Platelet Changes Following
Clinical And Experimental
Hepatic Homotransplantation

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DURING the last four years, ten patients with liver disease have been treated at the University of Colorado with either orthotopic or auxiliary hepatic homotransplantation.[1,2] A hemorrhagic diathesis, explained at least partially by increased circulating fibrinolysins,[3] frequently complicated the operative procedure. Derangements in other clotting factors are also known to occur,[3,4] but the presence of thrombocytopenia during the early postoperative period has not been well documented.

In the present study, platelet changes in the clinical cases are reviewed. In addition, the magnitude, timing, and duration of thrombocytopenia have been evaluated under controlled circumstances in untreated dogs subjected to orthotopic transplantation, as well as in comparable experiments in which various immunosuppressive agents were provided including antilymphocyte globulin, azathioprine, and prednisone.

Methods

Clinical Studies.—Three attempts at auxiliary transplantation were performed: One pa-

tient died two hours after operation and the other two lived for 22 and 34 days, respectively. One of the seven patients who received an orthotopic liver graft died of hemorrhage during operation and the others lived for 6.5, 7, 7.5, 10, 22 and 23 days, respectively. Before and after operation, platelet counts or platelet estimates were obtained. All recipients were treated with azathioprine, prednisone, and actinomycin C. In addition, the last two received intramuscular antilymphocyte globulin.

Experimental Studies.—Twenty-nine dogs weighing 12 to 19 kg (26 to 42 lb) received control or homotransplantation procedures while under pentobarbital anesthesia supplemented with phencyclodine hydrochloride (Sernylan). Nonrelated mongrel donors were used for the homotransplantations. The grafts were protected from ischemic injury by cooling with cold Ringer lactate except in group 5. Each recipient animal received 500 ml homologous blood during operation.

Platelet counts were performed with phase microscopy before, during, and after operation.[5] In selected experiments, platelet labelling studies were performed (36 hours before operation)[6] with di-isopropyl phosphorofluoridate (^{32}P); the tagged thrombocyte population was then followed throughout the next several days. In addition, fibrinogen levels were measured serially and fibrinogen degradation products were detected by agar gel immunoelectrophoresis.[8,9] Fibrinolytic activity was measured by the euglobulin lysis time[10] and fibrin plate methods.[11]

Group 1.—Control Operations.—In seven dogs, the liver was isolated from the circulation for 25 to 40 minutes by cross-clamping the su-

prahepatic and infrahepatic vena cava, the portal vein, and the hepatic artery. The caval and splanchnic venous beds were placed into communication with a temporary side-to-side portacaval anastomosis and then decompressed during the period of occlusion with a single plastic external bypass from the femoral to the jugular vein. After its isolation, the liver was quickly cooled by infusion through the portal vein with 1,500 to 2,000 ml chilled (4 C) lactated Ringer's solution. The fluid was allowed to escape from a venotomy in the inferior vena cava. Platelet counts were obtained before and after anesthesia, after portacaval shunt, during the anhepatic stage, after portal revascularization, after arterial revascularization, two hours postoperatively, and daily.

Group 2.—Nontreated Orthotopic Homotransplantation.—Six dogs were provided with orthotopic homografts.[12] Platelets were obtained before and each day after operation.

Group 3.—Homotransplantation and Treatment With ALG.—Five dogs were pretreated for 14 to 18 days with subcutaneous antilymphocyte globulin (ALG). After transplantation, daily injections were continued.

Group 4.—Homotransplantation and Treatment with ALG, Azathioprine, and Prednisone.—Six experiments were performed. The ALG was started 8 to 47 days before operation. The dose of azathioprine was determined on the basis of daily white blood cell counts (WBC). Prednisone was administered for five days in progressively decreasing daily doses starting at 2.5 mgm/kg of body weight on the day of operation.

Group 5.—Homotransplantation With Preserved Homografts and Combined Drug Therapy.—Five dogs received livers which had been preserved for eight to ten hours. The storage technique was the best one evaluated in a recent report and included hypothermia, hyperbaric oxygenation, and perfusion of both portal vein and hepatic artery with diluted homologous blood.[13] Immunosuppressive therapy was the same as in group 4.

