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COAGULATION PROBLEMS IN TRANSPLANTED ORGANS

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HUMORAL ANTIBODIES, BLOOD CELLS AND COAGULATION IN HYPERACUTE OR ACCELERATED RENAL HOMOGRAFT AND HETEROGRAFT REJECTION

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In recent years, increasing numbers of human renal homografts have been lost by hyperacute rejection. Although much has been learned about the complication, several aspects of its pathogenesis have remained unclear, either because of seemingly contradictory reports in the clinical literature or because of insufficient experimental information about the interlocking roles of humoral antibodies, coagulation, and formed blood elements in the process of destruction. Consequently, this paper will attempt to clarify the existing state of knowledge about hyperacute rejection. In so doing, considerable dependence will be placed upon investigations carried out at our institution on dogs following their deliberate immunization,[3, 30] after presumed or proved accidental sensitization of humans to antigens shared by their donors[34, 35] and after heterotransplantation to recipients who possess natural antidonor antibodies.[9, 10] Under all these circumstances, there has been evidence that coagulation is involved in the events of organ destruction.

THE ROLE OF PREFORMED ANTIBODIES

ABO Incompatibility

The first clear examples of hyperacute rejection of renal homografts were in patients who received kidneys from ABO blood group incompatible

donors.[32, 33] An effective blood flow to some of these transplants was not restored when the vascular anastomoses were opened (Fig. 7-1). The small vessels of the excised kidneys were demonstrated by angiography to be

Figure 7-1. Renal homograft from patient with A+ donor and transplanted to O+ recipient. The organ was removed three hours after revascularization. Note dark color and hemorrhagic zone at corticomedullary junction. (By permission of *Surg. Gynec. Obstet.*, *118*:819, 1964.)

closed (Fig. 7-2), and histopathologically, the arterioles and capillaries were plugged with formed blood elements, particularly erythrocytes (Fig. 7-3). A rational although partial immunologic explanation was available since the blood group substances which allow red cells to be typed are also found in other tissues including kidneys.[12, 37] Consequently, if the kidney

of an A, B, or AB donor were placed in a patient whose serum contained naturally occurring anti-A and/or anti-B isoagglutinins (an example would be a recipient with O blood type who would have both kinds of isoagglutinins), these antibodies might be predicted to bind with the renal red cell antigens. Serologic studies in some of our cases showed that falls in systemic isoagglutinin titers actually occurred.[32] Subsequent authors[24, 29, 40] have reached similar conclusions about the role of red cell isoagglutinins in precipitating accelerated rejections.

ABO Compatibility

It is unlikely that future organ transplantations will be carried out under the foregoing adverse conditions of ABO mismatching. However, hyperacute rejection in the presence of red cell group *compatibility* has been seen with increasing frequency and, in fact, this kind of rejection has become the chief cause of acute homograft loss in most major transplantation centers. The first case was described by Terasaki, Marchioro, and Starzl[38] in a patient whose serum contained lymphocytotoxic antibodies that killed donor cells. The authors speculated that in the course of being transfused prior to operation, the recipient had been immunized to white cells that shared histocompatibility antigens with the eventual renal donor. Since then, no one has seriously challenged this general hypothesis of presensitization. The concept has been indirectly supported by the high rate of hyperacute rejection with retransplantation in patients whose first homografts were rejected and who were thereby presumably immunized to some antigens also present in the second graft.

Subsequently, Kissmeyer-Nielsen and his associates[14] and many other authors[7, 22, 34, 39, 42, 43] have confirmed the adverse implications of preformed antidonor antibodies as detected with several techniques. The most commonly employed methods have measured lymphocytotoxins and leukoagglutinins, but the most sensitive examination has been said by Williams[43] and Milgrom[15, 20] to be the mixed agglutination test.

While certain tests may be more sensitive than others for the detection of the preimmunized state, no single antibody has yet been found to have unique predictive significance. In our laboratories,[3, 30] deliberate sensitization of dogs by repeated skin grafts led to the formation of a variety of antibodies, each with antidonor activity. However, the titer of these antibodies was often not well correlated with the rapidity of rejection of a kidney from the skin donor. Moreover, it has been emphasized in reports of clinical cases[34, 35] that hyperacute rejection which is presumably due to presensitization may occur even though antidonor antibodies cannot be found with any currently available technique, including the mixed agglutination method. Under these latter circumstances it has been necessary
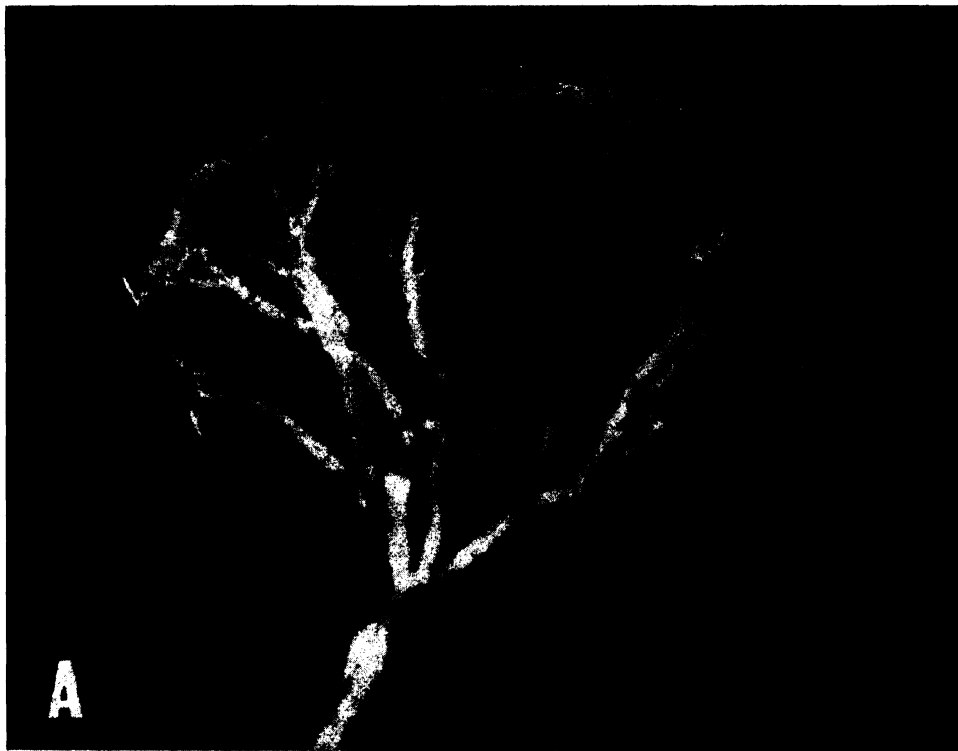
to assume[34,35] that an immediate, albeit undiscernible, immunologic reaction is the initiating event in the destructive process that follows.

