregate. Many of the platelets at the periphery are degranulated. There is extensive vascular damage (*arrow*) and platelet pseudopod formation. Platelet fragments (*F*) are also lying outside capillary lumen. Note the interstitial edema. *T* indicates tubule (× 17,500).

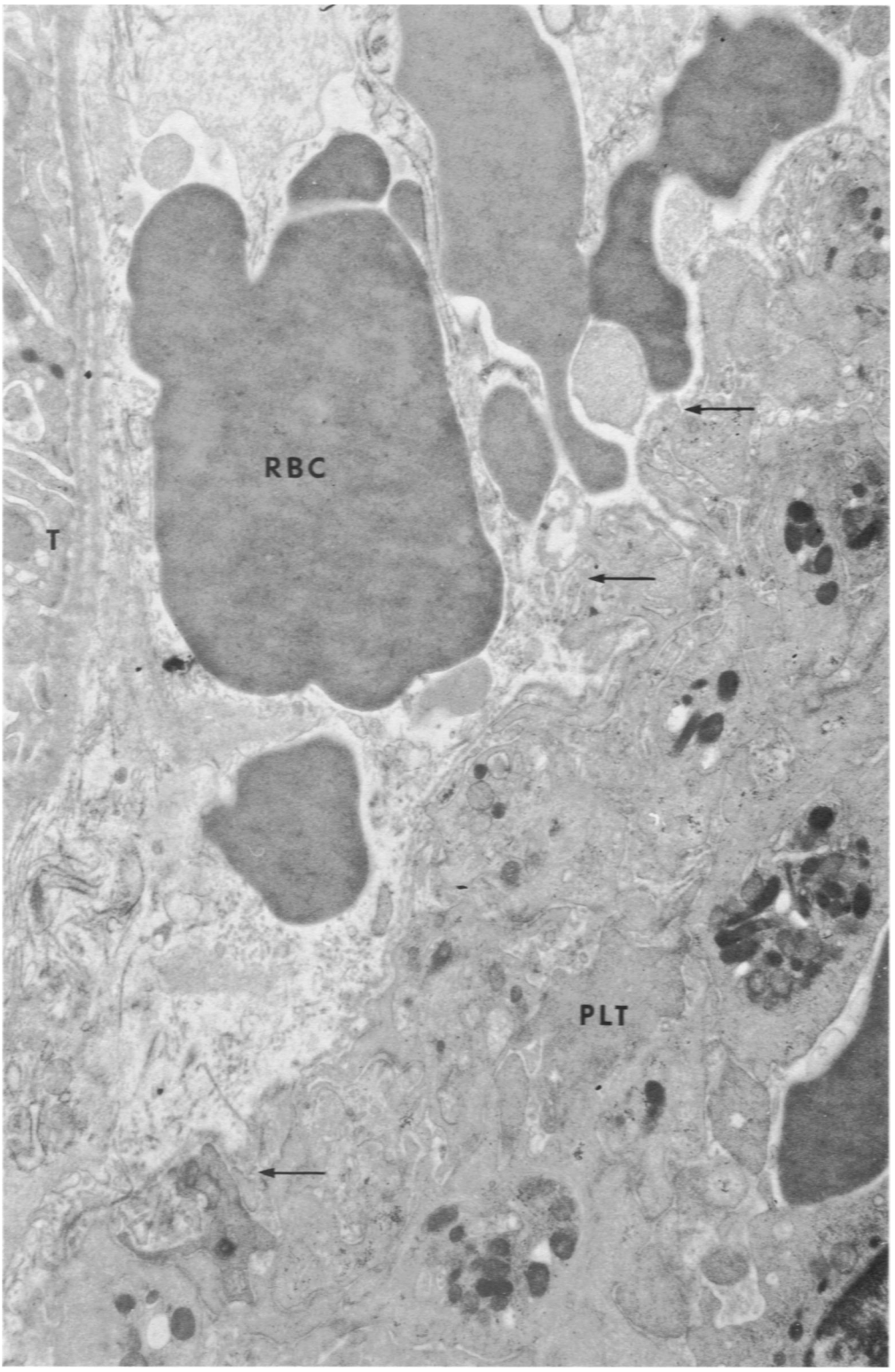

Fig 5—Peritubular capillary in control kidney, 10 minutes after transplantation, showing much more platelet (*PLT*) degranulation and red blood cells (*RBC*) in the interstitial space and area of vascular damage (*arrow*). *T* indicates tubule (× 15,548).