Pathologic Studies

The histopathologic changes were reviewed not only in the human and dog homotransplantations described above, but also in the homografts from more than 100 other previously reported canine experiments. The latter included six studies in which serial biopsies had been obtained in unmodified recipients for electron micrographic examination. Evidence of platelet trapping was

sought in azure II-stained, 0.5μ thick sections from material that had been fixed in formalin and then postfixed in osmium tetroxide and embedded in Epon. Where tissue had been primarily fixed in osmium for electron microscopy, ultrathin sections were cut, stained with lead hydroxide, and examined under an electron microscope (Siemens Elmiskop 1A).

Results

Clinical Cases.—Meaningful platelet studies were obtained in two recipients of auxiliary livers and in six of the orthotopic cases. All preoperative platelet counts were normal (200,000 to 400,000). There was an acute drop in platelet values to less than 100,000 (average 67,300/mm^3) in seven of the eight patients (five orthotopic and two auxiliary transplants) within the first three postoperative days. These findings did not seem to be the consequence of bone marrow depression since, at the same time, the WBC were normal or elevated (8,600 to 34,000; average 17,930).

In contrast, platelet depression beginning beyond the first postoperative week, was clearly associated with bone marrow depression since thrombocytopenia was then invariably accompanied by leukopenia of less than 5,000.

Experimental Studies

Group 1.—Control Operations.—A slight fall in platelet counts could usually be seen during the initial stages of the procedure which included the induction of anesthesia, portacaval anastomosis, external bypass, and liver isolation. However, these declines were severe in only two animals; one, 43%, and the other, 22% of control values. In the other five, they were less than 10%.

In contrast, the revascularization of the liver was invariably followed by a striking thrombocytopenia which developed within 15 to 20 minutes. Platelet counts fell to between 74,000 and 200,000 compared with preoperative values of 206,000 to 450,000. The average percentage decline from preanesthesia values was 41% (range 23% to 72%) and that from the level present just
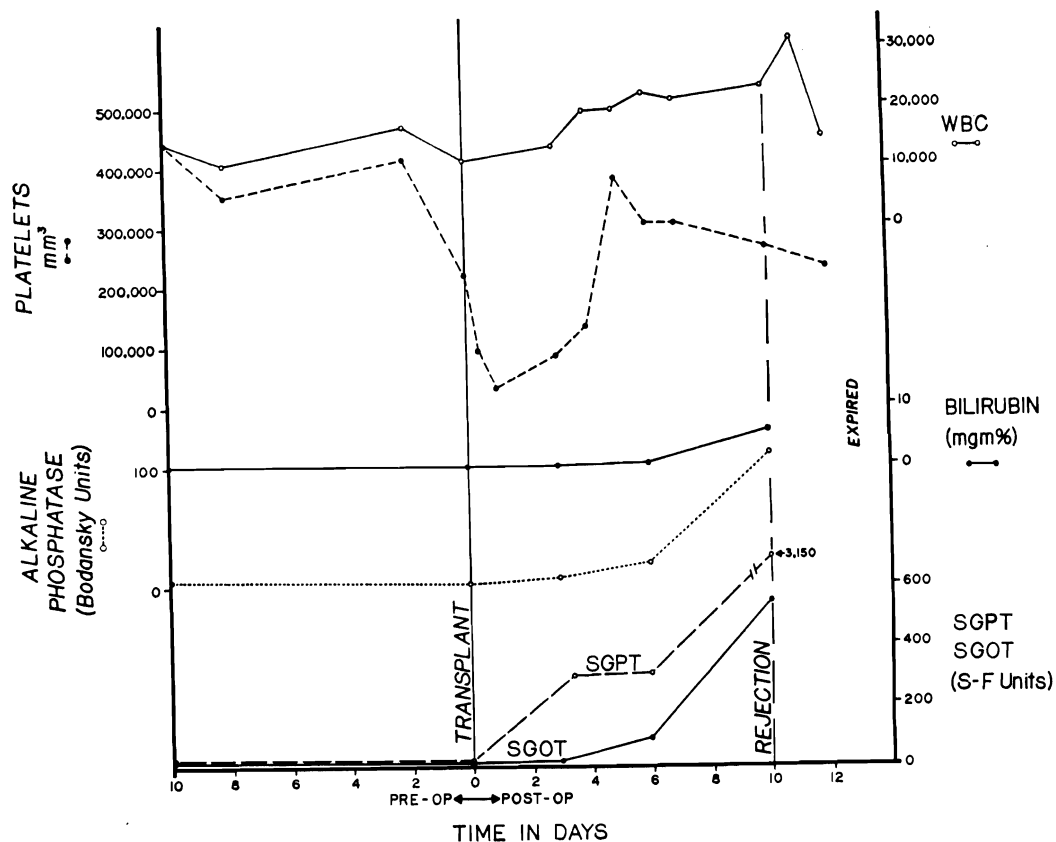
Fig 1.—Changes in platelet count, WBC, and liver function tests in nontreated dog that died of homotransplantation rejection.

