1955

Platelet transfusions

Ruth Alice Johnson
University of Nebraska Medical Center

PLATELET TRANSFUSIONS

Ruth Alice Johnson

Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

April 1, 1955

Omaha, Nebraska

TABLE OF CONTENTS

I. INTRODUCTION

Transfusions of platelets prepared in various ways are indicated for the control of bleeding in a number of conditions in which the hemorrhagic manifestations are due to a quantitative or qualitative platelet deficiency. These includ idiopathic thrombocytopenic purpura (slightly benificial); secondary "amegakaryocytic" thrombocytopenic purpura (before the development of resistance and platelet agglutinins); thrombocytoasthenias; before and during surgery in all patients with thrombocytopenia or thrombocytoasthenia; and for control of bleeding manifestations in thrombocytopenia due to myelosuppressive agents during the treatment of leukemias and lymphomas (drug induced thrombocytopenias).

It is the purpose of this paper to review the various techniques available at the present for collection, preservation, and transfusion of platelets; their therapeutic effect in various thrombocytopenic states and the factors that influence their preservation in vitro, and their survival in vivo. Platelet groups and types will also be presented.

II. METHODS OF COLLECTION, PRESERVATION, & TRANSFUSION OF PLATELETS

Many different methods of collection, preservation, and transfusion of platelets have been developed. Some methods are simple enough that they may be carried out in most any hospital with a minimum of special equipment. Other methods, while quite successful, are of research value only because of their intricacy and vast amount of special and expensive equipment necessary. The various methods in use today are as follows:

Method One--

Freeman (1) and Tullis (2) - Blood collecting and transfusion sets made of translucent plastic material (polyvinyl chloride acetate copolymer) and containing a column of ion exchange resin (sulfonated polysterone divinyl benzene copolymer) for making blood incoagulable by decalcification were used. Needles were coated with tris (2 hydroxyethyl) dodecylamine. The column of resin was contained in 28 mm. plastic tubing. The ion exchange consisted of 50 g of Dowex-50 beads on the sodium cycle. The resin was washed with saline solution and kept moist. Platelet suspensions were received in clean silicone lined flasks or bottles. The eluting fluid was an unbuffered solution of 0.85% NaCl in distilled H2O. At the end of the 500 cc donation, the resin container was cut free and the contents were washed with saline. If it is

desired to eliminate red blood cells caught in the resin, one can allow the suspension to settle in the cold for several hours or by light centrifugation at 1000 rpm for 5-8 minutes at 4° C. Furthur centrifugation can be carried out if morphology or individuality need not be preserved.

Method Two--

Dillard, Brecher, Cronkite (3) and Stefanini (4) - Sequesterene Na2, 1 gram and 0.7 gm. of NaCl were disolved in 100 cc of H_2O. Nine parts of blood were added to one part of sequesterene solution. Venous blood is collected by gravity flow through a coated 15 gauge nylon hub electro-polished needle, and plastic tubing into 200 ml. siliconed centrifuge bottles, each containing 20 cc. of sequesterene solution. The bottles are balanced and centrifuged at 30xG for 50 min. at 5° C and the supernatant siphoned off leaving the platelets as a loosely packed mass in the bottom of the tube. The platelets are then resuspended in an aliquot of the supernatant plasma.

Method Three--

Gardner, Howell, Hirsch (5) plus Thomas (6) - A plastic bag devised for whole blood collections is used with 30 cc. 3% Na2EDTA. All air is excluded from the plastic bag. All bags have 10 inches of tubing ending with a double-ended nylon luer adaptor covered by a plastic shield. A 15 Gauge arquad coated male hub fenwal needle is used. This needle is designed to prevent eddy currents

at this attachment of the plastic tubing. A # 15 needle allows a rapid flow so that a 600 cc. plastic bag can be filled in 8-10 minutes. Size 17 or 19 Fenwal needles coated with arquard may be used if the recipient has small veins.

a. Direct transfusion: Withdraw 10 cc. blood in a syringe to avoid tissue contamination. The blood flows into the bag by gravity. The bag is agitated by hand during the collection. Fresh whole blood may be infused rapidly, ie. 10-15 minutes with a #15 needle. With children, a small bag, 125 cc. is given.

b. Platelet rich plasma: The filled bag is centrifuged at 1,200 rpm. for 15 minutes at 4° C. The plasma is then siphoned off. The plasma from one liter of blood is put in one bag. The plasma is chilled with crushed ice till time of administration. It takes approximately 45-60 minutes to prepare the platelet rich plasma.

c. Platelet concentrates: The platelets in the plasma are concentrated by centrifugation in the plastic bag. Af ter centrifugation, the supernatant plasma is removed from the bag by positive pressure. No preparations of aggregates or clumped platelets has been given. The volume of platelet concentrate to be transfused is determined by weighing the plastic bag. One may anticipate platelet counts in the platelet rich concentrate of 1-5 million per mm3. It takes approximately one and one half hours to prepare platelet concentrates.

Method Four--

Minor, Burnett (7, 8) and Tullis (9) - A method usind ACD blood. Basis of this method: A plasma suspension of white blood cells and platelets may be obtained from fresh citrated blood by the addition of an agent which accelerates red blood cell sedimentation. This suspension will contain most of the platelets present in the whole blood, providing platelet agglutination does not occur in the presence of this agent. Furthurmore, the addition of a surface-active agent will permit complete resuspension of the platelets following prolonged centrifugation.

Siliconized apparatus is used. Four pints of blood are used in preparing one platelet concentrate (any blood groups). The blood is collected by gravity into ACD siliconized bottles. It is processed within three hours. Blood plus 60 cc. dextran (mw. 240,000 or above) is sedimented at room temperature for two hours. The supernatant is transferred into a bottle with 20 cc. 2% triton (a non-ionic sufrace active agent) and centrifuged at 2000 rpm. for 30 minutes. 40 cc. of saline is then added to the sediment remaining after all but 10 cc. of supernatant plasma has been removed. The sediments are then pooled and mixed by vigerous shaking. The concentrate is now ready for use. The final total volume is 200 cc. Saline, 160, plasma 21, ACD 11, dextran, 6, and triton 2 cc.

Method Five--

Tullis (9, 2, 10) - Fresh donor blood is passed over a cation-exchange resin column or collected in a plastic bag containing standard ACD (acid-citrate-dextrose). The platelets complexed by resin beads are eluted or the ACD plasma separated after red blood cell sedimentation, or resin treated plasma, are passed through a plastic falling film centrifuge bowl and the separated platelets resuspended in the preserving medium. Gravity flow is used throughout, and unnecessary agitation avoided, Promptly after withdrawal, the blood is chilled to 4' C and maintained at this temperature during preservation. Preservation is best in a protein-containing hypertonic solution as no buffer is required. The total salt concentration is maintained at hypertonic levels in an effort to inhibit the premature formation of blebbed and ballooned forms. Some changes of this type inevitably occur with prolonged preservation and probably represent the normal aging process. Platelets from 500 cc. of blood are added to 40cc. of the following solution: plastic bag containing NaCl 0.85 gm./100cc, sodium acetate 0.2 gm./100 cc, sterile gelatine solution (specially prepared commercially) 2 gm./100cc, and glucose 5 gm./100cc. On transfusion, the preserving gel was liquefied and added to a small volumn of IV saline. Non-wettable surfaces were maintained in all equipment, and only intra-ABO group transfusions were given.

Method Six--

Stefanini, Dameshek (11)

Blood collected by gravity in 0.1 vol. of Sequesterene Na2 in saline

↓

centrifugation at 4° C (1000 rpm/15 min.)

↓

separation of supernatant plasma

↓

0.1 vol. of 2% triton Wr 1339 solution added

↓

centrifugation at 4° C (1000 rpm/10 min.)

↓

platelet rich plasma, relatively free of rbc and wbc

↓

centrifugation at 4° C (3000 rpm for 20 min.)

