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STUDIES ON THE MECHANISM OF HYPERACUTE REJECTION OF RABBIT-TO-DOG LIVER TRANSPLANTS

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To study the immunological phenomena in a rapidly rejected organ xenograft, rabbit-to-dog liver transplants were performed in 15 dogs, and serological, morphological, and hemodynamic investigations were carried out. Livers were rejected within 5—7 minutes, retaining 88% of affluent platelets, 76% of neutrophils, as well as hemagglutinins and complement. Data obtained in animal experiments corroborate the findings in extracorporeal porcine liver perfusion in man. They indicate that a similar biological mechanism is responsible for destruction in both situations.

As has been reported in a previous paper on immunological aspects of heterologous liver perfusion in man (Olszewski et al., 1973), hyperacute rejection of porcine liver perfused with human blood seems to be responsible for the rapid deterioration of function of that organ and the decrease in its blood flow. To study the problem more thoroughly, an experimental protocol has been designed in which transplantation of the liver in a genetically discordant combination of animals, namely rabbit and dog, was performed. In this species combination rapidly developing rejection of vascularized transplants can be easily evoked. Technical details of transplantation and serological differences between both species have been described previously (Olszewski, 1973a). The purpose of the present paper was to investigate the morphological, hemodynamic and serological phenomena developing in the liver rabbit — to-dog transplant·

MATERIAL AND METHODS

The experiments were divided into 3 groups.

Group 1. Fifteen liver xenografts between rabbit and dogs were performed according to the previously described technique (Olszewski, 1973a). Liver rejection was judged by change in its gross appearance, decrease in blood flow below 0·05 ml/g/min, and loss of blood supply to the parenchyma as manifested by failure of its cut surface to bleed. Blood flow was

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measured by collecting and measuring the volume of entire hepatic veins effluent at 2 min intervals. Hepatic vascular resistance was calculated by the formula:

$$\frac{79\,920\ (\text{mean systemic pressure} - \text{mean venous pressure})}{\text{liver blood flow/ml/min.}}$$

Radiographic demonstration of the renal vasculature was accomplished by injecting the hepatic artery and portal vein at the rejection time with consecutively 1,3, and 5 ml of 80% Uromiro (Bracco), the artery under the pressure of 96 mm Hg, and the vein at 15 mm Hg.

Specimens of the graft tissue were taken for histology into 10% buffered hormaldehyde, embedded in paraffin, stained with hematoxylin-eosin and with phosphotungstic-hematoxylin acid (PTAH) for fibrin deposits. Specimens for electron microscopy were fixed in 2·5% glutaraldehyde and 2% osmium tetraoxide in Millonig buffer of pH 7·4, dehydrated, and embedded in Epon 812. A JEM 7A electron microscope was used.

Using appropriate donor cells, preformed anti-donor hemagglutinins, lymphoagglutinins, and lymphocytotoxins, whole complement activity, IgG and IgM were measured in the affluent and effluent blood according to the previously described techniques (Olszewski, 1973a). Platelet and leukocyte counts were done in Neubauer chamber.

Group 2. In this group 3 rabbit-to-rabbit heterotopic liver transplants were performed.

Group 3. Three dog-to-dog liver grafts were made with anastomosis to the recipient neck vessels. In both control groups the same hemodynamic, angiographic and morphological studies as in group 1 were carried out.

RESULTS

Organ survival. All 15 rabbit livers transplanted to the dog were destroyed within 5—7 minutes. Concomittantly with the change in gross appearence to the mottled dark-brown, blood flow decreased below 0·05 ml/g/min, which was considered to be consistent with total rejection. No such changes were observed in the control groups.

Hemodynamics. The mean arterial pressure of recipient dogs averaged 120 mm Hg. It usually decreased by 10—20 mm Hg two minutes after the revascularization of the graft. Blood flow through the graft never reached physiological levels, ranging at 2 min between 0·5 ml/g/min and 0·15 ml/g/min, and diminishing to 0·05 at 7 minutes. Liver arterial resistance rose rapidly to values of $63{,}984—177{,}733 \times 10^3$ dynes-sec/cm^5 at 7 minutes after revascularization.