before revascularization was 35.3%. The five animals which survived operation had a return of normal platelet counts by the second to the fourth postoperative day.

Plasma fibrinogen concentrations dropped 20% to 44% (average 34%) during operation in the four dogs in which it was measured, the principal fall occurring during devascularization at the same time when a transient increase was observed in fibrinolytic activity (Table). These changes were associated with appearance of breakdown products of fibrinogen as measured with immunoelectrophoresis. In three experiments, platelets labelled with di-isopropyl phosphofluoridate (^{32}P) disappeared from the blood in the same proportion as the reductions in

peripheral thrombocytes (Table 1). The tagged platelets did not return to the circulation when the thrombocytopenia later reversed.

Group 2.—Nontreated Orthotopic Homotransplantation.—In six dogs, the circulating platelets had dropped 30% to 73% by the morning after operation (average 52%). Subsequent survival was 3, 7, 8, 8, 13, and 22 days, respectively. Platelet counts had become normal by the fifth postoperative day in four of the five animals which lived for at least one week (Fig 1). In the longest survival, the values returned to normal only after 18 days. Throughout the experiments, the WBC were normal or elevated.
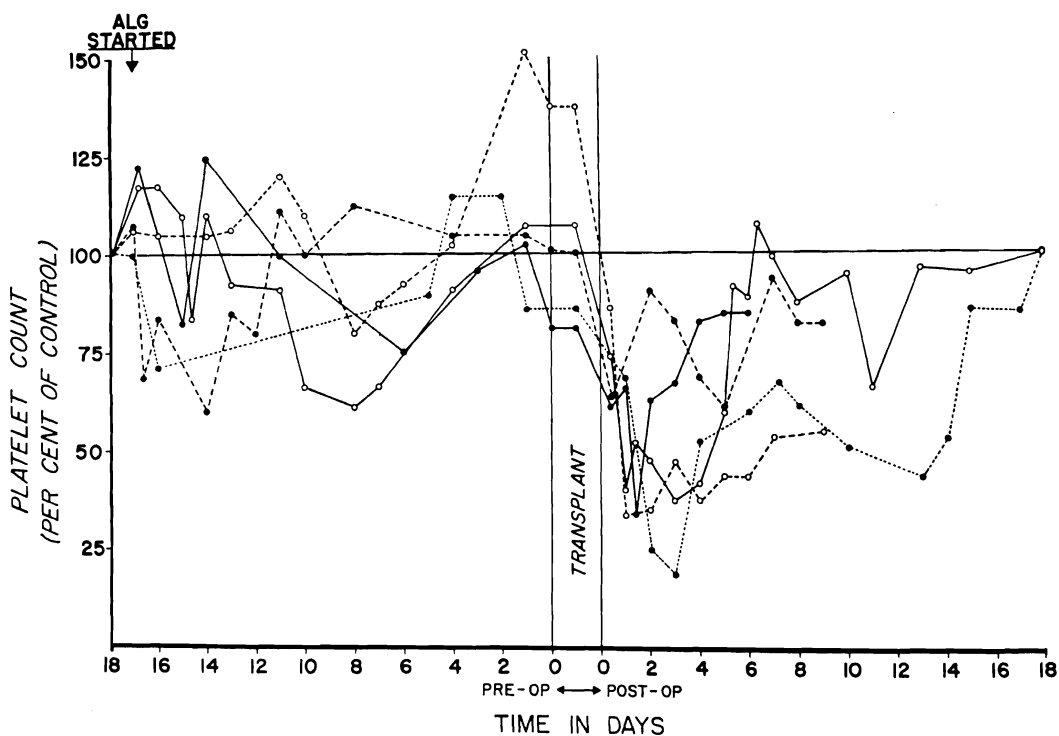
ORTHOTOPIC LIVER TRANSPLANTS WITH ALG