↓

platelet free supernatant plasma removed

↓

saline solution containing 0.1 vol. of 2% solution of Triton Wr1339 added in volumn equal to half the plasma originally collected

↓

centrifugation at 4° C (3000 rpm/20 min.)

↓

Packed platelets resuspended in saline solution or in other solution, added in vol. equal to 1/4 of the plasma originally collected

↓

final platelet preparation

Method Seven--

Hirsch and Gardner (12, 13) Sprague (14) Stefanini (15, 16)- Direct transfusion technique. Multiple silixone treated 50 to 100 cc. syringes are used, with # 15 Arquad coated needles. Each aliquot of blood remains outside the body 3-6 minutes, and a 500 cc. transfusion may be accomplished in 15 to 30 minutes. No clotting has been observed. Polycythemic blood is preferable.

III. EFFECT OF DIFFERENT METHODS OF COLLECTION ON THE NUMBER OF PLATELETS RECOVERED FROM WHOLE BLOOD

Dillard and Brecher (3) employing gravity flow of venous blood into siliconed centrifuge bottles via plastic tubing and sequesterene as an anticoagulant, were able to recover 70% of the platelets originally present in the whole blood. The supernatant after the first centrifugation, usually contained double the platelet count of the whole blood. A constant loss of supernatant plasma, 10%, was encountered since it could not be siphoned off without disturbing the buffy coat.

Freeman (1) collected platelets as a by-product of blood collection during which ion-exchange resin was used to prevent coagulation. Platelets were eluted with normal saline and received into siliconed lined flaskes via plastic tubing. The average concentration of recovered platelets was approximately 119,000 mm3 or about 40% of the total normal platelet complement. Actually, 88% of the platelets filtered from the blood were recovered. Between 90-95% of the total platelet complement is generally filtered out with four columns. When elution is carried out with smaller volumns of saline, ie. 25% of original blood volume, 70% of a possible total yield could be recovered. Purification and concentration were always performed at the sacrifice of absolute quantity.

Hirsch and GÅrdner (6) using platelets concentrated by centrifugation methods, Na citrate as anticoagulant, state that 50-90% of the platelets originally present in the whole blood were recovered. 1,000,000 platelets/mm3 (600 wbc and no rbc) can be prepared with this method.

Minor and Burnett (7) using platelets concentrated by the use of red blood cell sedimentation, anticoagulant ACD (accelerated by dextran), state that 80% of the platelets originally present in the ACD solution are saved. They are concentrated 8-10 times. An assay of the total volumn of concentrate, 200 cc., is 350 billion platelets. They furthur state in another paper that 85% of the platelets in normal blood may be separated out and concentrated by this method (8).

Tullis (2) states that sequestration of platelets onto resin beads (cation columns) provides the single chemical method for isolating one cell type from another. The final yield is about 30%.

The other methods of collection and preservation did not state the number of platelets recovered from the donor blood (4, 5, 11, 9, 10, 12, 13, 14, 15, 16).

IV. EFFECT OF DIFFERENT METHODS OF COLLECTION ON THE MORPHOLOGY OF THE RECOVERED PLATELETS

Platelets eluted from resin by the method described by Freeman (1) appeared entirely normal by visualization in the phase microscope. The elimination of high-speed centrifugation, washing, and resuspension, which are in present use, favors the natural state of the platelets. Also, the ease with which elution takes place and the physiological simplicity of the eluting fluid tend to maintain morphological and physiological integrity. If it is desired to eliminate red blood cells caught in the resin, the suspension may be allowed to settle in the cold for several hours or light centrifuging. Further centrifugation can be carried out at the sacrifice of morphology and individuality.

The tendencies of platelets to become sticky, to agglutinate rapidly, and to undergo morphologic alterations in shed blood are well known. Sequesterene Na2 minimized these changes whereas other anticoagulants including citrate and ACD were ineffective (3, 5, 6). Platelets prepared by the method of Dillard (3) utilizing sequesterene as an anticoagulant showed no morphologic alteration during processing, or in the final suspension, as proven by phase microscopy.

Minor and Burnett (7, 8) employing ACD blood, red blood cell sedimenting accelerator, and a non-ionic surface acting agent have recovered morphologically intact and discrete platelets.

Tullis (9, 10) commented extensively on the morphology of collected and preserved platelets. He believes isolation methods do influence morphologic change. He states that normal appearances persist lontest in platelets obtained by ACD plasma, and shortest in those obtained by resin bead elution. The significance of morphlogic changes is not clear to Tullis (9) as even the most damaged cells have some residual activity. He does state that clot retraction decreases with a decrease in size of fibrils. On studying platelets preserved up to one year in a protein containing media, Tullis (10) noted the following morphologic changes. The platelets gradually lost their filaments and sometimes there was a concurrent formation of a broad membrane around the platelet before the spicules fully disappeared. There membranous changes occured in only a small number of platelets. As storage proceeds, an asymetric bleb usually appears on the surface. These blebs generally multiply and continue to enlarge, and finally become confluent, giving the platelet a ballooned shape.

The other methods of collection and preservation did not comment on the morphology of the collected platelets (11, 12, 13, 14, 15, 16).

V. EFFECT OF DIFFERENT METHODS OF COLLECTION ON THE ACTIVITY OF THE PLATELETS IN VITRO AND VIVO

Blood collected in silicone bottles, with sequesterene Na2 as anticoagulant corrected the prolonged clotting time, prothrombin utilization, and clot retraction of platelet poor plasma (3).

Platelets collected with an ion-exchange resin returned to normal the prolonged clotting time of oxalated platelet poor plasma (1).

Hirsch and co-workers (12) believe that platelets are metabolically quite active. They therefore made every effort to chill the blood immediately on collection, during all manipulations, and during preservation to inhibit such activity.

Platelets collected using .006 M Na citrate and washed twice with Na citrate, did not appear viable in vivo (6). In vitro they produced clot retraction and normal clotting time in thrombocytopenic plasma. The serum prothrombin time, however, was lengthened far beyond that of fresh plasma containing the same number of platelets.

Washed platelets exhibit oxygen consumptiomand utilize glucose (6). They produce CO2 and lactic acid. On storage the platelets loose their ability to cause clot retraction within 24 hours. Their effect on clotting time and serum prothrombin time persists for weeks. Oxygen consumption decreased rapidly and was always zero after three days storage (6).

Platelets isolated by repeated centrifugation of ACD blood induce normal prothrombin consumption and clot retraction when added to platelet poor plasma or thrombocytopenic blood (7).

Platelets collected in ACD siliconized flasks and separated by sedimentation of red blood cells by a plasma expander are functionally active (8). On addition of 0.1 ml of concentrate to 3 ml of thrombocytopenic blood, normal prothrombin consumption and clot retraction were induced.

Tullis (9, 10) states that platelets have little metabolic activity and proven this by respiratory studies on platelets. He states that viability of a platelet can be assayed only by; retention of morphology, retention of physiologic activity, and ability to survive on reinfusion into platelet deficient recipients. Even the most damaged cells have some residual activity. Preserved platelets converted prothrombin to thrombin, and although this activity was gradually lost in storage, even badly damaged specimens retained some thromboplastic activity. When removed from the preservation medium to a saline acetate solution, the platelets deteriorated at the same rate as before preservation. There was a significant drop in activity a few days after preservation. A rapid loss in clot retraction was noted.

VI. EFFECT OF DIFFERENT METHODS OF COLLECTION ON YIELD AND LIFE SPAN IN THE RECIPIENT

Hirsch and Gardner (12) introduced human blood platelets into thrombocytopenic patients by transfusion of platelet rich polycythemic blood via silicone coated syringes and needles. Their yield per patient, average, was 90,000/mm3 platelets. The life span of the transfused platelets varied from a low of 24 hrs. (acute idiopathic thrombocytopenia) to 8 days (chronic idiopathic thrombocytopenia without splenomegaly).