In both control groups there was a slight increase in vascular resistance of the graft, from the normal values of 2000 to 3528—5514 dynes-sec/cm^5 (Fig. 1).

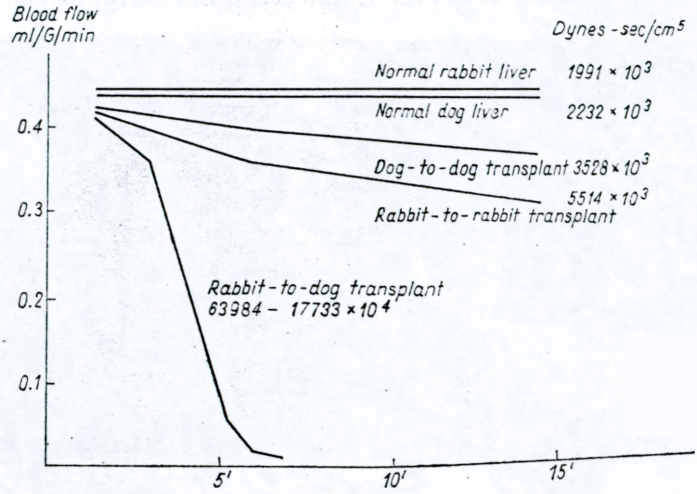

Fig. 1. Arterial resistance of the liver graft in: rabbit-to-dog, rabbit-to-rabbit, dog-to-dog transplantation. The upper two curves show normal values of vascular resistance of rabbit and dog livers.

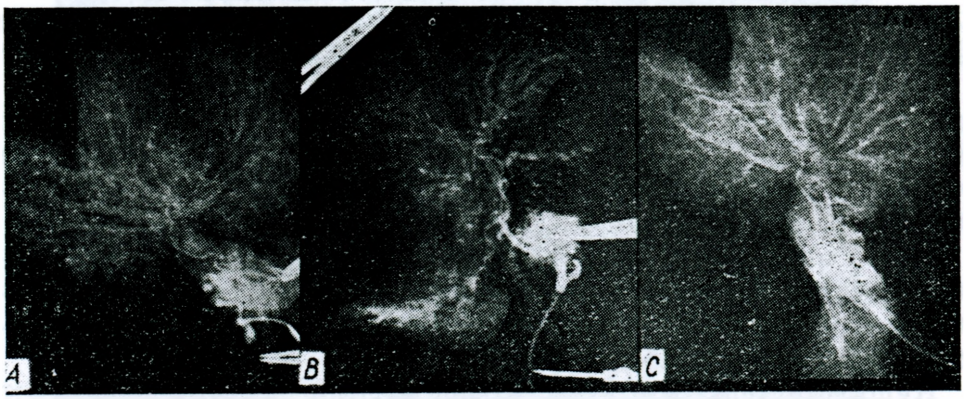

Fig. 2. Angiograms of transplanted livers at 7 min after revascularization. *A*. rabbit-to rabbit. *B*. rabbit-to-dog, *C*. normal rabbit. Note lack of filling of vessels at the periphery of the xenograft.

Angiograms. Arteriograms of rejected livers showed narrowing, elongation and tortuosity of segmental arteries. There was no filling of the peripheral arterial tributaries and no venous phase after 60 sec. The same pictures were obtained during liver venography (Fig. 2). Angio-

grams of the control allografts showed normal vascular pattern with an early venous phase.