As was speculated in an earlier publication,[34] it is conceivable that either an obvious or unobvious antigen-antibody union could occur within or outside the homograft. However, subsequent investigations from our laboratory[30] and elsewhere[4,26] have suggested that the precipitating immunologic events of hyperacute rejection probably almost always occur within the transplant. With or without demonstrable antibodies in the recipient serum, the immunoglobulin and/or complement depositions in the transplants may be in such small quantities that their specificity as judged by strictly morphologic criteria in immunofluorescence studies could be open to question,[34,35] even though on other grounds it is reasonable to believe they are significant. In Figure 7-4B is shown an example. Although this kidney was hyperacutely rejected, the quantities of immunoglobulins were not particularly striking. However, there was easily detectable complement.

Since it is often difficult to see immunoglobulins in hyperacutely rejected kidneys studied by immunofluorescence, it is not surprising that arteriovenous gradients of antibodies across these organs do not always become detectable. Nevertheless, in many animal experiments in which canine recipients are presensitized to donor tissues, gradients can be seen.[5,30] Moreover, if two successive organs are placed from the same donor there may be enough absorption of antibodies by the homografts to cause a significant reduction of the systemic titers (Fig. 7-5). As will be mentioned again below, the same kinds of falls of white cell and platelet counts are even more regularly observed (Fig. 7-5).
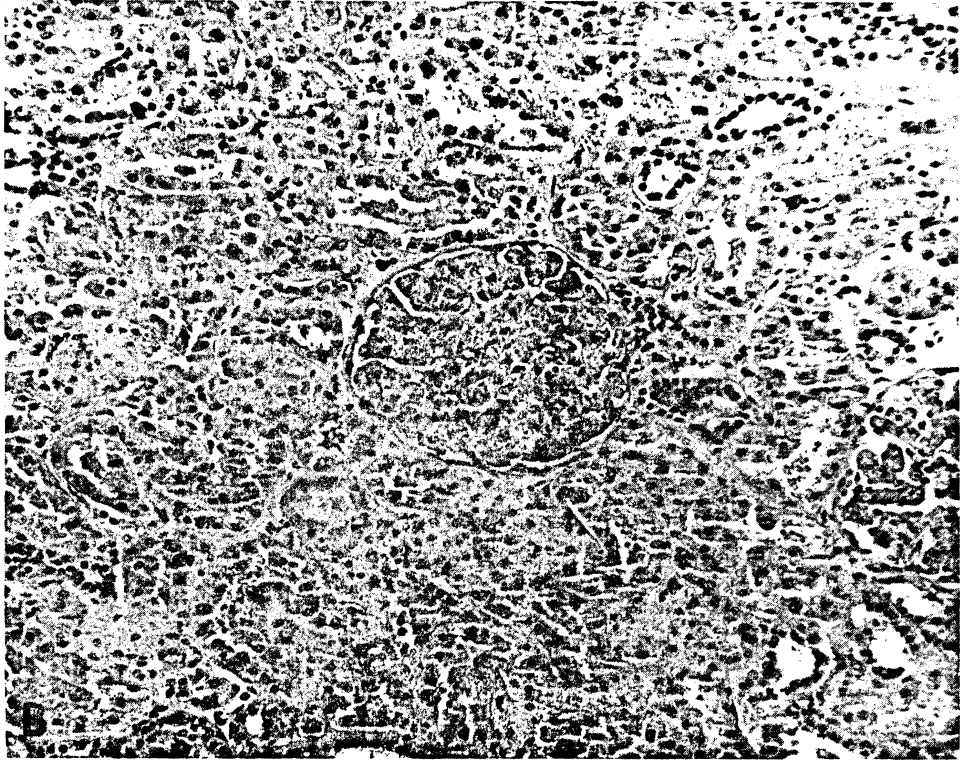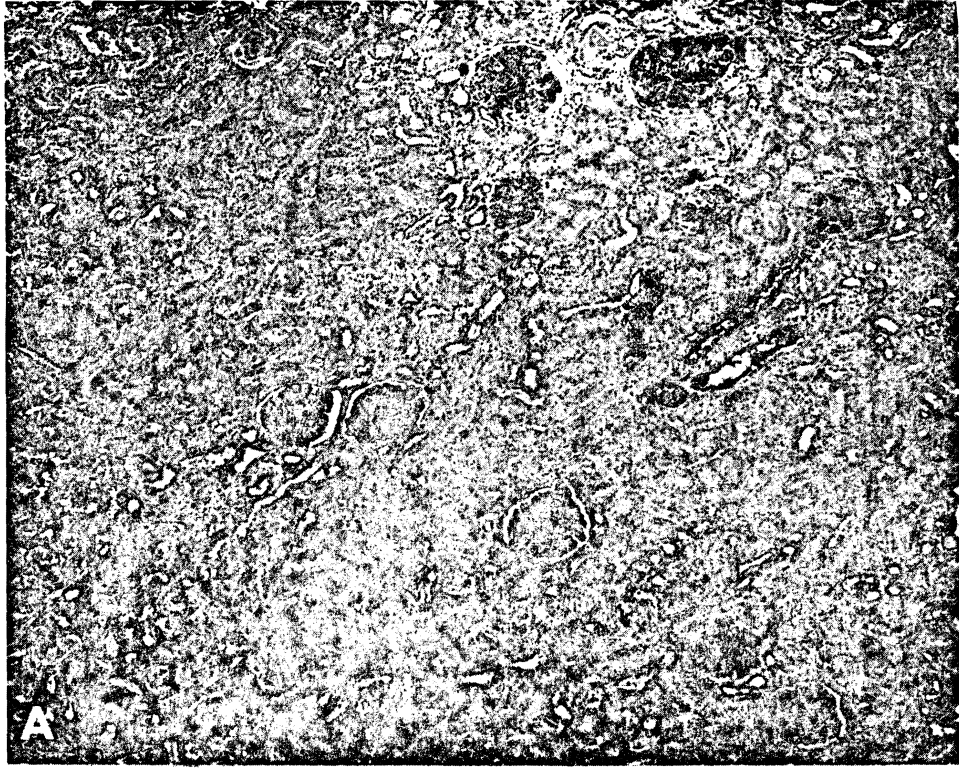
FORMED BLOOD ELEMENTS

A simplistic view of hyperacute rejection might be that the antidonor antibodies discussed in the preceding section were destructive of renal homografts by their direct nephrotoxicity. The observations already cited in the ABO incompatible cases were not consistent with such a conclusion since the most obvious lesion in the rapidly repudiated kidneys was occlusion of their blood supply, apparently by formed blood elements.[32,33]