Hirsch and Gardner (13) on 42 transfusions on 35 patients, noted their highest yields and longest platelet survivals in patients with aplastic anemia, acute leukemia, and pancytopenia. Lowest yields and shortest platelet survival times were seen in acute thrombocytopenia. In chronic idiopathic thrombocytopenia, yields were uniformly high, but platelet survival varied.

Gardner and Howell (5) presented data on platelet transfusions of three types - direct transfusion of whole blood, platelet rich plasma, and platelet rich concentrates. As a basis for comparison an original study was done using 100 ml. silicone treated syringes. In silicone coated syringes, the average yield in recipients was 73% (55-96%). In the transfusions with whole blood the average yield was 74%. Platelet rich plasma gave yields averaging 66% with average survival of 4-6 days. Platelet concentrates gave a

mean yield of 52% with normal survival times. The platelets from silicone coated syringes and plastic bag whole blood disappeared most rapidly during the first 24 hours. The higher platelet percentage retained for the first day after transfusion with platelet concentrates suggests that this proceedure may be of greater therapeutic value and has the added advantage that less volume need be given.

Stefanini (16) on transfusing 22 cases of thrombocytopenic purpura, 14 idiopathic and 8 secondary, with platelet rich polycythemic blood via multiple silicone coated syringes noted a survival time from 1/2 to 96 hours. Sprague and Harrington (14) using polycythemic blood in silicone coated bottles noted a survival time of 4-6 days in aplastic anemia, and only a few hours in idiopathic-thrombocytopenia. Stefanini (18) using a similar apparatus noted a survival time of three hours in idiopathic thrombocytopenic purpura; 96 hours in secondary thrombocytopenic purpura.

Tullis (10) states an average yield in patients with no antibodies of 50% of theoretical yield. These platelets are preserved in gelatin at 4' C. The life span was three days.

Stefanini and Dameshek (11) state that the length of survival depends in large measure on the immediate elevation of the platelet count induced by the transfusion, and therefore on the number of platelets successfully transfused. When a comparable

number of platelets administered or collected with various technics are transfused int such patients. With secondary thrombocytopenic purpura, the resultant yields and survival time are as shown in Figure I (11).

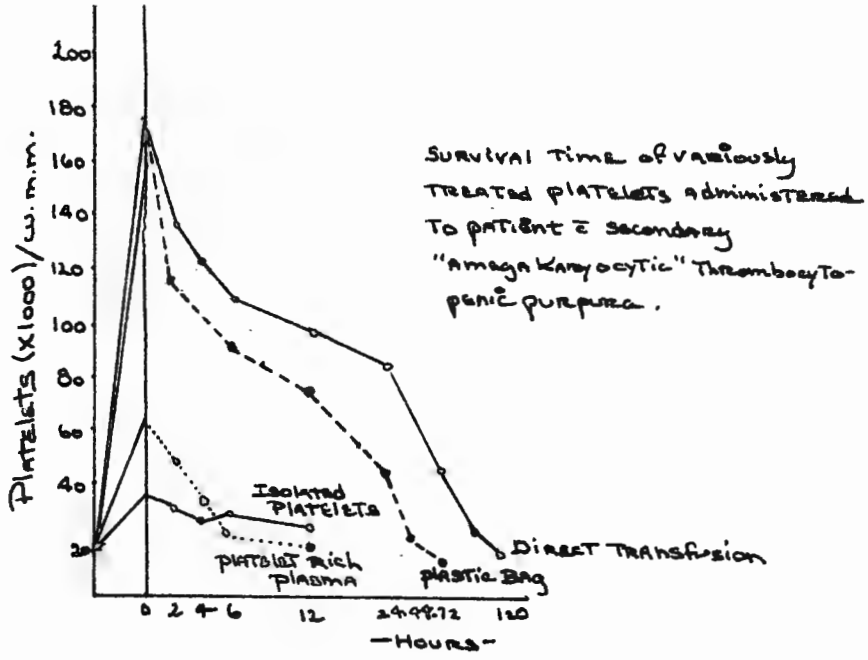


Figure I.

The other methods of collection and preservation did not state their effect on yield and life span in the recipient (1, 2, 3, 6, 7, 8, 9).

VII. PLATELET TYPES, GROUPS, & AGGLUTININS

Since 1905, when Marion (17) established the hereroantigenicity of platelets, many investigators have experimented with "typing" of platelets (17, 18, 19, 20, 21, 22, 23).

In 1923, Toda (23) tried to investigate the presence of different antigenic structures in platelets by the use of anti red blood cell human sera. After testing 40 random patients, Toda concluded that the platelets of human blood are not capable of being relegated to four groups by means of rbc grouping sera. As recently as 1954 an investigator attempted to correlate platelet groups wit h the ABO rbc grouping system, and states that platelets do correspond to and can be typed with ABO typing sera. This is the only report, however, that states a relationship does exist. Stefanini (17) who proposes 6 types and 4 groups of platelets has disproved the ABO correlation to the satisfaction of most investigators.

Harrington and associates in 1953 discussed the existence of different platelet types (20). Eight individual types were established by these authors, and one - Type I - 18% incidence - was clearly identified by absorption studies in vitro and immunization studies in vivo. More recent observations suggest the iso and auto antigenicity of blood platelets as well. A thrombocytopenic factor has been demonstrated in the plasma of some patients with thrombocytopenic purpura, idiopathic, and platelet iso and auto agglutinins

have been detected in the plasma of these patients (4, 20, 24). In one patient a platelet agglutinin titer of 1:1280 was noted. This agglutinin had continued activity after submitting the serum to acidification or alkalinization; temperatures 4° to 66° C; absorption with gels, filtration through Seitz and dialysis; absorption with human rbc and wbc, beef rbc, and guina pig kidney; storage at 4° C. The agglutinin was present in the gamma glovulin fraction of serum. When injected in humans the plasma induced; severe, sustained thrombocytopenia, with circulating large bizarre platelets, increase in the size and number of megakaryocytes with vacuolization and lack of platelet production; demonstrable platelet agglutinin in recipients serum for 12-14 days. 7 of 11 of these patients showed significant Forssman like antibodies against sheep erythrocytes.

Stefanini and associates (17, 22) show evidence for the existence of several platelet groups and types in man. By direct cross-testing techniques it was possible to determine the existence of two naturally occuring platelet agglutinins in human beings. Four groups probably exist as determined on the basis of the antigens found in platelets: I, II, III(I & II) and IV (O: no antigen). These incidences have been determined in 285 white individuals. See Table I (22).

Platelet-Groups	I	II	III (I+II)	IV - O
Platelet agglutinin -Anti I - 3.5%	+	—	+	—
Platelet agglutinin -Anti II - 2.5%	—	+	+	—
Incidence - %	10.2	4.6	3.7	81.5

Table I.

Agglutinin absorption tests and in vivo immunization tests have confirmed the presence of such groups. They are unrelated, but immunologically have the same significance as the ABO antigenic system of the rbc, since they are detected by the use of naturally occuring agglutinins.

By the use of "immune plasmas" (plasma from patients who had received repeated platelet transfusions) it has been possible to distinguish 6 serologically different platelet types (23, 24).

See Table II

Platelet Types	1	2	3	4	5	6
Plasm 1	+	+				+
2	+			+	+	
3	+			+		
4	+				+	
5	+	+				
6	+					
Incidence - %	34.42	10.28	18.43	18.28	16.16	2.43

Table II.

By agglutinin absorption techniques and in vivo immunization studies, only 3 serologic types have been confirmed, 1, 3, 6.

Since they are detected with "immune" plasma, platelet types correspond to the Rh-Hr system of the red blood cells. No antigens comparable to the M-N system of the red blood cells have been thus far detected in the platelets.