Morphology. Light microscopy revealed marked engorgment od portal tributaries, sinusoids, and partially central veins. There was accumulation in the sinusoids of erythrocytes and of amorphous material stained slightly with PTAH (Fig. 3). Electron micrographs showed aggre-

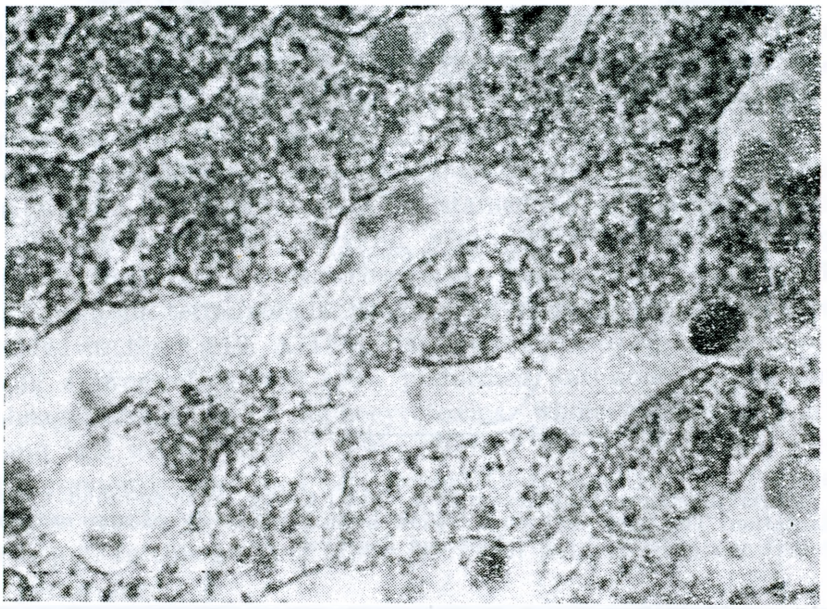

Fig. 3. Histological pattern of a specimen of rabbit-to-dog liver graft at the time of rejection. Note engorgement of sinusoids and aggregates of a cell-free mass in their lumen No evident deposition of fibrin. PTAH, × 960.

gation of thrombocytes in the lumen of sinusoids (Fig. 4a), loss of platelet gralunomer, and adhesion of platelets with pseudopods to the membrane of endothelial cells (Fig. 4b). Disintegration and desquamation of endothelial cells into the sinusoids was a common finding.

In the control groups, histological studies revealed only slight dilatation of sinusoids. Electron micrographs showed some damage to the endothelial cells and adhesion of single thrombocytes to their surface. No platelet aggregates were seen.

Hematological studies. A striking decrease in platelet and PMN cell counts across the xenograft was found in all experiments (Fig. 5). The platelet count dropped from 293,000 ± 84,900 to 65,000 ± 16,400. This means that approximately 88% of platelets were retained in the xenograft. Strangely enough, in the control allografts there was trapping of platelets, in the rabbit-to-rabbit group 45%, and in the dog-to-dog group 35%.