In cases with red cell compatibility, there was also evidence of sequestra-

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Figure 7-2. Homografts removed a few hours after revascularization. Both recipients were O+ blood type and the donors were A+ and B+ respectively. The arteriogram in A is from the same specimen as shown in Figure 7-1, and the dye staining at the corticomedullary junction corresponds to the hemorrhagic area seen in the gross specimen. The cortical devascularization in B is not so extreme. (By permission of *Surg. Gynec. Obstet.*, 118:819, 1964.)

tion of formed blood elements, since white cells, platelets, and red cells constituted a morphologically prominent component of the vascular plugs in hyperacutely rejecting renal homografts. Williams *et al.*[12] were the first to draw attention to the dramatic appearance of polymorphonuclear leukocytes (PMN's) in such kidneys. Their observations, since amply confirmed,[22,34] were made possible by systematic biopsies of homografts about one hour after revascularization. In some instances the PMN's appeared before any other histopathologic findings were evident. That the participation of these cells in the ultimate destruction was not immunologically specific was illustrated by the canine experiments of Clark[4] and Robertshaw,[26] which showed that autologous PMN's could be effective intermediaries of hyperacute rejection following exposure to the antigraft antibodies of hyperimmune serum.

More recently, observations of actual arteriovenous gradients across hyperacutely rejecting renal homografts have been made in humans[35] and in presensitized dogs.[3,30] In both species, there was unequivocal evidence of entrapment of leukocytes and platelets, the major sequestration occurring within a few minutes after revascularization (Fig. 7-6). After 30 to 60 minutes, the arteriovenous gradients sometimes became reversed so that the concentrations of white cells and platelets leaving the organ now were higher than those in the arterial blood (Fig. 7-6). The interpretation of the latter finding was that these initially sequestered formed blood elements were subsequently leaving the transplant.

COAGULATION

Within the last three years, it has been accepted that the essential physiologic event of hyperacute rejection is abrupt devascularization of the homograft. When Kissmeyer-Nielsen described the histopathology of two hyperacutely rejected kidneys,[14] he noted that the glomerular capillaries and the arterioles were full of microthrombi, making the morphologic features indistinguishable from those of a generalized Shwartzman reaction. Similar observations were made in our own first cases.[34] The diagnostic changes were well seen with light microscopy, using the appropriate tissue stains. They consisted of fibrin deposits in the small vessels (Fig. 7-7), usually accompanied by a consequent cortical necrosis.[34] With immunofluorescent examination, it was confirmed that fibrin was a prominent component of the obstructing intravascular thrombi (Fig. 7-4A). Although

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Figure 7-3. Histologic appearance of the two unsuccessful homografts seen in Figure 7-2. *A* and *B* are in the same order. Note aggregation of red cells, particularly in glomeruli and in small arteries. *A*. H & E, × 32. *B*. H & E, × 80.

these histopathologic findings suggested that coagulation changes had occurred, clotting studies were not available to determine if the alterations


7-4A

$\longrightarrow$

were systemic or if they were confined to the actual homograft. The first published efforts to obtain such information were completely negative.[7, 25]

More recently, evidence has been published from our institution indicating that coagulation changes are an integral feature of hyperacute rejection, in the presensitized canine model[3, 30] as well as in man.[35] In the dogs which were exposed to multiple skin grafts from the eventual organ donor, the subsequently transplanted kidney, spleen, or liver always consumed clotting factors and platelets locally, in the same way as shown in Figure 7-6 for a human kidney. One of the objectives of these animal investigations was to see if transplantation of consecutive organs from the same

donor would mitigate the rejection of the second graft. It was found that the second transplant was briefly protected, possibly by the prior deple-
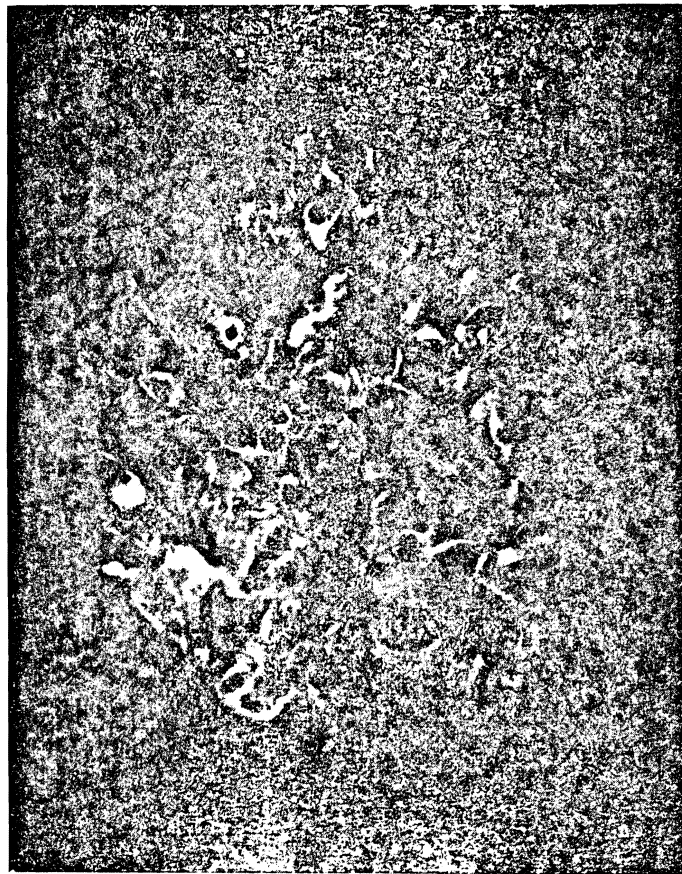
7-4B

Figure 7-4. Glomerulus from a hyperacutely rejected kidney which was removed 146 minutes after its revascularization. × 250. A. Stained with fluorescein-labeled antihuman fibrinogen showing irregularly shaped deposits with glomerular capillary lumens. B. Stained with fluorescein-labeled antihuman C_3 ($\beta_1 C$ globulin) showing patchy deposits in an irregular distribution along the glomerular basement membrane.

tion of either humoral antibodies, clotting factors, or formed blood elements (Fig. 7-8). In time, however, the final organ suffered the same fate as the first one.

