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IMMUNOLOGICAL PHENOMENA IN THE PORCINE LIVER PERFUSED WITH HUMAN BLOOD

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In order to study the immunological phenomena developing in the porcine livers perfused with human blood, investigations were carried out on 2 patients during extracorporeal liver perfusion and 3 in vitro perfusions. Trapping of platelets, leukocytes, and retention of hemagglutinins to pig erythrocytes and lymphocytes was found in the livers. This occurred simultaneously with deterioration of liver function and increase in its blood flow resistance.

Isolated porcine liver perfusion has been used for extracorporeal hepatic assist of patients suffering from acute hepatic necrosis and in several cases resulted in considerable but temporary improvement of such patients (Eiseman et al., 1965; Abouna et al., 1969; Nielubowicz et al., 1973). The effect of perfusion depends on the function of the heterologous liver. Unfortunately, its function deteriorates within 2—4 hours and perfusion has to be discontinued. The heterologous liver changes its appearance, becomes edematous and dark-bluish on its surface. The vascular resistance of the organ increases (Hickman et al., 1971), bile production decreases, as well as oxygen consumption. Serum AspAT and AlAT activities rise steadily, and there is a considerable, wash-out of K from the liver (Hickman et al., 1971). The nature of the damage resulting from the perfusion of porcine liver with human blood is not clear. Heterologous liver perfused by a patient is a temporary xenograft which may be hyperacutely rejected. The purpose of the present study was to evaluate the immunological response of the pig's liver perfused with human blood by a patient with hepatic coma, and also in an artificial perfusion system, without patient, filled with fresh human blood.

MATERIAL AND METHODS

The studies were carried out in two groups. In group 1 two patients with hepatic coma due to acute viral hepatitis were investigated. In group 2 three porcine livers were perfused for 2 hours in a perfusion system

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filled with fresh O Rh positive heparinized human blood, consisting of a roller pump, bubble oxygenator, and heat exchanger.

The following parameters were measured in the human blood inflowing and outflowing from the porcine liver: a) platelet count measured in a hemocytometer using ammonium oxalate as diluent, b) leukocyte count, c) human serum heteroantibodies to porcine erythrocytes and lymphocytes: hemagglutinins and hemolysins (Ślopek, 1970), lymphocytotoxins and lymphoagglutinins (Histocompatibility Testing. Nat. Res. Council, Washington 1965) and, d) total complement (Kabat, 1961). At the end of the perfusion a speciment was taken from the liver for light and electron microscopy.

RESULTS

Group 1. Detailed results of one patient will be presented. Platelet and leukocyte count. There was a significant decrease in platelet and leukocyte counts in the peripheral blood of the patient and an evident

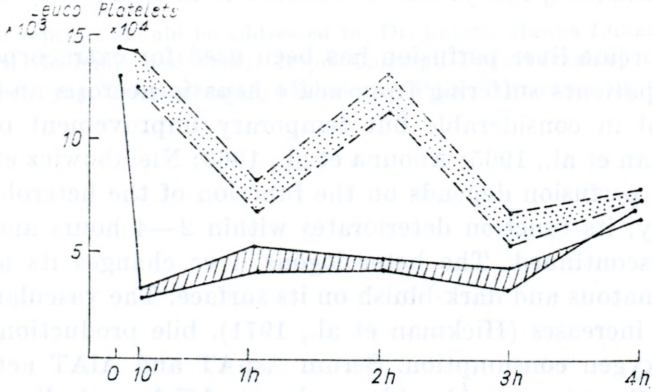

Fig. 1. Platelet (dotted line) and leukocyte (continuous line) counts in the affluent (upper line) and effluent (lower line) blood in a patient during ex-vivo porcien liver perfusion.

gradient across the porcine liver. The most pronounced changes developed within the first hour of perfusion. Platelet count decreased from the initial 15×10^4 per cu mm to 7×10^4 at 4 hr, with a constant gradient of 2×10^4 (Fig. 1). Leukocyte counts dropped in the first 10 minutes from 13×10^3 to $2{\cdot}5 \times 10^3$, and remained at that level until the end of perfusion. There was a constant gradient of leukocytes across the liver of $1{\cdot}5 \times 10^3$ cells (Fig. 1).