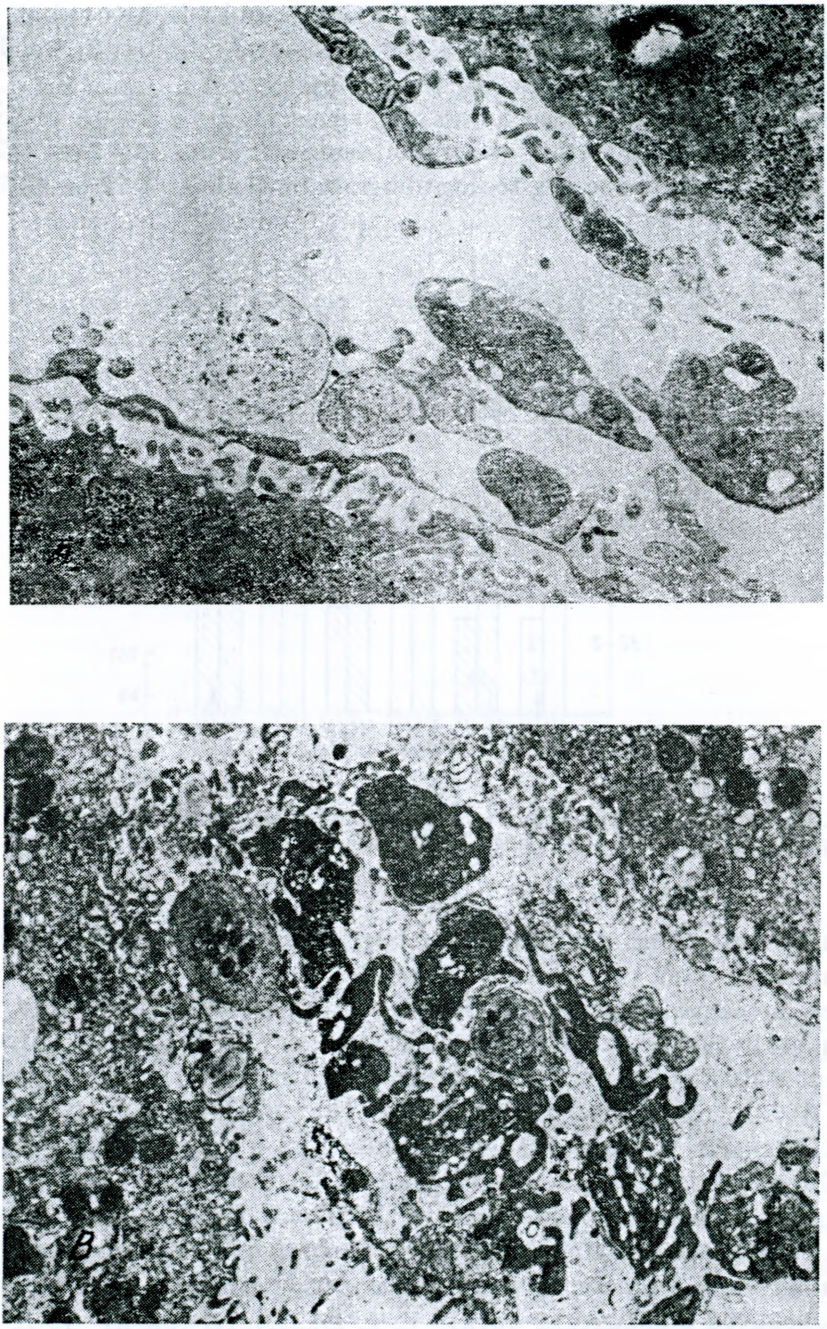

Fig. 4. Electron micrographs of rejected liver xenografts. *A*. The wall of a sinusoid lined with aggregated and disintegrated platelets. *B*. Aggregation of thrombocytes in the sinusoids. Adhesion of a platelet with pseudopods to the endothelial cell membrane (arrow).

The PMN cell count fell across the xenograft from 10,041 ± 3937 to 2400 ± 1995, that is by 76% (Fig. 5). In the control rabbit-to-rabbit group it decreased from 7400 ± 2646 to 3500 ± 1905 (by 53%), but in the dog-to, dog group there was practically no change.

The hematocrit increased across the xenograft from 45 ± 5·46 to 48 ± 7·44, but decreased across the rabbit-to-rabbit allograft from 37 ± 3·8 to 31 ± 4·85, and in the dog-to-dog allograft from 46·5 ± 3·72 to 44·7 ± 6·7

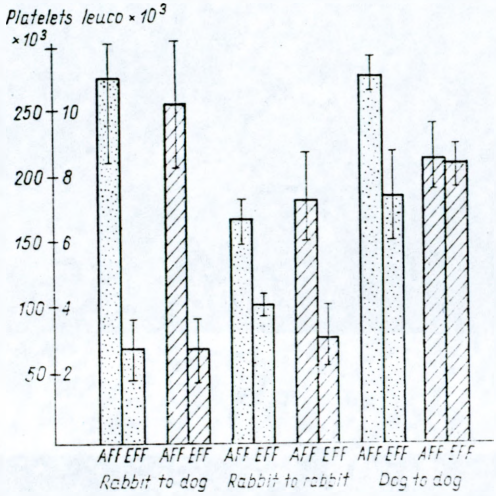

Fig. 5. Platelet and leukocyte counts in affluent and effluent blood of liver grafts: rabbit-to-dog, rabbit-to-rabbit, dog-to-dog. Dotted areas-platelets. striped areas-leukocytes.