All of the sensitized canine recipients in the above study developed evidence of local consumption. In addition, a minority of animals also had
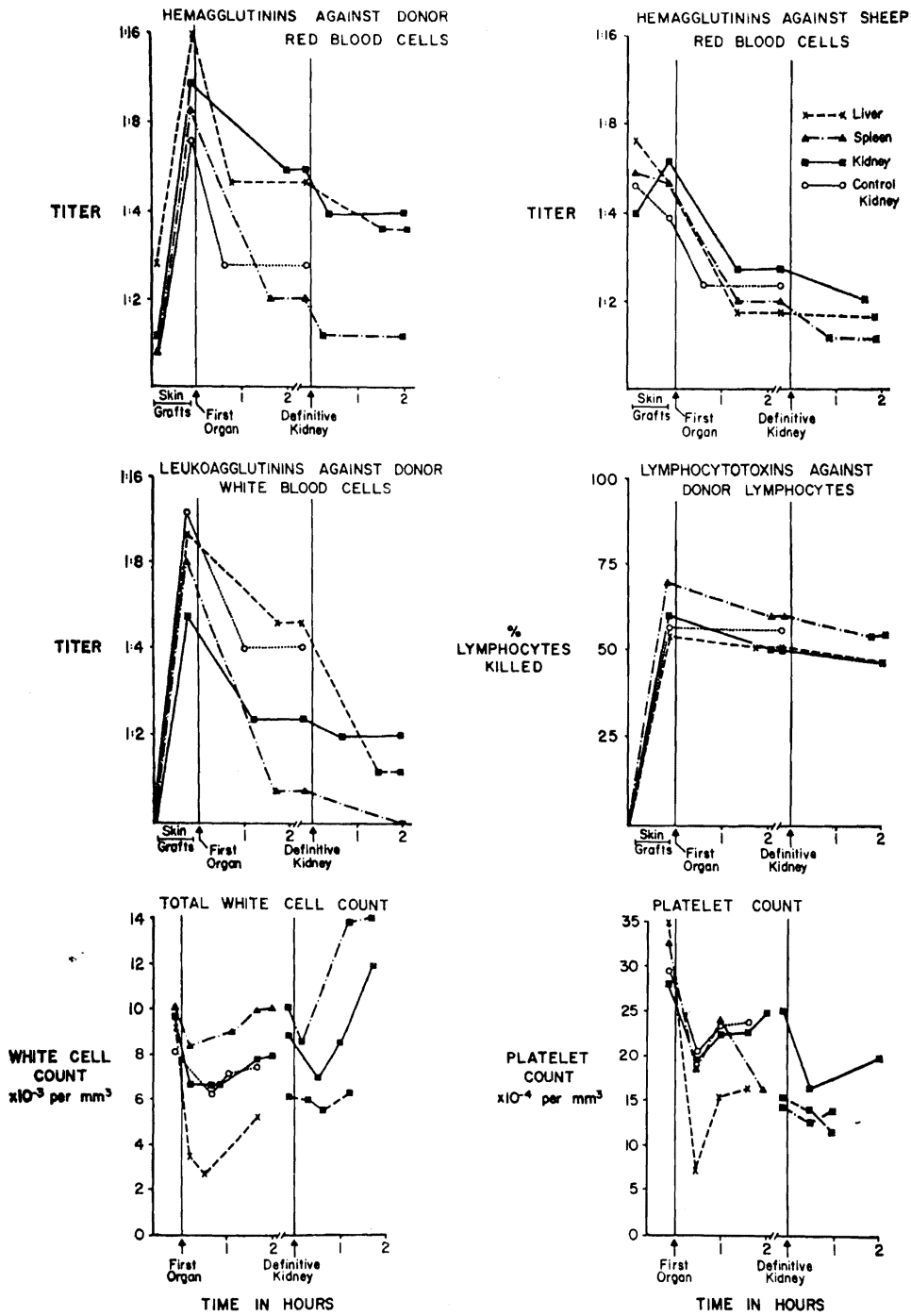
Figure 7-5. Changes in humoral antibodies, peripheral white cell counts, and platelet counts during the period of multiple skin grafting and at the time of a first and second whole-organ transplantation from the same donor. Codes for the different kinds of first organs (kidney, spleen, and liver) are identified in the upper right graph. All second organs were kidneys. (By permission of *Surgery, 68*:77, 1970.)

profound systemic coagulation changes which were like those of disseminated intravascular coagulation (DIC).[18, 19] The same kinds of observations
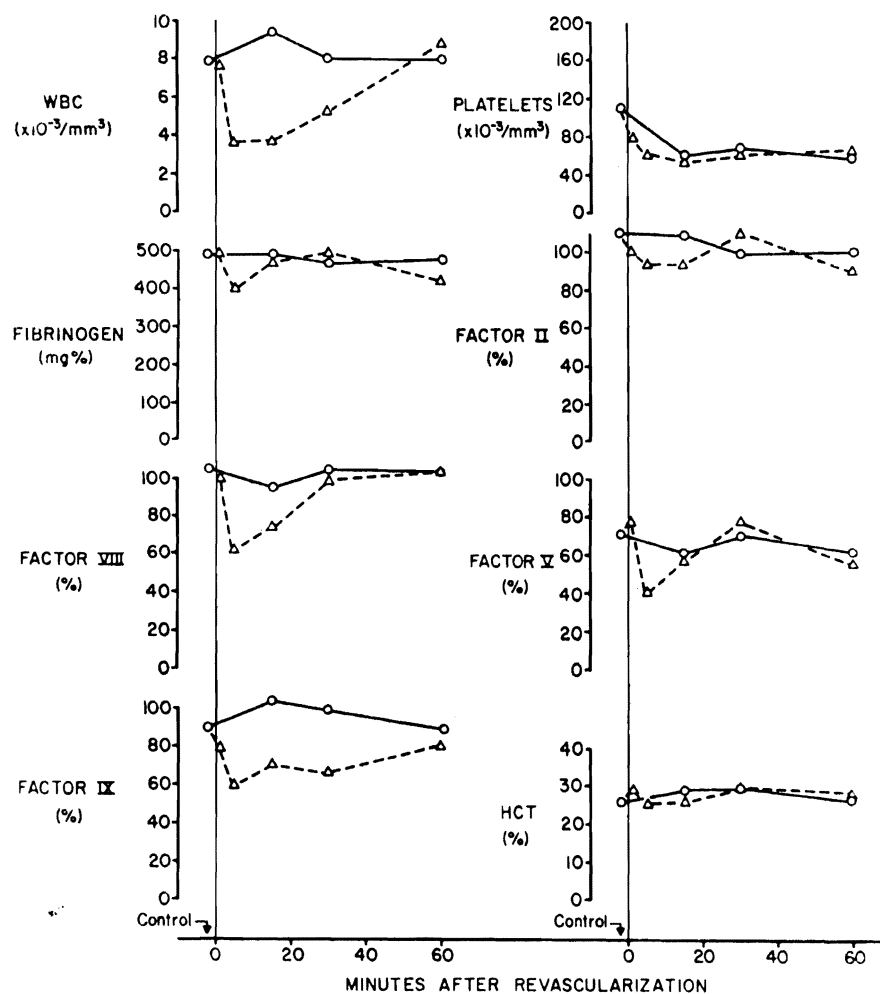

Figure 7-6. Hematologic and coagulation changes in renal arterial (solid lines) and renal venous (dashed lines) blood after the extracorporeal transplantation of a child kidney. Note the local consumption but with no detectable systemic effect. (By permission of *New Eng. J. Med.*, 283:383, 1970.)

have been made on patients after renal homotransplantation with a consequent severe or even fatal bleeding diathesis.[35] Thus, although the clotting aberrations of hyperacute rejection are usually confined to the graft insofar as can be measured, there is now little reason to doubt that profound systemic changes may follow.