These group and type differences in platelets have important practicle aspects. One has been indicated by Harrington and co-workers (20) with the description of a type of neo-natal thrombocytopenia due to mother-fetus platelet incompatibility. Another is the all significant problem as to wheather the administration of group and type compatible platelets to thrombocytopenic patients may reduce the incidence of development of platelet agglutinins in patients receiving multiple platelet transfusions, a complication which threatens to nullify efforts toward preservation and administration of platelets to some thrombocytopenic patients for hemostatic purposes (14, 17, 22, 25). Stefanini and Dameshek (25) studied the rate of platelet disappearance or platelet survival time at regular intervals in four patients with hypoplastic and aplastic anemia receiving repeated transfusions of platelet rich polycythemic blood or of isolated platelets as supportive therapy (platelets not typed) (25). As transfusions were repeated, in three of these patients survival time of platelets became progressively shorter, administration of platelets was less and less effective in the control of the bleeding manifestations.

In one patient a platelet agglutinin was detected in the serum.

See Figure II. (25)

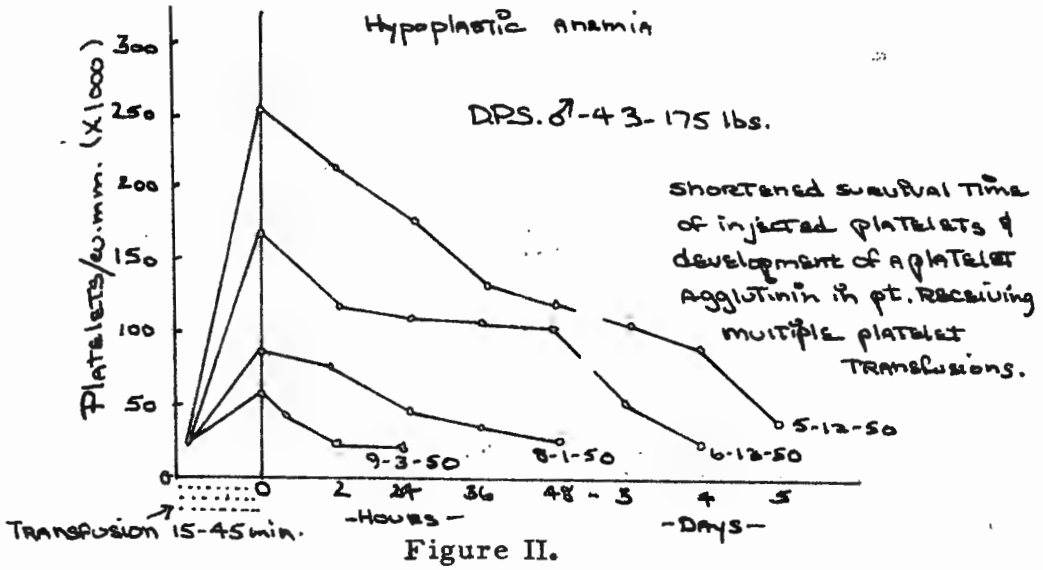


Figure II.

Hirsch and Gardner (13) also present a case having multiple platelet transfusions with subsequent decrease in yield, shortened survival time, and decreased clinical effectiveness. They do not state if an agglutinin was demonstrated in the patient's serum. See Figure III.

Development of platelet agglutinins was not prevented by ACTH and occured more promptly when viable well preserved platelets were given (17). Remissions have been produced with ACTH or cortisone and seemed to be associated with disappearance of the agglutinins (20).

It remains to be established wheather the administration of group and type compatible platelets would materially reduce the occurence of platelet iso-sensitization in thrombocytopenic states. Stefanini

(22) has noted, however, that repeated administration of compatible platelets to normal humans failed to induce iso-immunization. Otherwise normal plasma infused into a platelet incompatible recipient produced striking thrombocytopenia.

Harrington and co-workers (20) have demonstrated platelet agglutinins in the plasma of patients with chronic idiopathic thrombocytopenic purpura. Transfusions of whole blood or plasma from patients with chronic idiopathic thrombocytopenic purpara into normal recipients caused a precipitous drop in the platelet count, 16 in 26 instances. The depressed platelet level persisted 4-7 days. They also noted the marked decrease in platelet survival time of platelets injected into idiopathic thrombocytopenic purpura patients and they attributed this phenomenon to the platelet agglutinins present.

Stefanini, Dameshek, and Chatterjea (4) reported a case of idiopathic thrombocytopenic purpura, M.K., with a potent platelet agglutinin present. It was noted that the agglutinin was found in the beta 2 globulin area and represented 9.33% of the entire serum protein. M.K.'s plasma injected into a normal recipient caused striking degenerative changes of the bone marrow megakaryocytes with lack of formation of platelets, an extreme degree of platelet reduction with the development of hemorrhagic phenomenon, and a detectable platelet agglutinin in the patients serum for 12-14 days.

Adelson and Stefanini (24) demonstrated a heterologous (Forssman) platelet agglutinin in a patient with thrombotic thrombocytopenic purpura. Its significance was not determined. Stefanini also noted the presence of a Forssman like antibody in 7 of 11 patients with idiopathic thrombocytopenic purpura in 1952.

Stefanini and Chatterjea (21, 22) also report on a thrombocytopenic factor in normal human blood, plasma, or serum. They noted a temporary but significant thrombocytopenia in 32 of 36 individuals receiving ABO compatible blood plasma or serum from non thrombocytopenic donors. No spontaneous bleeding manifestations, alterations of the various hemostatic mechanisms, changes in number or activity of the bone marrow megakaryocytes, alterations of function or morphology of the remaining platelets, agglutinating or lysing activity of the recipients serum against normal platelets were noted. This thrombocytopenic effect was apparently due to a component of plasma stable at 56° C, not absorbed by Seitz filters nor by ion exchange resins, but absorbed on Ca3(PO4)2 gel from which it could be eluted with Na citrate solution.

VIII. NUMERICAL RESPONSE OR YIELD NOTED IN VARIOUS THROMBOCYTOPENIC STATES FOLLOWING TRANSFUSION

Platelets administered to two patients with leukemia raised the counts from 28,000/mm3 to 42,000/mm3, and 22,000/mm3 to 40,000/mm3. Although these yields were not great, a definate clinical response was noted (9).

On administration of repeated transfusions to a patient with aplastic anemia, no difference was noted in yield on the first three transfusions (13). However, on subsequent platelet transfusions a marked decrease in yield was noted, along with a diminution of clinical effectiveness of the transfusions. This same phenomenon was noted by Stefanini and workers (19). See Figure III. also, Figure II.

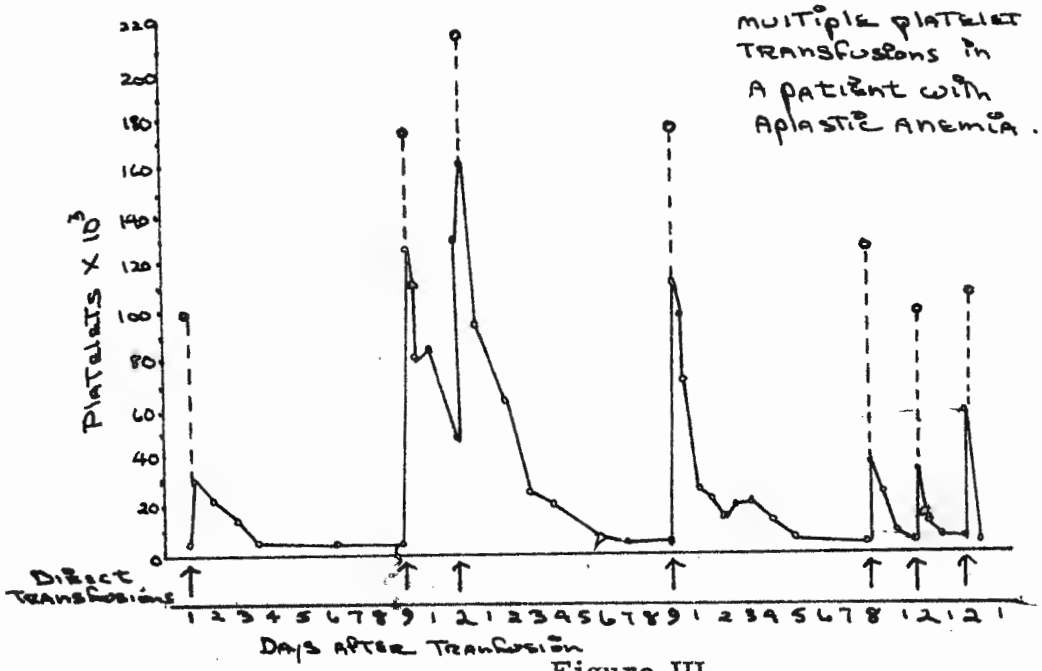


Figure III.