Heteroantibodies. An initial titer of hemagglutinins of 1:32 was found (Fig. 2). It decreased to 1:8 at 10 minutes. At that time the gradient

across the liver was two dilutions. At 1 hr the titer in the inflow line was still 1:4, but there was already no gradient, and this persisted until the end of the procedure. No hemolysins to porcine red cells were found. The human anti-pig lymphoagglutinin titer was 1:2 (Fig. 2). It remained at that level with an evident gradient across the liver. No lymphoagglutinins

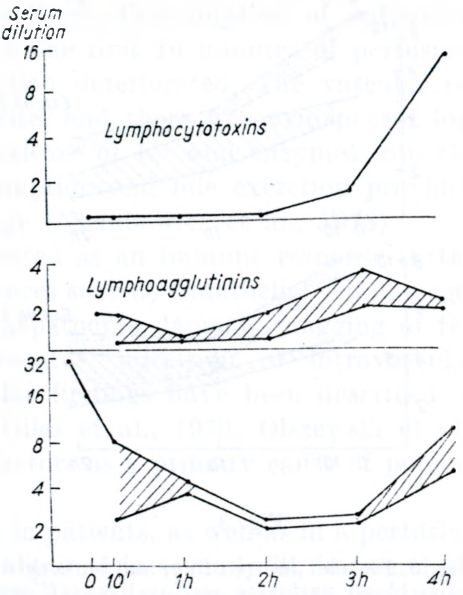

Fig. 2. Human serum heteroantibody titers to porcine lympho- and erythrocyte antigens in a patient during ex-vivo pig liver perfusion (affluent blood-upper line, effluent blood-lower line).

were found in the effluent blood. No lymphocytotoxins to porcine lymphocytes were found in patients blood.

Total complement. Total complement titer decreased at 10 minutes of perfusion from the initial 40 u/ml to 27 u/ml and remained unchanged at that level.

Group 2. Platelet and leukocyte count. There was a contant tendency for both platelets and leukocytes to decrease during the perfusion (Fig. 3). Most rapid changes developed in the first hours of the study. There was a high gradient of both types of cells across the liver.

Heteroantibodies. The most striking was a rapid drop in hemagglutinin titer, which was evident already at 10 minutes (Fig. 4). The titers decreased from 1:64, 1:8, and 1:4 to 1:4, 1:2, and 0. No hemolysins were found. There was also a decrease in lymphoagglutinin titer with a constant gradient across the liver (Fig. 4). The titers decreased from 1:8, 1:8, and 1:2 to 1:4, 0, and 0. No lymphocytotoxicity was found in human serum for porcine lymphocytes.

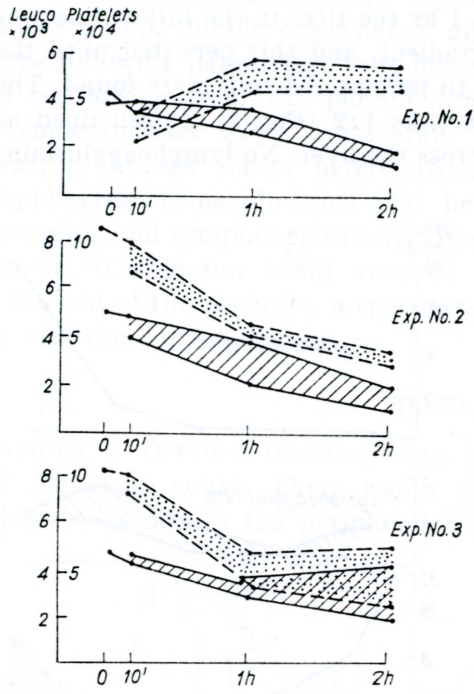

Fig. 3.