Fig 2.—Changes in platelet counts in five dogs treated with
ALG which survived transplantation for more than five days.

Group 3.—Homotransplantation and Treatment With ALG.—During preoperative ALG therapy, platelet counts usually dropped slightly (Figure 2). Survival of platelets tagged with di-isopropyl phosphofluoridate (^{32}P) slightly but consistently decreased. The baseline half-life in four dogs was 5.0, 3.0, 5.4, and 3.2 days, respectively. The tagged platelet half-life after 10 to 14 days of treatment was 3.0, 2.1, 4.8, and 3.0 days, respectively.

The five dogs died of homotransplantation rejection on postoperative day 6, 7, 9, 29 and 152, respectively. On the morning after operation, each had a drop in platelet count (Fig 2) of 36% to 60% of preoperative values (average 45%). The thrombocytes had begun to rise by the fourth or fifth postoperative day. The two longest surviving animals had full restoration of peripheral platelets after 10 to 20 days. The onset of rejection did not interfere with this recovery (Fig 3). The platelet survival during rejection remained stable in two dogs at 2.2 and

2.8 days, respectively. All animals in this series had normal or elevated WBC.

Group 4.—Homotransplantation and Treatment With ALG, Azathioprine, and Prednisone.—Platelet counts were depressed 20% to 76% the day after operation (average 57%). Two of the 6 dogs died with hepatic artery thrombosis after 4 and 8 days. The others died of rejection after 45, 95, 97, and 121 days, respectively. Three of the four dogs which lived beyond the 20th day developed platelet counts higher than before operation. Furthermore, these animals all passed through rejections with platelet values that were 90% to 146% of the preoperative values. The fourth animal that died of rejection after 45 days had persistent thrombocytopenia. In this animal, the platelets were still depressed by 64% in comparison to pretransplantation levels.

Group 5.—Homotransplantation With Preserved Homografts and Combined Drug Therapy.—The five dogs had a platelet drop

Hematologic Changes During Simulated Autotransplantation

	Platelets/cu mm	Drug-tagged* Counts /ml Blood	Fibrinogen mg/100 cc	Euglobulin Lysis time (hr)	Fibrin Plate Assay (sq mm)
Preoperatively	299,000	49	455	4	100
During devascularization of the liver	·185,000	·0·	292	2	125
Ten minutes after revascularization of portal vein	131,000	·0·	234	1	280
One hour after total revascularization	240,000	·0·	306	4	100
Three hours after total revascularization	300,000	24	379	4	·0·
Twenty-four hours after total revascularization	211,000	21	464	·0·	·0·

*Isoflurophate tagged with radioactive phosphorus (^{32}P).