Hirsch (12) on transfusing a patient with aplastic anemia, noted an increase in platelet count of 90,000/mm3.

A consistantly low yield has been noted on transfusing patients with idiopathic thrombopathic purpura (4, 12, 16, 28). In one patient, the platelet count was below pretransfusion levels before the platelet transfusion was complete (4).

Hirsch (13) on transfusing 35 patients with 42 transfusions noted highest yields in patients with aplastic anemia, acute leukemia, and pan cytopenia. His lowest yields were seen in acute thrombocytopenia, and in thrombocytopenias associated with massive splenomegaly. In chronic idiopathic thrombocytopenic purpura, yields were uniformly high. See Figures III, VIII, IX, X, XI.

IX. CLINICAL EFFECTIVENESS OF PLATELET TRANSFUSIONS IN VARIOUS THROMBOCYTOPENIC STATES

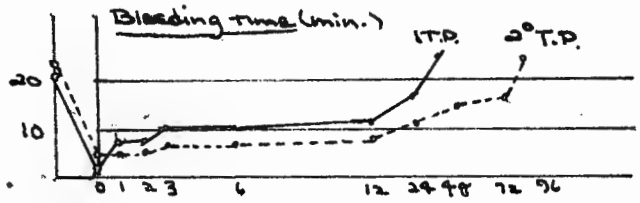
Dillard and Brecker (3) administered platelet concentrates to 3 irradiated dogs who had prolonged clotting time (30 min. to hours), a fragile, non-retractable clot and whose blood failed to utilize prothrombin. The clotting time was reduced to less than 2 minutes, the clot retraction became normal, and prothrombin was utilized. Woods, Gamble, Furth, Bigelow (26) state that the appearance of large numbers of rbc in lymph channels of dogs and rats exposed to massive doses of ionizing radiation is partially or completely prevented by transfusions of concentrate suspensions of intact homologous blood platelets. The anemia of irradiation has several factors; depression of erythropoietic centers, diversion of rbc into lymph spaces, and destruction of rbc. Platelet transfusions will not correct the first, but will certainly correct the second and perhaps some of the third. Since depression of the rbc mass amounts to only 1% even anemia will not reach critical levels if the hemorrhagic state is corrected. They state that clearing of the bloody lymph is noted within 1 hour after transfusion

Mond and Singer (27) noted that a concentrated homogenate of platelets shortened the coagulation time of hemophilic blood. The prothrombin consumption time was also increased, but this was not constant.

Many workers noted the prompt cessation of hemorrhage following a platelet transfusion (7, 9, 11, 12, 13, 14, 16, 27). They noted also that the hemostatic effect often lasted from 1 to 10 days following the return of the platelet count to pre transfusion levels. Hirsch (13) noted tha striking result of dramatic cessation of oozing from the gums and the site of needle punctures and bone marrow aspirations on transfusion of platelets. He also noted cessation of hematuria or amelioration of vaginal bleeding within 24 hours after transfusion in all patients in whom such findings were present. In one case, intracranial bleeding was stopped by a direct transfusion. When transfused platelets remained in the circulation for at least 4 days, no new petechiae and ecchymoses appeared during the life span of the transfused platelets. Bleeding from needle punctures did not resume after the platelets had dropped to their previously low levels. Similarly hematuria and menorrhagia were often relieved beyond the life span of the transfused platelets. This excellant correlation between platelet survival and the duration of improvement suggests that improvement of the hemorrhagic state was due to transfused platelets and not to some other factor in the blood.

Various laboratory tests were also altered by the transfusion of platelets to patients with thrombocytopenia (11, 12, 13, 14, 15, 16, 27). The bleeding time, Figure IV, prothrombin conversion

time, Figure V, clot retraction, Figure VI, and vascular fragility were noted to return to normal following platelet transfusions.



FigureIV.

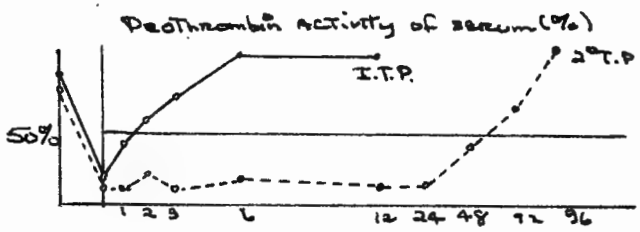


Figure V.

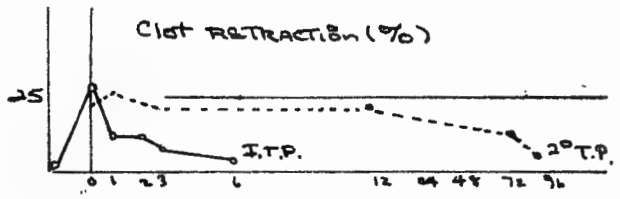


Figure VI.

The prothrombin conversion time and clot retraction were noted to closely paralled the platelet levels, again becoming abnormal with the return of the platelet count to pre-transfusion levels (15, 16). The bleeding time and vascular fragility remained normal long after (24-48 hours) the return of the platelet count to pre transfusion levels (15, 16).

Tullis (9) stated that the clinical effectiveness of platelet transfusions is far greater than would be expected from the slight rise

in total platelet count following their administration.

Remissions were noted in two of Stefanini"s (16) patients with idiopathic thrombocytopenic purpura transfused with polycythemic blood. The platelets disappeared promptly after transfusion, but within 24 hours, the platelet counts began to rise and a long-sustained remission became established. This finding suggests the possibility that polycythemic blood may contain a stimulatory principle which can determine or accelerate a spontaneous remission.

In the experience of Hirsch and Gardner (12) platelet transfusions were beneficial in acute and chronic thrombocytopenias, controlling acute hemorrhagic episodes and preparing patients for surgery.

Sprague and Harrington (14) state that on transfusion of platelets into idiopathic thrombocytopenic purpura patients, the platelets are gone in hours. The bleeding tendencies were temporarily helped, but the effect was so transitory that it would not be a satisfactory form of medical management for patients with idiopathic thrombocytopenic purpura. It might occasionally be beneficial while preparations are being made for splenectomy. The platelet survival time of 4-6 days following transfusion into patients with aplastic anemia makes platelet transfusion of practical value in helping to support them during temporary depression

in platelet formation. They are limited, however, by the fact that platelet antibodies seem to develop, so that with repeated transfusions, the platelet survival becomes progressively shorter (14, 17, 22, 25).

X. LIFE SPAN OF TRANSFUSED PLATELETS IN NORMAL & THROMBOCYTOPENIC STATES

The normal life span of transfused platelets has been determined by many methods and authors to be 5-6 days in length (5, 11, 12, 13, 28). The survival rate of transfused platelets in thrombocytopenic patients varies greatly, depending on the etiology of the thrombocytopenia. See Figure VII. (11).