Although systemic coagulation changes in either dogs or humans occur in a small minority of cases, recognition of this complication is important
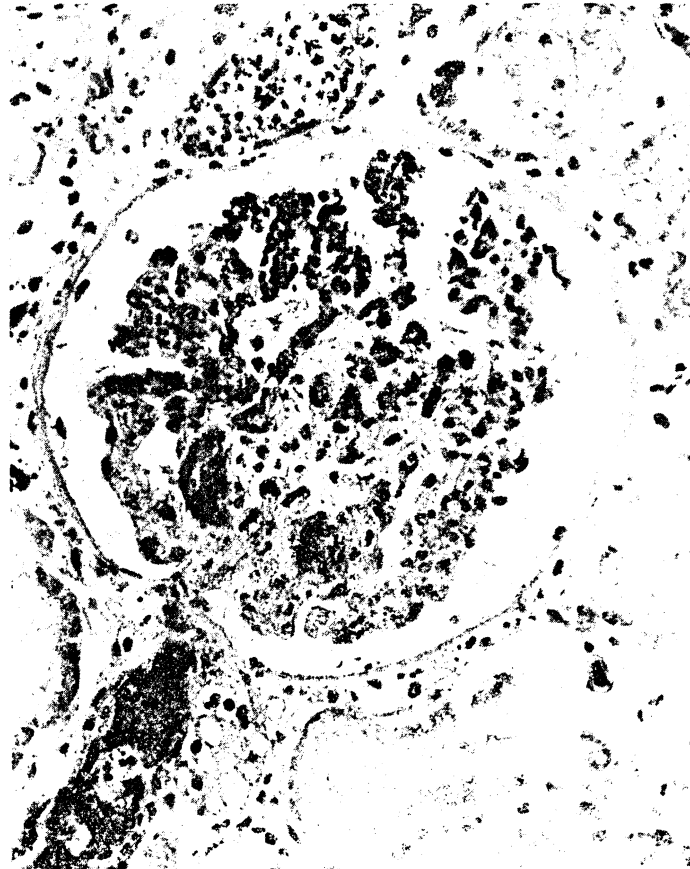

Figure 7-7. Glomerulus from a hyperacutely rejected renal homograft. Phosphotungstic-acid–hematoxylin stain. Note the fibrin deposits in the afferent arteriole and glomerulus.

because of its potentially lethal nature.[35] In Figure 7-9 is shown the course of a patient who died of the consequences of a bleeding diathesis which developed within a few hours after renal transplantation. Changes occurred that were diagnostic[19] of disseminated intravascular coagulation (DIC). There was severe consumption of clotting factors as well as fibrinolysis. Ultimately a major bleeding diathesis ensued. Further depletion of the various clotting factors continued during the next day until heparin was administered. As the bleeding diathesis developed there were also gross signs of

pathologic clotting, including the appearance of large necrotic patches of skin on the chest and abdomen and the disappearance of pulses in the right

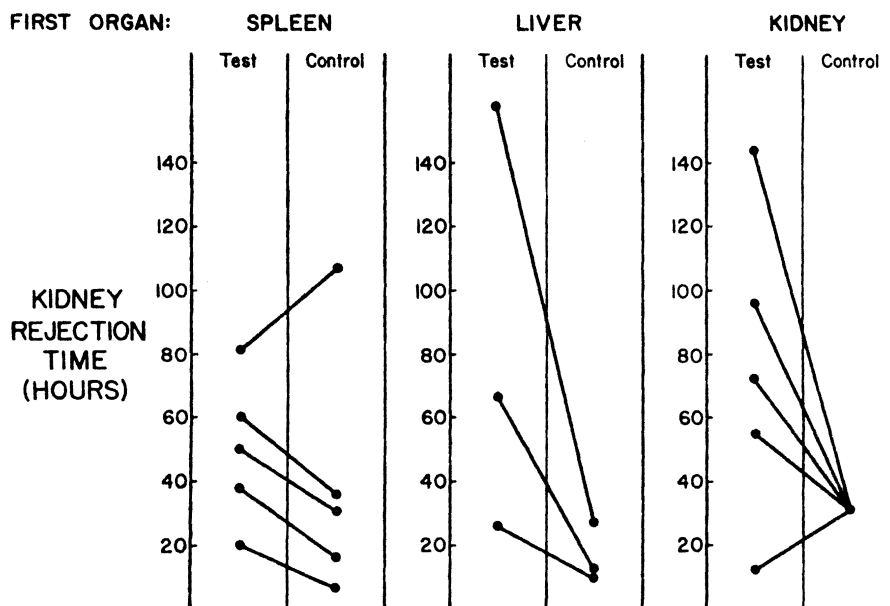REJECTION TIMES OF MATCHED PAIRS OF KIDNEYS FROM SAME DONOR

Figure 7-8. Functional times of kidney homografts after transplantation to sensitized unmodified recipients (control) compared to the functional intervals when kidneys were inserted into comparably sensitized recipients but as second organs following donor spleens, livers, or contralateral kidneys. Note that the secondarily transplanted kidneys usually functioned longer than the first kidneys, but that the prolongation was very transient. (By permission of *Surgery, 68*:77, 1970.)

arm and the left leg with pregangrenous changes in the distal portions of these extremities. The findings at reoperation and at autopsy, two and four days respectively after transplantation, were also consistent with the diagnosis of disseminated intravascular coagulation with sizable fibrin deposits in the liver and associated midzonal hepatic necrosis and with coagulative necrosis of the gastric mucosa. This case and another similar one have been reported in detail.[35] Myburgh et al.[22] and Moriau[21] have also seen apparent examples of DIC in human transplant recipients.