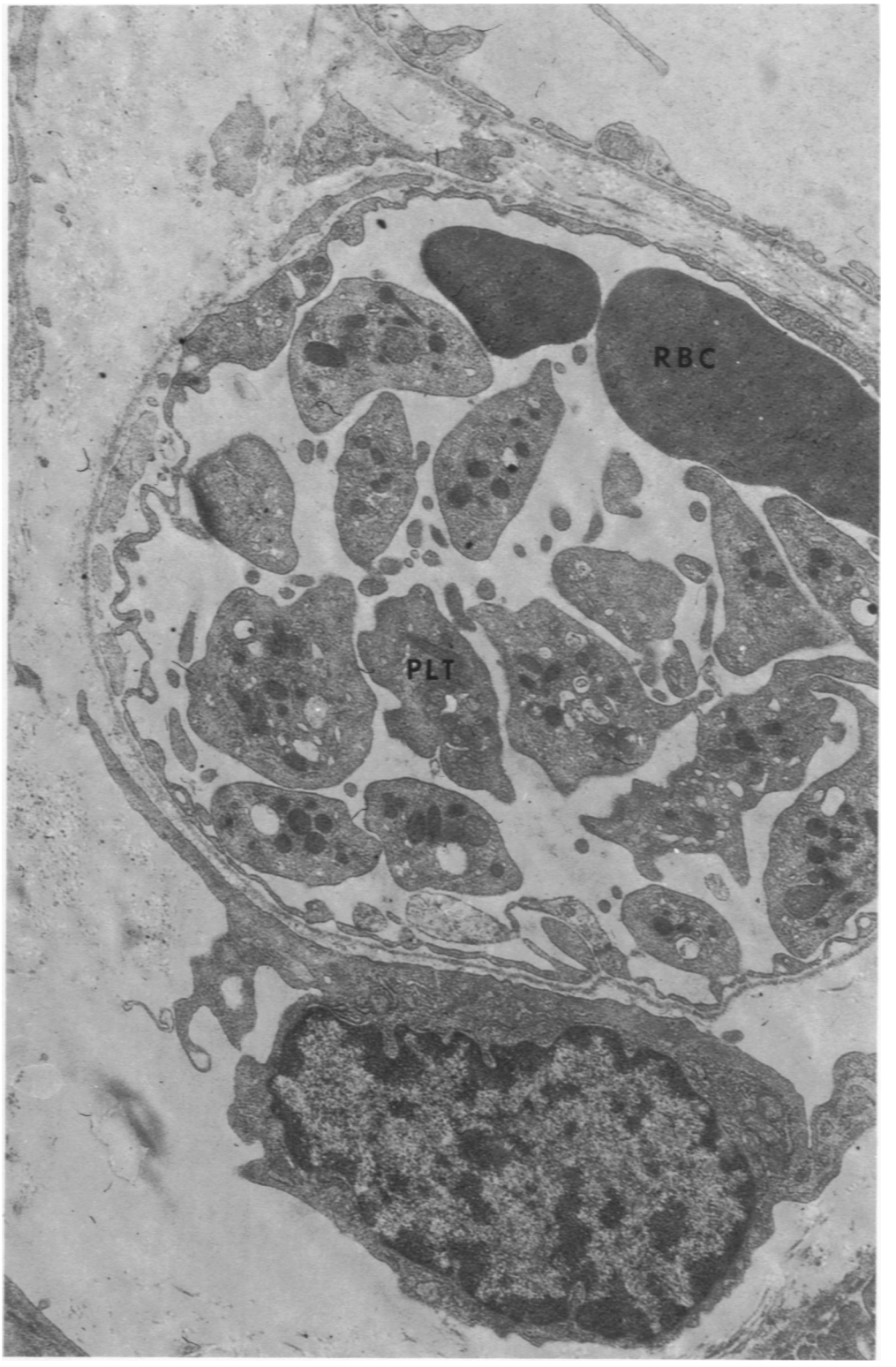

Fig 6—Peritubular capillary in treated kidney, 7 minutes after transplantation, shows loose platelet collection (*PLT*). Most of the platelet granules are intact. *RBC* indicates red blood cell (× 9600).