PALS 4
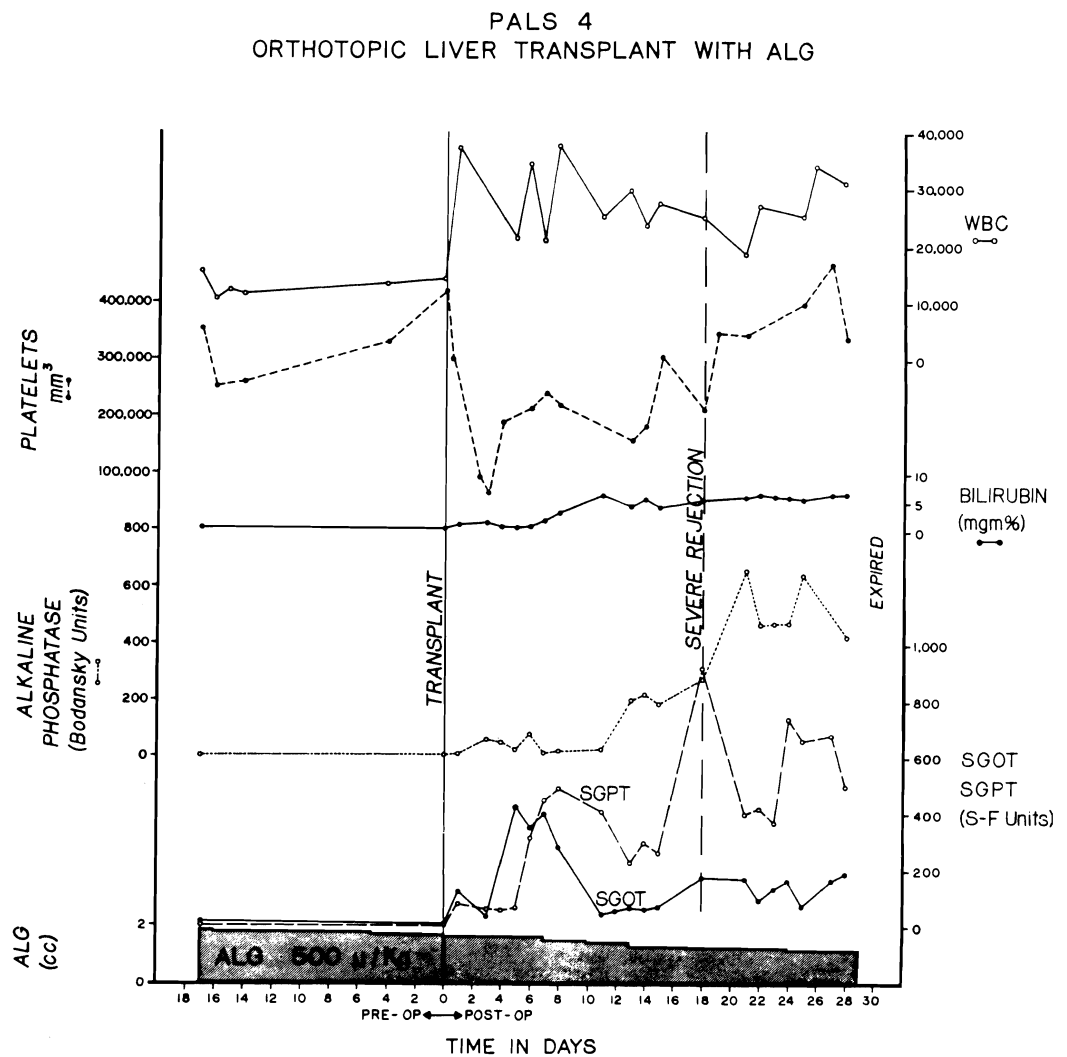## ORTHOTOPIC LIVER TRANSPLANT WITH ALG

Fig 3.—Changes in platelet count, WBC, and liver function tests in dog undergoing homo-transplantation rejection while treated with ALG. Note thrombocytopenia just after operation. Recovery from this continued during a later severe rejection.

on the first postoperative day of 26%, 46%, 64%, 70%, and 83% (average 59%). The rebound of platelet count was delayed. One dog which died on the 17th postoperative day of rejection and liver abscess still had a 40% depression. The other four all had platelet increases of 26% to 227% above normal at some time after the 25th postoperative day.