INTERRELATIONSHIPS

To date it must be conceded that most of the information about hyper-acute homograft rejection has been restricted to observations of events
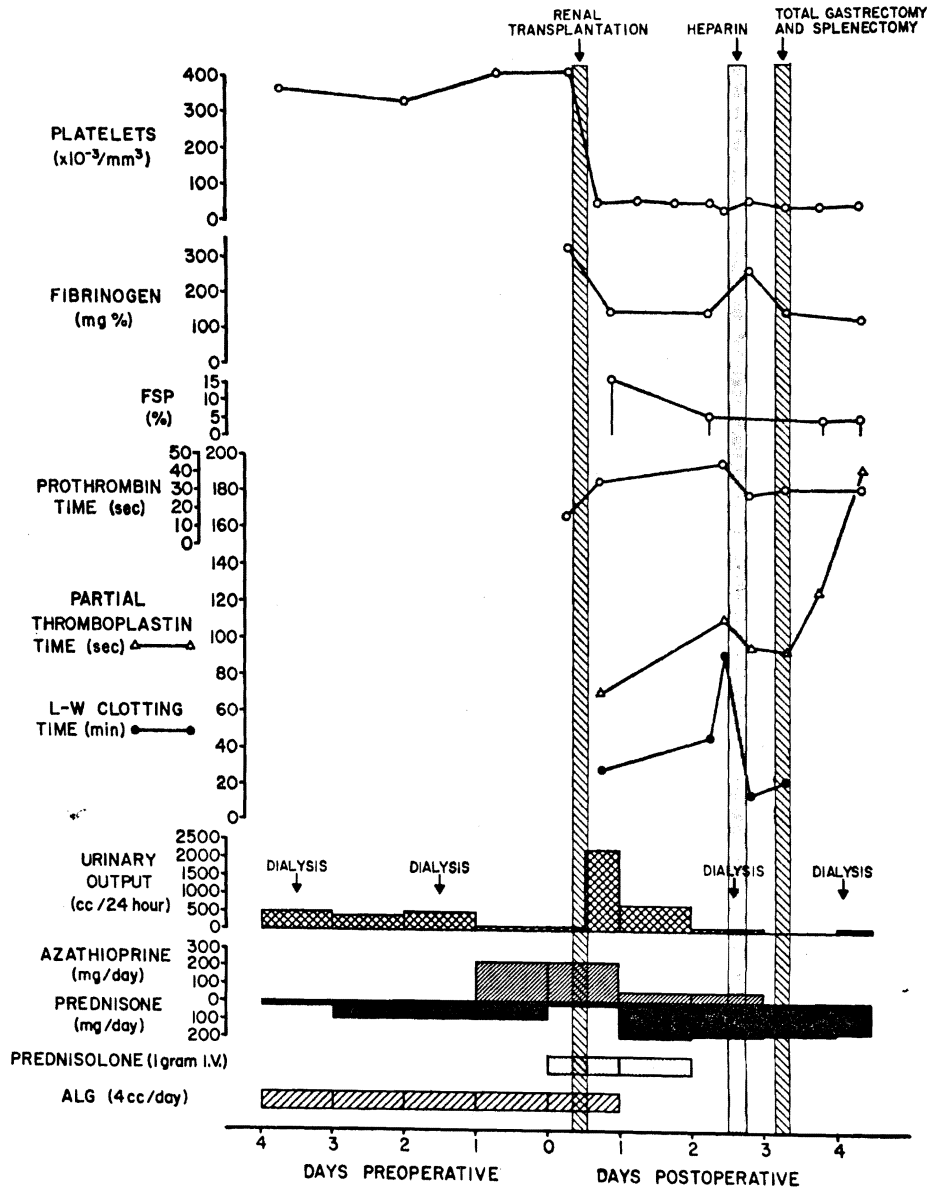

Figure 7-9. Urinary output, immunosuppressive treatment, and coagulation studies in a 27-year-old recipient whose renal homograft underwent a violent early rejection. The patient developed a disseminated intravascular coagulation, fibrinolysis, and a bleeding diathesis. FSP: fibrin split products. (By permission of *New Eng. J. Med.,* 283:383, 1970.)

rather than to a real definition of the pathogenesis. The sequestration in the homografts of antigraft humoral antibodies, platelets, white cells, and clotting factors is an established fact. With this kind of information it is not difficult to envision likely mechanisms by which the process of hyperacute rejection could be initiated immunologically and then carried to completion with the collaboration of formed blood elements and coagulation. Since the preformed antibodies appear to react with antigens in the transplants, clotting could be induced directly by the antibody-antigen reaction [1, 11, 25] or by the consequent tissue injury. In addition, antibody-antigen reactions attract PMN leukocytes via C' activation.[41] These white cells appear capable of inducing clotting [13] and are an important if not essential intermediary in the causation of the thrombosis seen in the local [36] and generalized [13] Shwartzman reactions.

Unfortunately, efforts to dissect out the sequential events that are implied by the foregoing comments have not been successful. In our attempts to separate hyperacute rejection into antibody, formed blood element, and coagulation phases,[3] renal homografts were transplanted to six presensitized dogs. Arteriovenous gradients were then determined across these kidneys at very frequent intervals starting 30 seconds after revascularization. Insofar as could be determined, the removal of the various substances occurred simultaneously (Fig. 7-10). This observation cannot be taken as evidence against the initiation of the process by immunologic means. It probably signifies only that the steps transpire so quickly that it is beyond the ability of the experimental method to separate them.

An interesting observation was made in the experiments described above.[3] The greatest arteriovenous differences of all the various measured substances developed within a few seconds to a few minutes (Fig. 7-10) and then diminished in the next half hour. During this time, it was not possible to be certain that hyperacute rejection was occurring since, grossly, the organs still had a circulation. Nevertheless, fibrin deposits could be seen with immunofluorescence examination of biopsies (Fig. 7-11). Later, as the organs underwent obvious gross rejection, these gradients became very much smaller or sometimes were absent altogether (Fig. 7-10). Nevertheless, special isotope studies of platelet and fibrinogen half-life indicated that consumption of these clotting substances was continuing at a very accelerated rate in spite of the failure to detect this fact with simple gradient measures. With increased consumption of fibrinogen products, there was often also evidence of fibrinolysis, especially within the transplants. The resulting unstable situation (with coincident fibrin deposition and removal) may have accounted for the differences in fibrin layering that were sometimes observed in serial biopsies from the same kidney (compare A and B of Fig. 7-11).

