☐ Christian Leithner; Hildegunde Piza-Katzer, Second Department of Internal Medicine; Paul Kruzik, Department of Biology and Technical Microscopy; Maya Winter, Second Department of Internal Medicine; Wolfgang R. Mayr, First Department of Surgery; Herbert Stachelberger, Department of Blood Group Serology, School of Medicine, University of Vienna; Helmut Sinzinger, Second Department of Internal Medicine, Technical University of Vienna, Vienna, Austria

A Study of Hyperacute Rejection of Renal Transplants Using Scanning Electron Microscopy

The scanning electron micrographic features of hyperacute transplant rejection in a rat model are presented. Endothelial lesions can be identified as early as one minute after unclamping the vascular anastomoses. Earliest changes consist of adherence of platelets and fibrin fibers to the endothelium. At two minutes, fibrin networks are found which contain erythrocytes and platelets. These networks enlarge over a period of time resulting in complete renal arterial thrombosis at 30-60 minutes. It is concluded that the immunologically-caused endothelial lesions are followed by a localized intravascular coagulation.

The rare but important phenomenon of hyperacute rejection occurs immediately or soon after opening the vascular anastomoses. Within hours, the kidney becomes bluish-red, swollen, and necrotic. This event seems to be caused by preformed antibodies which circulate in the blood of sensitized recipients.[1] Experimental findings indicate that cellular immune mechanisms are also involved.[2]

The morphological alterations of this kind of rejection have been described by several authors using light and transmission electron microscopy.[2-5] A scanning electron microscopic study (SEM) which may provide further information has—as far as we know—not been published.[6] This technique seems to be useful to demonstrate the features of hyperacute rejection in a very obvious way.

Methods. Renal transplants were performed between inbred rats with a strong histoincompatibility.[7,8] The recipients were 20 male Lewis rats, the donors, 20 (Lewis x BN) f_1 rats. After presensitization by three consecutive skin grafts, serum samples were obtained from the recipients for lymphocytotoxic antibody assays. After flushing the donor kidneys with cold Collins' solution, transplantations were performed using microsurgical techniques.[9] One, two, three, five, seven, 10, 15, 30, 60, and 120 minutes after unclamping the vascular anastomoses, the animals were sacrificed by perfusion fixation with 2.5% gluteraldehyde solution (pH 7.4). An equal series of Lewis-to-Lewis renal isografts was handled in the same way. The kidneys were excised, washed in cacodylate buffer, and cut into small tissue samples. These were dehydrated, fractured under liquid nitrogen, critical point dried with CO_2 and sputtered with gold-palladium. SEM was performed on a Jeol 100-C.

Results. Before kidney transplantation, the sensitized rats had significant titers of cytotoxic antibodies in a range of 1:256–1:1024. These animals underwent hyperacute rejection following transplantation. One minute after opening the vascular anastomoses, the endothelium of the intrarenal arteries was granular and rough in appearance. Fibrin fibers and platelets could be ob-

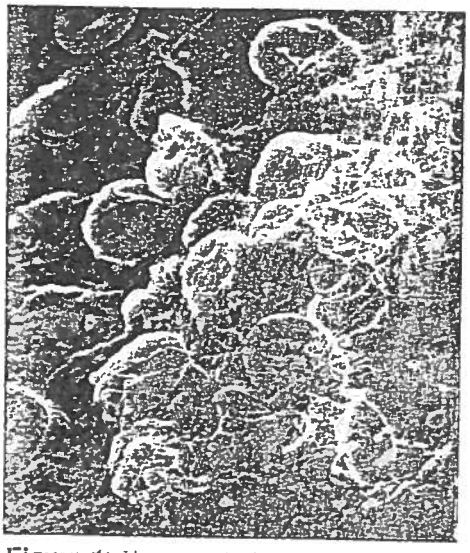

Figure 1: *Hyperacute transplant rejection (HTR) at minute 2; a fibrin network adhering to the arterial endothelium contains platelets and erythrocytes; original magnification x 5,000. (By permission of Pitman Medical Ref. No. 6.)*