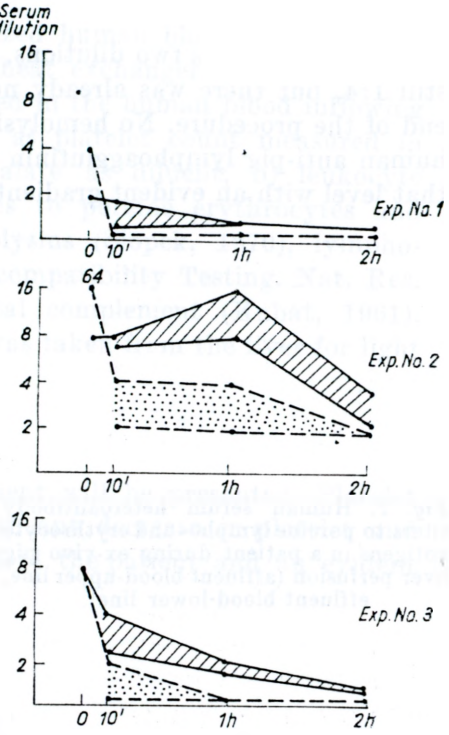

Fig. 4.

Fig. 3. Platelet (dotted line) and leukocyte (continuous line) counts in human O Rh positive blood perfusing porcine liver (affluent blood-upper line, effluent blood-lower line) Three experiments.

Fig. 4. Human serum heteroantibodies to porcine lymphocyte and erythrocyte antigens during porcine liver perfusion. Hemagglutinins-dotted line, lymphoagglutinins-continuous line (upper line-affluent blood, lower line-effluent blood).

Total complement. The total complement titers dropped rapidly in the first hour of the perfusion from values 30, 28, and 27 u./ml to 13, 12, and 6·5 u./ml at 2 hr.

MORPHOLOGICAL STUDIES

In both groups of livers light microscopy revealed dilatation of portal veins in portal areas and sinusoids, engorgement of erythrocytes and leukocytes in sinusoids, but no evident changes in hepatocytes.

Electron microscopy showed aggregation of platelets within the sinusoids, adhesiveness of platelets to the endothelial cells, and desquamation of endothelial cells. For details see page 57.

DISCUSSION

The results of our studies show that during the first hour of perfusion of porcine liver with human blood, by the patient as well as in an artificial perfusion system, a striking decrease occurred in platelet and leukocyte levels across the liver. There was also an evident decline of human anti-pig hemagglutinin and lymphoagglutinin titers. Consumption of antibodies and complement was most evident in the first 10 minutes of perfusion. Simultaneously with this, liver function deteriorated. The vascular resistance of the organ continued to rise, and there was evidence of loss of integrity of cell membrane with release of K^+ and enzymes into the circulation. Decline in oxygen consumption and bile excretion provided further proof of growing liver damage (Nielubowicz et al., 1973).

These observations may be interpreted as an immune response in the xenogeneic liver with all the consequences such as endothelial cell damage, increased permeability of sinusoids, hepatocyte damage, plugging of the microcirculation with platelet aggregates, initiation of intravascular nonenzymatic coagulation, etc. Similar findings have been described in experimental xenograft procedures (Giles et al., 1970; Olszewski et al., 1973) which also points to immune factors as a primary cause of porcine liver destruction.

Our investigations were carried out in patients, as well as in a perfusion system. We thought that the results obtained in severely ill, immunologically deficient, virusinfected and multitransfused patient might be misleading and modified by the basic disease. That is why we carried out the studies in a perfusion set with fresh blood of healthy individuals. The data obtained in group 2 do not differ from those of group 1, indicating that the basic immunological inter-species discordance seems to be responsible for the rapid liver damage. The immune reaction in the porcine liver considerably limits indications for the use of the method of heterologous liver perfusion in clinical practice.

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