With an increased understanding of the pathogenesis of hyperacute rejection, it may become possible to evolve effective techniques of therapy. Such developments have become increasingly needed as more and more

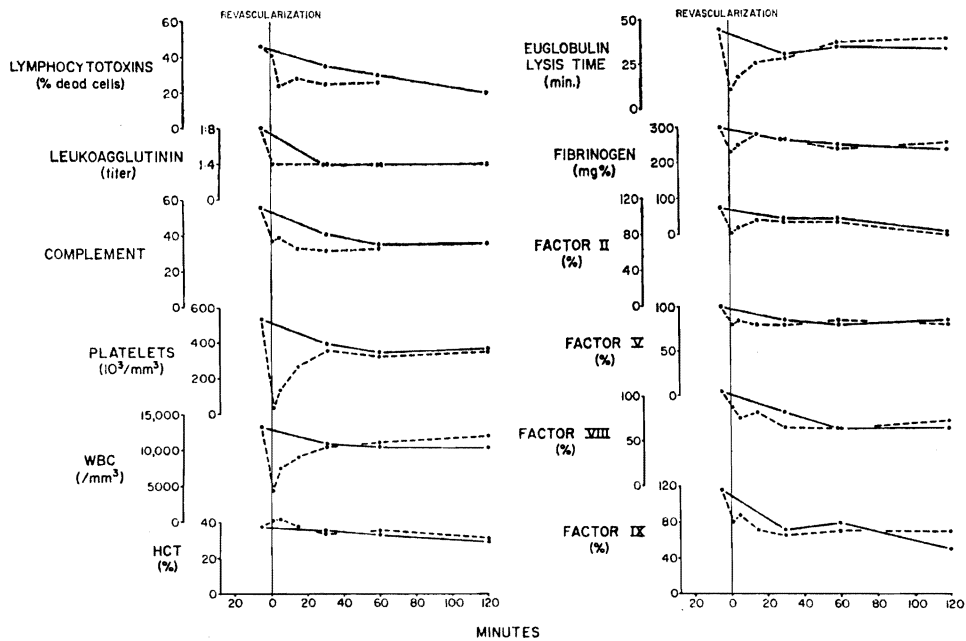RENAL HOMOTRANSPLANTATION TO A PRESENSITIZED CANINE RECIPIENT

Figure 7-10. Homograft A-V gradient studies after renal homotransplantation to a dog which had been sensitized to donor skin and kidney tissue. Arterial values are represented by solid lines; the venous results are shown in dashed lines. Note the gradients of clotting factors, antibodies, platelets, and white cells. The apparent intrarenal concentration of red cells reflected by transient rise in hematocrit has been seen much more clearly after heterotransplantation between divergent species.[8]

potential recipients have become noncandidates for transplantation by virtue of their presensitization. The most extreme example has proved-to be the patient who has rejected a first or second homograft and who has developed antibodies against essentially all members of the human population.

Two directions of inquiry would seem worthwhile pursuing. First, it may be useful to interfere with the coagulation process as was speculated upon several years ago.[34] Recently, MacDonald and his associates[17] published evidence that this approach could be valuable under some circumstances. In hypersensitized dogs, they were regularly able to prevent immediate destruction of renal grafts by the simple expedient of prophylactic

total-body heparinization. Other means of interfering with the clotting process have not been systematically investigated under similar experimental conditions.
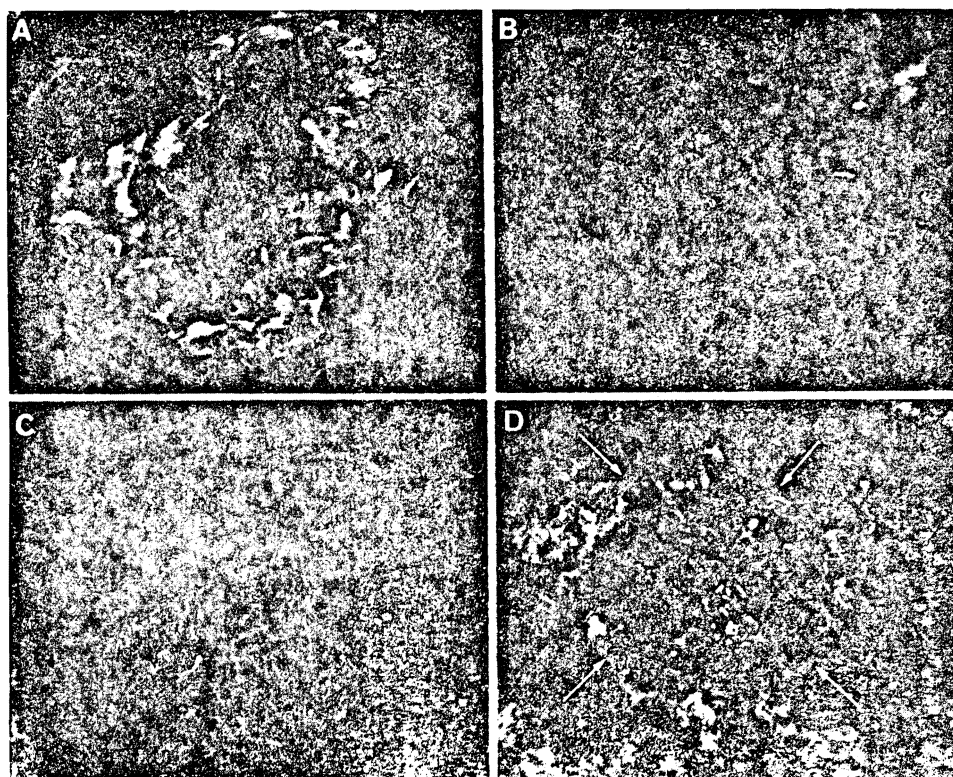

Figure 7-11. The patterns of renal fibrin deposition detected by fluorescein conjugated rabbit antidog fibrin. Original magnification × 400. *A.* A glomerulus from a sensitized dog studied ten minutes following vascularization of the second of two renal homografts from a common donor. Moderate amounts of fibrin are present in an irregular pattern along the glomerular capillary walls. *B.* A glomerulus from the same kidney as in *A,* 60 minutes after vascularization. Most of the fibrin which was seen at ten minutes has disappeared. *C.* A glomerulus (ten minutes) is shown from a homograft placed in a nonsensitized control dog. A small amount of fine, irregular fibrin is deposited along the glomerular capillary walls. This was the most extensive fibrin deposition seen in any of a series of control homografts transplanted to nonsensitized recipients. *D.* A glomerulus (arrows) and the surrounding renal tissue are shown from a homograft, 24 hours after placement in a sensitized dog. Moderate glomerular and heavy peritubular fibrin deposits are evident.