Pathologic Studies

In none of the livers studied was there evidence of platelet aggregation in the sinusoids or larger vessels. Electron microscopy, however, did show platelets in the space of Disse in many of the homografts biopsied shortly after transplantation. The platelets were single, undamaged, and lay in the increased quantities of fluid found in the perisinusoidal spaces at this time. Macrophages were also often present in the widened space of Disse and in the spaces around the small central and portal venous tributaries. These larger mononuclear cells often contained cell fragments and occasionally ingested whole platelets.

Comment

The role of platelet sequestration in organ transplantation has been the subject of speculation. Recent electron microscopic observations have shown platelet clumps in the glomerular capillaries of human renal homografts biopsied during acute rejection crises.[14] These thrombocyte aggregations were near γ-globulin deposits on glomerular basement membranes, raising the possibility that the clumping had been induced by contact with antigenantibody complexes.

These events may prove to be important in the pathogenesis of rejection, but the predominant changes described in the present study do not appear to have such an immunologic basis. During simulated autotransplantation, there was an initial slow decline in the numbers of circulating thrombocytes similar to that which has been noted during a variety of other major surgical procedures.[15] In addition, however, there was a more profound acute thrombocyte depletion which occurred within a few minutes after graft revascularization. This was accompanied by declines in plasma fibrinogen which began during devascularization of the liver. Such changes were even more prominent in the homotransplantations, but they were not qualitatively different. In the latter experiments, the depressions seemed to be of longest duration in the most severely damaged organs, namely those which had been preserved for some hours.

In the homotransplantation experiments, the subsequent onset of rejection was not accompanied by delayed thrombocytopenia, decreased platelet survival, or reduction in fibrinogen levels. Indeed, the converse was usually true since platelet counts were often normal or even supernormal at that time.

The results thus suggest that the primary cause of at least the early thrombocytopenia is mechanical entrapment of the platelets within physically injured grafts. The swiftness with which this occurs has been shown in recent preliminary experiments in which a 55% reduction occurred within minutes in the platelet concentration of blood entering and leaving the liver (unpublished data). The gradient began to disappear in a half hour but systemic thrombocytopenia persisted. A similar platelet clearing has been demonstrated in cat kidneys initially cooled in vivo to a temperature of 20 C; some persistence of the effect was noted even after rewarming.[16]

The mechanism in both instances could be related to that responsible for the widespread formation of microthrombi[17] in experimental shock[18,19] or prolonged systemic hypothermia.[20,21] This possibility, which cannot be excluded, even derives some credence from the fact that decreases in fibrinogen concentration often accompanied the thrombocytopenia. However, there was no confirmatory histologic evidence of intravascular coagulation. Instead, the pathologic findings suggest that injury to the sinusoidal endothelium leads to extravasation of fluid accompanied by migration of platelets and macrophages into the space of Disse. Platelets are apparently then engulfed by the macrophages and destroyed. This would explain their failure to return to the pool of circulating platelets.

Summary

Within the first few days after orthotopic transplantation of the liver in dogs, there is transient thrombocytopenia. This occurs with the use of either simulated autografts, or with the placement of homografts into either treated or untreated recipients. At the same time there is often a decrease in plasma fibrinogen and an increase in fibrinolysins. These early changes usually had returned to or toward normal by the time of subsequent rejection. The latter process did not cause a recurrence of the platelet depression.

The hematologic data, in conjunction with histologic studies, suggest that there is accelerated platelet clearing due to thrombocyte entrapment in the freshly transplanted liver. There was no morphologic evidence of microthrombi in the hepatic tissue. Instead, platelets seemed to have extravasated into the spaces of Disse.

Similar observations have been made in five and two patients who received orthotopic and auxiliary liver homografts, respectively.

Generic and Trade Names of Drugs

Prednisone—*Deltasone, Deltra, Meticorten, Paracourt.*

Pentobarbital—*Nembutal.*

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