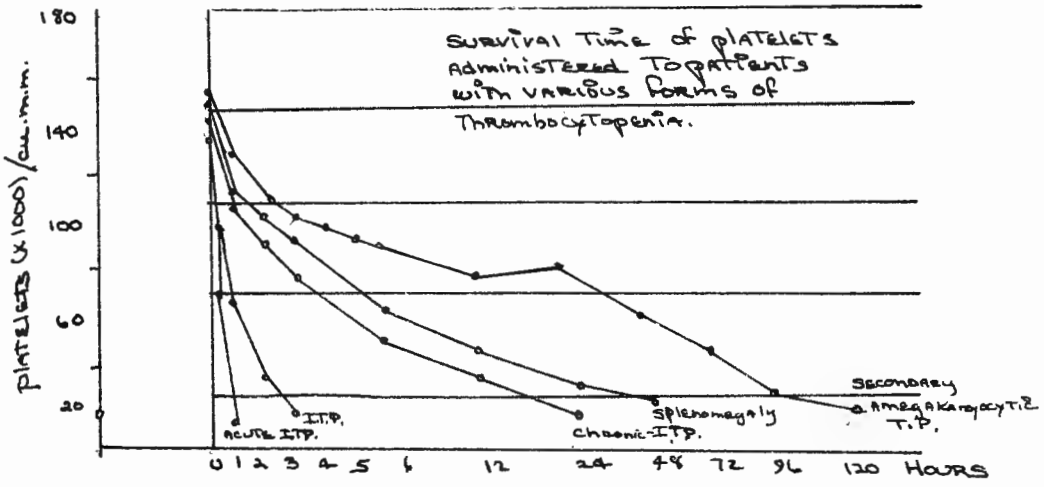


Figure VII.

The survival rate of platelets administered directly as platelet rich polycythemic blood into patients with secondary thrombocytopenic purpura is 4-5 days. It is however drastically reduced when a transfusion reaction occurs; when acute uncontrollable bleeding is present; in patients with marked splenomegaly; in cases of iodopathic thrombocytopenic purpura (especially acute); and as a result of the development of platelet agglutinins in

patients who have received multiple platelet rransfusions (11). When the platelet antibody titer is high, platelet destruction occured even in the absence of the spleen (20).

Hirsch and Gardner (13) administered 42 direct transfusiont to 35 thrombocytopenic individuals. Highest yields and longest platelet survivals were noted in the patients with aplastic anemia, Figure III, acute leukemia, and pancytopenia with normal bone marrow, Figure VIII, life span being 5-6 days. Lowest yields and shortest platelet survivals were seen in acute thrombocytopenia, Figure IX, and in thrombocytopenias associated with massive splenomegaly, Figure X. In chronic idiopathic thrombocytopenic purpura, yields were uniformly high, but platelet survival varied. It was as short as in acute thrombocytopenia or thrombocytopenia with massive splenomegaly, but platelet survival was never as long as in aplastic anemia (Figure XI). Three patients were transfused repeatedly, two of these showed decreasing yields, shortened platelet survival and lessening of clinical effect , Figures III, and VIII.

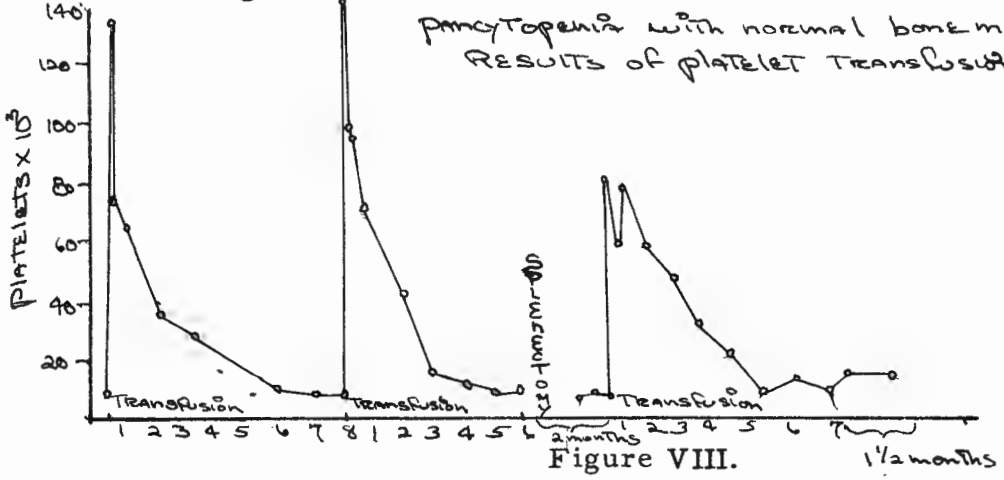


Figure VIII.

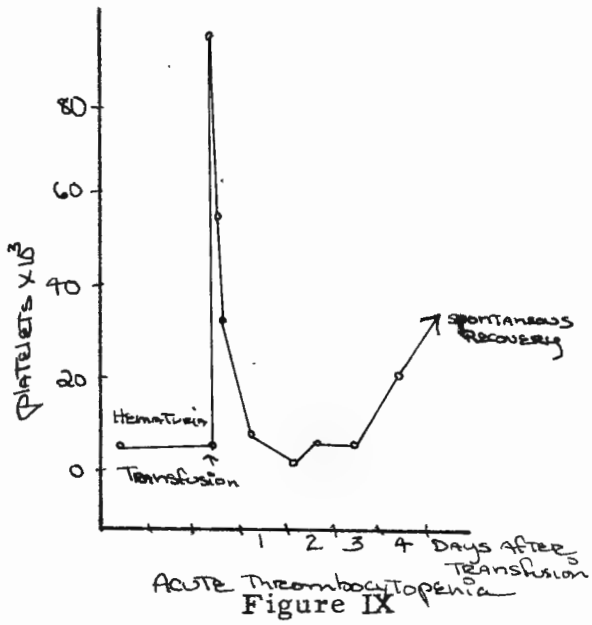


Figure IX

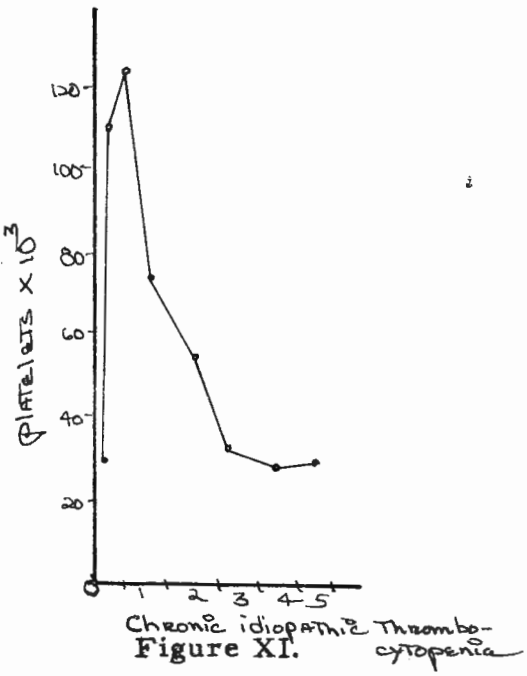


Figure XI.

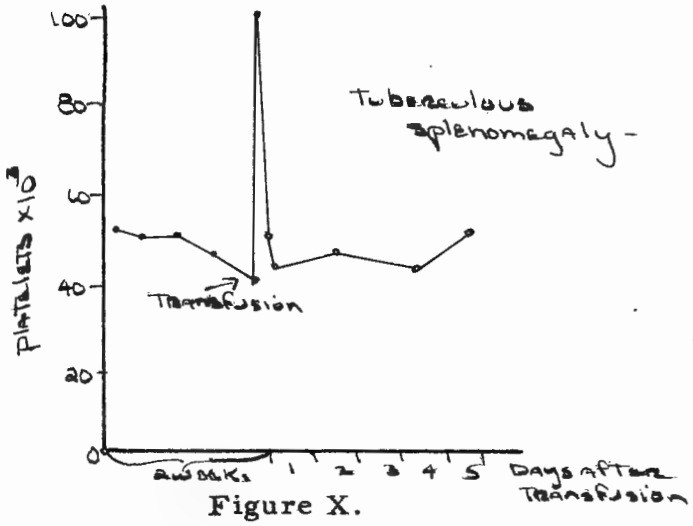


Figure X.