The logical alternative approach would be to eliminate the preformed antibodies, an undertaking which is not practical at present. That the principle may be sound is indicated by the prolongation of both homografts[30]

and heterografts[9] that has been obtained by transplanting successive organs from the same donor. Presumably, the protection to the final graft was achieved by absorbing the antibodies on the first (or screening) organ. In addition, mitigation of heterograft rejection has been described after removal of immunoglobulins by plasmapheresis.[2] The possible relevance of such heterotransplantation experiments is discussed below.

HYPERACUTE HETEROGRAFT REJECTION

In recent years, it has been thought on the basis of indirect evidence that the violent rejection occurring after heterotransplantation between divergent species was initiated by the action of preformed heterospecific antibodies. Support for the hypothesis included the fact that antidonor antibodies of several kinds were often demonstrable by preoperative *in vitro* testing of the recipient animal's serum;[4, 23] that such antibodies were cleared by organs transplanted from this donor;[5, 6, 8, 16, 23] that the vascularization of successive kidneys from the same donor (or donors of the same species) usually prolonged the function of the last organ, presumably by antibody depletion,[6, 16] and that physiochemical removal of immunoglobulins[2] or the inactivation of complement[8, 31] in the recipient sometimes increased heterograft survival.

It has been of considerable interest to compare the events of hyperacute heterograft rejection with those which lead abruptly by unquestionably immunologic mechanisms to the destruction of homografts that are placed into recipients deliberately sensitized to donor tissue (see earlier). The observations have been so similar in each circumstance that progress in ameliorating hyperacute rejection would be expected to be applicable to both situations. For example, after pig to dog transplantation, Rosenberg *et al.*[24] as well as Giles of our own group[9] have shown the sequestration of platelets and clotting factors in patterns quite analogous to those after homotransplantation under conditions of sensitization. In this species combination, Giles[9] has also demonstrated the entrapment of leukocytes of heterospecific antibodies in whole-organ grafts (Fig. 7-12). The same kinds of observations were made by Giles[9] after dog to pig grafting of livers, spleens, and kidneys.

Summary

Hyperacute rejection has been described on the basis of studies in human and canine recipients of renal homografts. This complication ordinarily is a manifestation of a presensitized host state. The events of the abrupt homograft repudiation involve sequestration by the transplanted organ of antibodies, platelets, white cells, and clotting factors and the consequent occlusion of the vessels of the graft. Although the factors contribu-

tory to hyperacute rejection have been well defined, the precise mechanism of the destructive process remains obscure. In particular, the pathogenetic
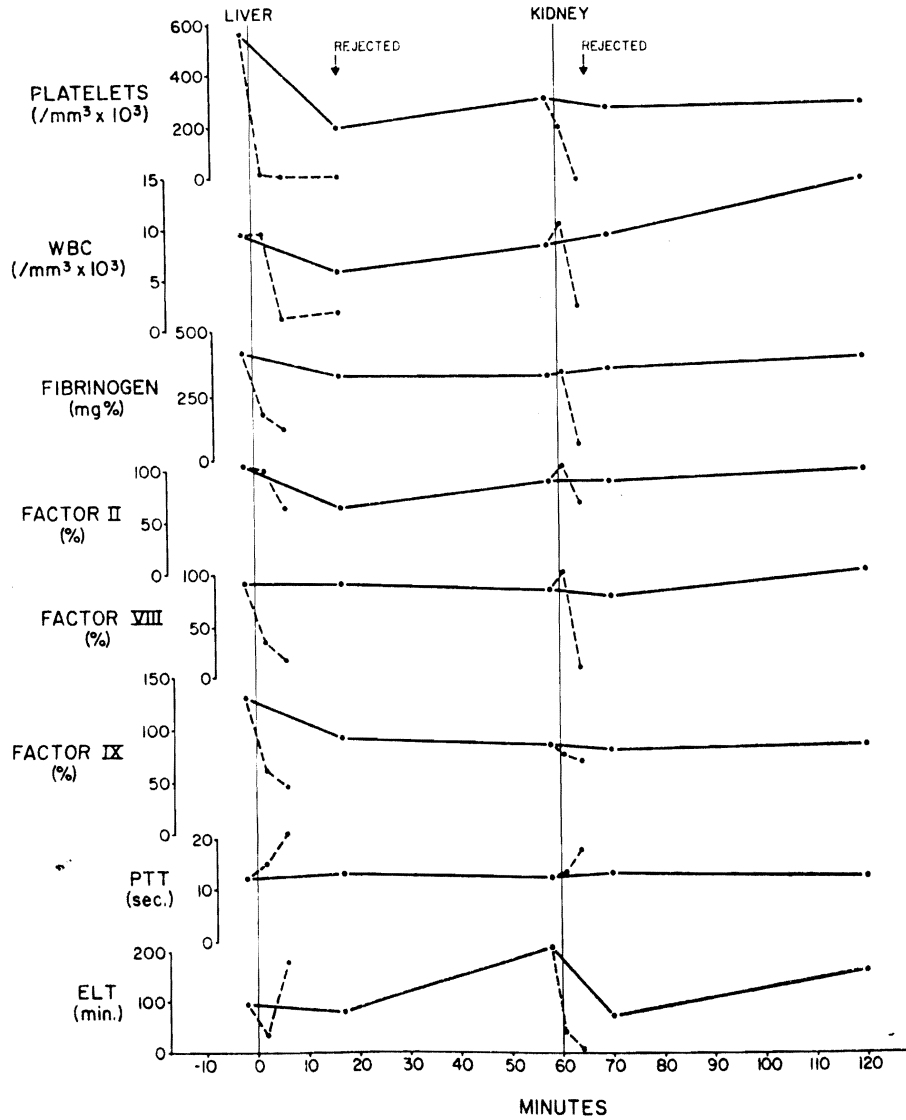

Figure 7-12. Pig to dog heterotransplantation. Arteriovenous gradients of platelets, white cells, and clotting parameters across a liver and across a subsequently transplanted kidney from the same donor. PTT: partial thromboplastin time. ELT: euglobulin lysis time. The solid lines represent arterial values and the broken lines depict the results in the heterograft venous blood. (By permission of *Transplant. Proc.*, 2:522, 1970.)

interrelationships of antibodies, formed blood elements, and clotting factors have not been well defined. On the basis of heterotransplantation experiments between dogs and pigs, it has also been suggested that hyperacute heterograft rejection is quite similar to that of homografts transferred to presensitized hosts.

Discussion Remark

R. Colman: Our findings suggest that heparin must be given prior to renal transplantation in a presensitized recipient because of the extremely rapid and severe changes occurring following revascularization. In one patient, whose graft showed renal cortical necrosis, one dose of heparin given 12 minutes following revascularization proved too late to avert this catastrophe.

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