Figure 2: *HTR at minute 2; platelets can be observed at the endothelium partly forming aggregates (top right); original magnification x 6,600. (E = erythrocytes.)*

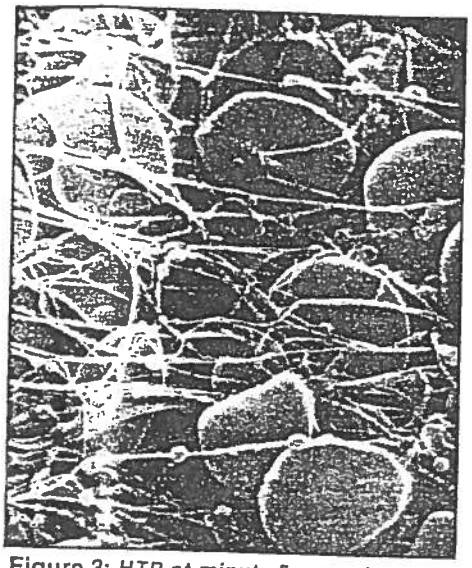

Figure 3: *HTR at minute 5; vascular wall is covered by tight fibrin network, containing erythrocytes; original magnification x 5,000.*

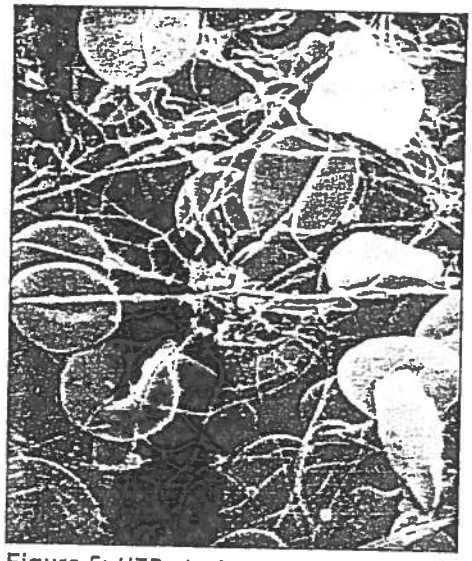

Figure 5: *HTR at minute 5; in the center of the fibrin net, a lymphocyte can be identified; original magnification x 4,000.*

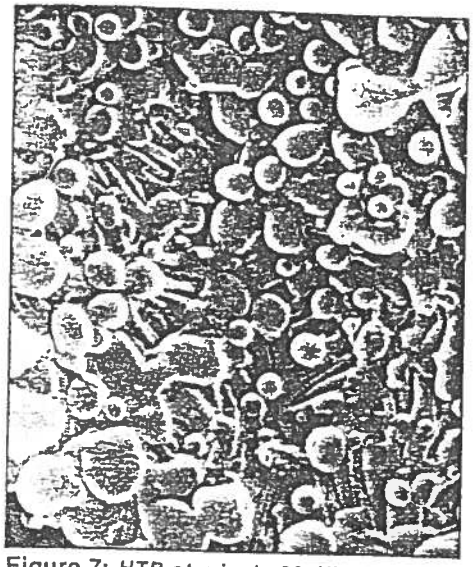

Figure 7: *HTR at minute 30. Visceral layer of Bowman's capsule with the podocyte processes is severely altered by hemispheric protrusions; original magnification x 10,000. (By permission of Pitman Medical Ref. No. 6.)*

sequed.

At two minutes the endothelial layer seems to be desquamating. Limited fibrin networks, in which platelets and erythrocytes were caught, were being found (*Figure 1*). The numerical relation between platelets and fibrin fibers varied markedly from one locale to another. The platelets were often observed in juxtaposition to erythrocytes, frequently forming aggregates (*Figure 2*).