Harrington (20) noted normal platelets transfused into patients with bone marrow aplasia survived 4-6 days, while those infused into idiopathic thrombocytopenic purpura patients survived only a few hours.

Many workers have noted a normal survival of transfused platelets in patients with bone marrow aplasia, thrombopathic thrombocyto-

penia, aplastic anemia, acute leukemia, hypoplastic anemia, and some with chronic idiopathic thrombocytopenic puppura. (6, 9, 11, 12, 13, 14, 15, 20, 28). Short survival times of transfused platelets, some as low as two hours are noted in acute idiopathic thrombocytopenic purpura, idiopathic thrombocytopenic purpura with splenomegaly, and secondary thrombocytopenic purpura with splenomegaly (4, 11, 12, 13, 15, 16, 20, 24).

Rapid platelet destruction apparently occurs in all types of acute thrombocytopenias wheather post-infectious, idiopathic, or due to drug sensitivity (13).

Stefanini and Dameshek (4) presented a case of acute idiopathic thrombocytopenic purpura, MK, in whom the platelet level began to fall during the transfusion of platelets.

Hirsch and Workers (28) were able to accurately determine the normal platelet survival time by transfusing normal platelets into a patient with morphologically abnormal platelets (thrombopathic thrombocytopenia), and evaluating the response by stained smears. They arrived at a normal survival of 5-6 days. They also studied the normal survival time in patients with aplastic anemia, by noting the return of the platelet count to pre transfusion levels.

Stefanini (15) on studying platelet survival time in 13 cases, gave a preliminary fresh blood transfusion to limit errors due to possible utilization of platelets by platelet famished tissues.

Stefanini and Chatterjea, and Dameshek (29) also studied the role of the lung in removing platelets from the circulation in idiopathic thrombocytopenic purpura. No difference could be noted in the platelet level between the samples collected from the arterial and venous side of the pulmonary circuit.

Many other authors have also noted the decrease in life span and effectiveness of repeated platelet transfusions. (11, 12, 13, 17, 22, 25).

In patients with normal platelet survival, even widspread hemorrhages and in one case splenectomy did not appear to effect the life span of the transfused platelets (12).

XI. DISCUSSION

The technics for collection of platelets vary from simple fractional sedimentation requiring no equipment, when viable cells are not necessary, to complex, refrigerated centrifuge equipment when cells in their true state are desired.

The most common available methods for administration of platelets are as follows: Direct transfusion of polycythemic (preferably) or normal blood with silicone-coated syringes and arquad 2 C-coated needles. Administration of polycythemic (preferably) or normal blood collected by gravity into plastic bags containing acd 3.8% solution or Sequesterene 1% through plastic tubing. Administration, through plastic tuving, of platelet rich plasma obtained by low-speed centrifugation, or sedimentation of rbc after addition of plasma volume expanders. Administration of concentrated platelets, isolated by multiple low-speed centrifugations, with or without preliminary use of plasma-volume expanders, and resuspended in a small volume of their own plasma, in saline ot other isotonic preservative solutions, with the help of surface active agents, Tween 80 or Triton 1339, to prevent their irreversable agglutination. Administration of platelets collected on ion exchange resin columns and subsequently eluted from them with theuse of metal complexing agents (sodium citrate, sequesterene Na2).

Differential centrifugation is used in practically all separation methods, with the exception of the resin column technic. The resin teechnic has the advantage of collecting platelets as a by-product of blood collection, leaving the rest of the blood for other administration, but the disadvantage of a low recovery (30% as opposed to 70-80% with methods based on centrifugation). By using platelet rich plasma, or platelet concentrates, it is possible to administer larger concentrations of platelets to a patient without raising the total blood volume materially.

Na2EDTA (Sequesterene, or disodium ethylene diamine tetra acetate dihydrate) is the anticoagulant of choice in the collection of platelets. It prevents clumping and adherance to surfaces of platelets. Preservation of platelets is best in a protein-con taining hypertonic solution. This helps maintain normal platelet morphology longer, and prevents premature formation of blebbed and ballooned forms of platelets.

A progression of morphological changes occurs in stored platelets, probably representing a normal aging process. The various methods of collection etc. noted in this paper do not alter materially the morphology of the collected platelets.

Activity of the platelets in vitro and in vivo was not altered by the different methods of platelet collection. A rapid loss in clot retraction, though, on long preservation of platelets was noted.

Platelets collected by different methods have the same survival rates in comparable thrombocytopenic states. Although Stefanini (25) offers a graph, Figure I, to illustrate that various methods of collection do effect the survival time of infused platelets, it must be noted that his different transfusions were all done on the same patient, It is noted elsewhere in this paper that a decrease in the survival time of platelets on multiple transfusions is to be expected, if no platelet typing was done. Also, the yield in the recipient with different methods of transfusion varies greatly, and this would markedly effect the survival rate of infused platelets. This variation of yield is probably on the basis of a decreased yield noted on multiple platelet transfusions.
Six platelet types and four platelet groups have been proven. The four platelet groups are immunologically similar but unrelated to the ABO antigenic system of the rbc, since they are detected by the use of naturally occuring agglutinins. The six platelet types are detedted by "immune plasma", thus corresponding to the Rh-Hr system of the rbc. These group and type differences are clinically important, as the formation of potent platelet agglutinins have been reported to have occured following multiple whole blood and platelet transfusions. A thrombocytopenic factor was proven to be present in the blood, serum and plasma of 32 of 36 normal, previously untransfused people. Potent platelet

agglutinins have been noted in some patients with idiopathic thrombocytopenic purpura (this may be the etiology of their disease).

The numerical response or yield noted in various thrombocytopenic states varies somewhat. The yield is low in all cases when compared to the clinical responses noted. The yields are highest in leukemia, and aplastic anemia, lowest in acute idiopathic thrombocytopenic purpura. Yields are also low following multiple transfusions. Possible mechanisms leading to the lower than expected yields in all thrombocytopenic purpura states are; destruction or loss during actual transfusion, an increase in the patients blood volumn, therefore a relatively low count, trapping of transfused platelets in a reservoir, ie. lung or spleen, platelets utilized immediately to satisfy backlog of need for platelets for hemostasis, possible destruction of patients own platelest on infusion of type and group different platelets.

Platelet transfusions are clinically effective in all thrombocytopenic states, but the duration of effectiveness varies with the length of platelet survival, and the specific disease state being treated. On transfusion, a hemostatic effect is noted immediately, and persists from one to ten days following the return of the platelet count to pre transfusion levels. The bleeding time, prothrombin conversion time, clot retraction, and vascular fragility are returned to normal

with platelet transfusions. The prothrombin conversion and clot retraction parallel the platelet levels, becoming abnormal as the platelet count returns to pre transfusion levels. The bleeding time, and vascular fragility remain normal long after (24-48) hours) the return of the count to pretransfusion levels. Platelet transfusions are therefore beneficial in acute and chronic thrombocytopenias, controlling acute hemorrhagic episodes, and in preparing patients for surgery.

The normal life span of transfused platelets is 5-6 days. The life span of transfused platelets is markedly reduced when a transfusion reaction occurs; when acute uncontrollable bleeding is present; in patients with marked splenomegaly; in cases of idiopathic thrombocytopenic purpura (especially acute); and as a result of the development of platelet agglutinins in patients who have received multiple transfusions. Rapid platelet destruction apparently occurs in all types of acute thrombocytopenias wheather post-infectious, idiopathic, or drug induced.

XII. SUMMARY

One

Various methods of collection, preservation, and transfusion of platelets are outlined.

Two

The effect of these different methods on the number of platelets recovered from whole blood, platelet morphology, platelet activity in vitro and vivo, life span of transfused platelets, and platelet yields in the recipient are discussed.