Immunological studies. All preoperative dog sera exhibited activity against rabbit red cells. The titers of the preformed heterospecific antibodies, hemagglutinins and hemolysins, varied from dog to dog by as much as 2 tubes. After revascularization of the xenograft, there was evident reduction of antibodies in the effluent blood in all experiments (Fig. 6).

All dog sera contained slight amounts of lymphoagglutinating and lymphocytotoxic antibodies to rabbit lymphocytes. The lymphooagglutinating titers in the affluent blood ranged from 1:2 to 1:16. The results from the effluent blood, however, were inconsistent. In some cases there was a decrease, an in others even increase in titer (Fig. 7). The cytotoxic titers behaved similarly.

In the control allograft groups, no antibodies were found in recipient sera to donor red and white cells.

The total complement activity decreased in the xenograft group at 7 min after revascularization from $17{\cdot}6 \pm 3{\cdot}5$ u./ml in the affluent blood to $4{\cdot}7 \pm 2{\cdot}2$ u./ml in the effluent. The IgG concentration was respectively 5·2 mg/ml and 4·8 mg/ml, and IGM 2·1 mg/ml and 2·1 mg/ml.

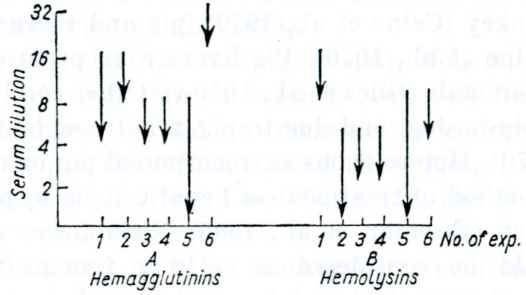

Fig. 6. Gradient of dog heteroagglutinins (*A*) and hemolysins (*B*) to rabbit erythrocytes across a liver xenograft. Upper end of the arrow indicates titer in affluent serum, lower in the effluent.

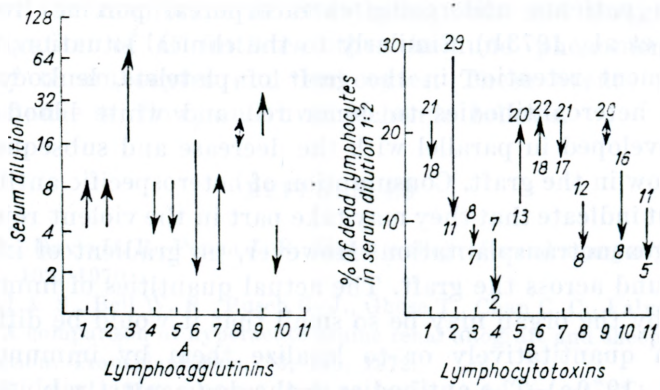

Fig. 7. Heterolymphoagglutinin (*A*), and heterolymphocytotoxin (*B*) titers in the affluent and effluent serum of a liver xenograft. Arrows show increasing or decreasing titers. In the case of lymphocytotoxins, numbers represent percentage of dead lymphocytes and not titers. Note variability of results.