At three minutes, the number of fibrin fibers and platelets was further increased. Larger intrarenal arteries showed a pleat of the intimal layer which could be interpreted as a consequence of vasoconstriction. At five minutes, the vascular walls were covered with a tight fibrin network which contained erythrocytes (*Figures 3–5*), platelets (*Figure 4*), platelet aggregates, and different kinds of leukocytes, including lymphocytes (*Figures 5, 6*). Within the glomerular capillaries, the thrombotic alterations developed the same way. At 10 and 15 minutes, the intravascular lesions were further developed. Complete thrombotic occlusion progressed from the cortex to the hilus, which resulted in total kidney thrombosis at 30 or 60 minutes.

After 30 minutes, the visceral layer of Bowman's capsule lost its regular structure. The podocyte processes were altered by hemispheric protrusions, presumably manifestations of severe dystrophy (*Figure 7*).

In contrast to the aforementioned allografts, the isotransplanted kidneys (Lewis-to-Lewis) had a normal, rosy color after opening the anastomoses and soon began to produce urine. SEM revealed rare minor arterial lesions. This could be regarded as a sequel of ischemia. Glomerular capillaries showed the typical, normal fenestrated endothelium (*Figure 8*). The visceral layer of Bowman's capsule was unaltered also and consisted of normal, fern-like podocytes (*Figure 9*).

Discussion. In hyperacute rejection, contact occurs between preformed antibodies of the recipient and the endothelium of the transplant immediately after renal perfusion is established. This induces an activation of the complement system[10,11] and results in severe endothelial lesions. Following this, there is a localized intravascular coagulation.[12-14] These harmful events are intensified by vasoconstriction and cellular immune mechanisms.[2] On the other hand, activation of the plasmin and kallikrein system can be regarded as protective, albeit unsuccessful, mechanisms.[13,15]

Under experimental conditions, hyperacute rejection can be modified very little. Heparin and platelet inhibitors have been tested, as well as other sub-

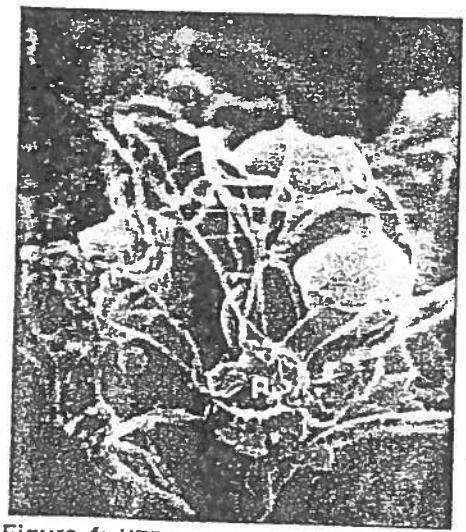

Figure 4: *HTR at minute 5; one deformed erythrocyte (E) and two platelets (P) in the fibrin network; original magnification x 10,000.*

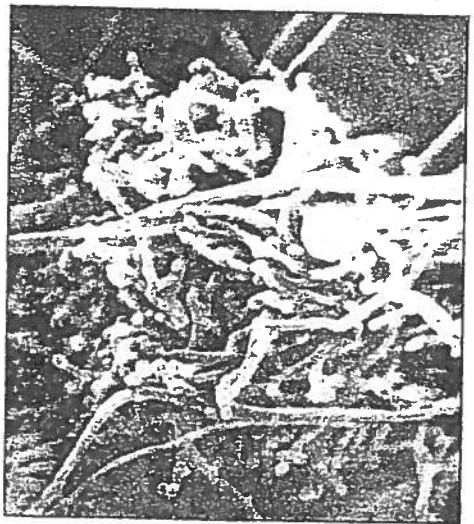

Figure 6: *Higher magnification (x 20,000) of the same lymphocyte which shows a surface rich in microvilli.*

Figure 8: *Isotransplantation (Lewis-to-Lewis) at minute 30, normal endothelium of a glomerular capillary showing the typically fenestrated structure; original magnification x 40,000.*

stances such as cytosine arabinoside, methylprednisolone, azathioprine, and phenoxybenzamine.[13,15-17] The best results have been achieved by using anticomplementary compounds such as sodium citrate and cobra venom factor.[10,11,18] In spite of all these endeavors, we are far from having a successful therapy which can be applied to patients.