Three

The life span of transfused platelets in different thrombocytopenic states is noted.

Four

The clinical effectiveness of single and multiple platelet transfusions is presented.

Five

A brief resume on platelet groups and types, platelet agglutinins in diseased and normal patients and their effect on platelet transfusions is given.

BIBLIOGRAPHY

1. Freeman, G.: Method for obtaining large yields of human platelets as a by-product of blood collection. Science 114:527, 1951.

2. Tullis, James, L.: Separation and purification of leucocytes and platelets. Blood 7.2:891, 1952.

3. Dillard, G.H.L., Brecher, G. & Cronkite, E.P.: Separation, concentration & transfusion of platelets. Proc. Soc. Exper. Bibl. & Med. 78:796, 1951.

4. Stefanini, M., Dameshek, W., Chatterjea, J.B., Adelson, E., Medincoff, I.B.: Studies on Platelets IX. Observations on the properties & mechanism of action of a potent platelet agglutinin detected in the serum of a patient with idiopathic thrombocytopenic purpura. Blood 8:26, 1953.

5. Gardner, F.H., Howell, D., Hirsch, E.O.: Platelet transfusions utilizing plastic equipment. J. Lab. & Clin. Med. 43:196, 1954.

6. Hirsch, E.O., Gardner, F.H., THomas, E.D.: Isolation & concentration of human blood platelets. J. Clin. Invest. 31:638, 1952.

7. Minor, A.H., Burnett, L.: A method for separating & concentrating platelets from normal human blood. Blood 7:693, 1952.

8. Minor, A.H., & Burnett, L.: Separation, concentration & transfusion of platelets from normal human blood. Federation Proc. 11:422, 1952.

9. Tullis, James L.: Preservation of platelets. Am. J. M. Sc. 226:191, 1953.

10. Tullis, James L.: The platelets of human blood: their morphology, interactions, & preservation. Blood cells & Plasma Proteins, J.L. Tullis editor, New York Academic Press, 1953.

11. Stefanini, M., Dameshek, W.: Collection, preservation & transfusion of platelets. New England J. Med. 248:797, 1953.

12. Hirsch, E.O., Gardner, F.H.: The life span of transfused human platelets. J. Clin. Investigation 30:649, 1951.

13. Hirsch, E.O., Gardner, F.H.: The transfusion of human blood platelets. J. Lab. & Clin. Med. 39:556, 1952.

14. Sprague, C.C., Harrington, W.J., Moore, C.V.: Platelet transfusions & the pathogenesis of idiopathic thrombocytopenic purpura. J.A.M.A. 150:1193, 1952.

15. Stefanini, M. & Chatterjea, J.B.: Rate of platelet survival in thrombocytopenia. J. Clin. Investigation 30:676, 1951.

16. Stefanini, M., & Chatterjea, J.B., Dameshek, W., Zannos, L., Perez, Santiago E.; Studies on platelets II. The effect of transfusion of platelet-rich polycythemic blood on the platelets & hemostatic function in idiopathic and secondary thrombocytopenic purpura. Blood:7:53, 1952.

17. Stefanini, Plitman, Dameshek, Chatterjea, Mednicoff: Studies in platelets XI. Antigenicity of platelets & evidence of platelet groups and types in man. J.Lab. & Clin. Med. 42:723, 1953.

18. Adelson, E. & Stefanini, M.: Studies on platelets VI. Demonstration and characterization of a heterologous (Forssman) platelet agglutinin. Blood 7:700, 1952.

19. Gurevitch, J., Nelken, D.: ABO groups in blood platelets. Nature 173:356, 1954.

20. Harrington, W.J., Sprague, C.C., Minnich, V., Moore, C.V., Aulvin, R.C., Dubach, R.: Immunologic mechanisms in idiopathic and neonatal thrombocytopenic purpura. Ann. Int. Med. 38:433, 1953.

21. Stefanini, M., Chatterjea, J.B.: Studies on Platelets IV. A thrombocytopenic factor in normal human blood, plasma, or serum. Proc. Soc. Exper. Biol. & Med. 79:623, 1952.

22. Stefanini, M., Plitman, G.: Antigenicity of platelets & evidence for platelet groups. J. Clin. Investigation 32:606, 1953.

23. Toda, T.: The relationship of the blood platelets & RBC; an attempt to group human platelets with RBC grouping sera. J. Path & Bact. 26:303, 1923.

24. Stefanini, M., Chatterjea, J.B., Adelson, E.: Immunologic aspects of idiopathic thrombocytopenic purpura. J. Clin. Investigation 31: 665, 1952.

25. Stefanini, M., Dameshek, W., Adelson, E.: Studies on platelets VII. Shortened survival time & development of platelet agglutinins following multiple platelet transfusions. Proc. Soc. Exper. Biol. & Med. 80:230, 1952.

26. Woods, M.C., Gamble, F.N., Furth, J., Bigelow, R.R.: Control of the postirradiation hemorrhagic state by platelet transfusions. Blood 8:545, 1953.

27. Mond, E., Singer, K.: Effect of platelet extract of hemophilic blood. J. Clin. Investigation 30:77, 1951.

28. Hirsch, E.O., Faure, Gilly, J., Dameshek, W.: Thrombopathic thrombocytopenia: Successful transfusion of blood platelets. Blood 5:568, 1950.

29. Stefanini, M., Chatterjea, J.B. & Dameshek, W.: Studies on platelets V. Observations on the removal by the pulmonary circulation of platelets injected into patients with idiopathic thrombocytopenic purpura. J. Lab. & Clin. Med. 39:865, 1952.

ADDENDUM: ILLUSTRATIONS OF A BRAND OF COMMERCIALLY AVAILABLE PLATELET TRANSFUSION EQUIPMENT.

Fenwal Platelet-Pack
Syringe & Needle

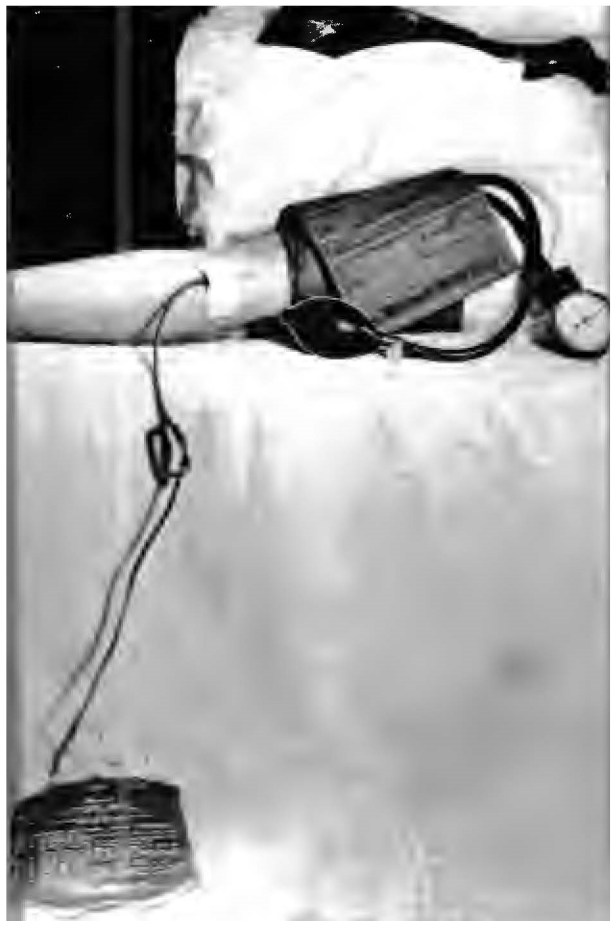
Needle Pack
Platelet Recipient Set

Platlet-Pack Syrlnge

Filled & Centrifuged Pack in Pack Rack

Transfer Pack

Platelet Reciplent Set Needle Pack



COLLECTION OF BLOOD IN PLATELET-PACK