DISCUSSION

The typical pattern of hyperacute rejection of vascularized xenografts, according to most authors (Marceau et al., 1965; Rosenberg et al., 1969; Giles et al., 1970a; MacDonald et al., 1972), consists in a rapid decrease in graft blood perfusion, uptake of platelets, leukocytes, heteroantibodies to donor erythrocytes and lymphocytes, and complement by the graft, also morphological changes as endothelial damage, platelet aggregation,

and subsequent intravascular coagulopathy. Most of these data come from experiments with kidney xenotransplants. The experimental model of organ xenografts has been used by many investigators as a prospective method in clinical transplantation, and also as a model for studies on the role of preformed circulating antibodies in the process of hypercute rejection. Xenografting of the liver was performed twice clinically (Starzl, 1969; Giles et al., 1970b), and experimentally between the rhesus and-cynamolgus monkey (Calne et al., 1970), pig and rhesus monkey, pig and chimpanzee (Calne et al., 1970). Pig livers were perfused with dog blood from the living animals (Giles et al., 1970a). Other combinations including rabbit-to-dog, pig-to-dog, and dog-to-pig xenotransplantations were made (Giles et al., 1970). Heterologous extracorporeal pig or calf liver perfusion was used as a method of treatment of hepatic coma in patients with acute liver insufficiency (Eiseman et al., 1965; Nielubowicz et al., 1973). This procedure should be considered as a liver xenografting. Our clinical experience with this method and in particular the problem of rapid deterioration of liver function and hemodynamic disturbances in that organ prompted us to study the immunological aspects of liver perfusion with blood of other species. The results of the present studies corroborate the findings in patients undergoing extracorporeal porcine liver perfusion (Olszewski et al., 1973b). Similarly to the clinical situation, there was in the experiment retention in the graft of platelets, leukocytes, complement, and heteroantibodies to donor red and white blood cells. These changes developed in parallel with the decrease and subsequent cessation of blood flow in the graft. Consumption of heterospecific antibodies and of complement indicate that they may take part in the violent rejection occurring during xenotransplantation. However, no gradient of immunoglobulins was found across the graft. The actual quantities of immunoglobulins picked up by the organ may be so small that it would be difficult to estimate them quantitatively or to localize them by immunofluorescence (Giles et al., 1970a). The antibodies in the dog against rabbit cells may be very active and their slightest amount may be sufficient to initiate pronounced intravascular immune reaction.

Light and electron-microscopic studies of our grafts showed that endothelial cells bear the brunt of the cytotoxic effect of antigen-antibody-complement interaction. It seems very likely tat the initial rise in vascular resistance to flow developing in the graft is caused by endothelial swelling, sloughing into the lumen, and subsequent edema of the tissue. It is aggravated by plugging of the microvasculature with platelet and leukocyte aggregates. Ischemia which follows may be responsible for the destruction of the organ. It should be stressed, however, that platelet and leukocyte retention in the graft may be an unspecific nonimmune phenomenon caused

by mechanical damage to the microvasculature by ischemia and wash-out procedure. In the two control groups of allografts there was considerable retention of these cells without evident deterioration of perfusion of the graft.

The contribution of the coagulation process to the destruction of the graft is controversial (Giles et al., 1970a; Rosenberg et al., 1971; MacDonald et al., 1972; Łukasiewicz et al., 1973).

Damage to the vascular wall and vasoconstriction may be caused also by direct action of vasoactive substances released from disintegrated platelets and leukocytes. We did not measure the concentration of histamine, seratonin, etc., in the effluent blood. Rosenberg (1971), who did so in pig-to-dog kidney grafts, found no changes in the level of vasoactive substances in the renal vein effluent.

Basing on our as well as other authors' observations, the following sequence of events can be postulated to account for cessation of blood flow in liver xenografts. The heterospecific antibodies directed against donor cell antigens react with antigenic sites on the surface of endothelial cells. This is followed by immediate complement fixation with subsequent disruption of the membrane of endothelial cells and sloughing of the intima. Activation of C' initiates other immune phenomena such as adherence of thrombocytes and leukocytes. These events produce increased resistance to flow, and liver ischemia follows.

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