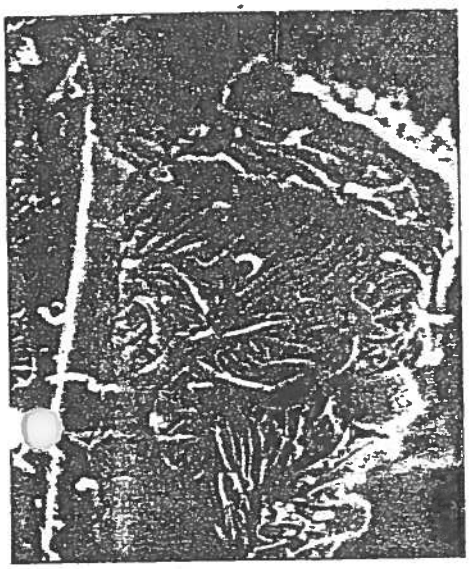

Figure 9: *Isotransplantation at minute 30, normal structure of podocytes of the visceral layer of Bowman's capsule. Compare this normal surface with the severely altered one of Figure 7 occurring in HTR; original magnification x 10,000.*

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DIALYSIS TECHNICIAN

Decreasing the Possibility of Aerosol-Borne Hepatitis

Jim McCammon, B.S., C.H.T.
Chief Technician
Fort Sanders Kidney Center, Inc.
Knoxville, Tennessee

Can hepatitis be spread by aerosol? The answer depends on who you talk to; most tests have been inconclusive or insignificant. Due to risk in the dialysis clinic setting, it was decided to minimize the sources of blood aerosol in our facility. Steps taken to minimize risk included reducing needle sticks into blood lines, covering all needles with 4 x 4s when withdrawing a needle from a blood line, discontinuation of venting used needles with a syringe into the air, and, last, changing our take-off and rinsing procedure.

Prior to this, we used a saline-air rinse. The blood would be followed with the desired amount of saline (usually 200 cc), then air would follow the saline. When the air reached the venous trap, the venous line was clamped below the trap to stop the flow of blood-saline mix. Then air was vented out of the trap with a needle and syringe to bleed as much of the blood-saline mixture as possible from the kidney. The venting of the air which had been inside the blood circuit into the atmosphere was the step which was considered to be the highest risk in exposing staff and patients to the aerosol.

We used this procedure because we found it the most efficient way of returning the maximum amount of blood to the patient. We were aware that the proximity to the printed residual blood volumes, as given by dialyzer manufacturers, is solely dependent on the take-off procedure. Several procedures were tried to find the one which would maximize the RBC return without increasing the amount of saline being given. An alteration to our original procedure was finally chosen as the method of choice. The take-off procedure is as follows:

- Clamp venous monitoring line.
- Clamp arterial needle.
- Turn blood pump off.
- Clamp arterial blood tubing line and disconnect from arterial access line.
- Turn blood pump on.
- Unclamp arterial blood line, then reclamp when the blood reaches the saline "Y."
- Open saline line and allow desired amount (150–200 cc) of normal saline to infuse. After infusing this amount, disconnect saline line from saline "Y" and allow air to be drawn in.
- While saline is infusing, disconnect venous monitoring line and discard transducer filter.
- Clamp and unclamp venous line several times to allow a slight buildup of pressure inside the kidney.
- Clamp venous line below the drip chamber when air enters the trap and before it empties of blood completely.