HEMORRHAGE IN LEUKEMIA.
WITH SPECIAL REFERENCE TO PLATELET TRANSFUSIONS.

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Episodes of bleeding from mucous membranes or into the skin are a common manifestation in patients with all types of leukemia. Hemorrhage is the major cause of death in about 50 per cent of patients with acute leukemia.⁽¹⁾ The most common site of fatal hemorrhage is intracranial^(2,3) with subarachnoidal and subdural bleeding being most frequent. Although thrombocytopenia is the most common cause of bleeding in leukemia, all four basic mechanisms of hemorrhage have been found operative in this disorder: increased vascular fragility, clotting factor deficiencies, activated fibrinolysis, and thrombocytopenia.

Vascular hemorrhage caused by parietal and perivascular infiltrates of leukemic cells was particularly studied by Frugoni of the University of Rome around the turn of the century.^(4a) It was later postulated that capillary damage in leukemia may also result from lysins^(4b) and anoxemia.^(4c) Bleeding due to infiltration of blood vessels is, however, comparatively rare in leukemia.⁽⁷⁾

Although intracranial hemorrhage in leukemia may have various mechanisms, it is more frequent in patients with white cell counts higher than 100,000 to 300,000 per cu mm.^(2,3) The risk of intracranial bleeding increases in patients with a white cell count above 100,000 per cu mm, even if the platelet count is normal. The height of the preterminal white cell count correlates well with the amount of intravascular leukostasis and infarction in the cerebrum observed at autopsy.⁽⁵⁾ The peculiar structure of in-

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tracranial blood vessels seems to predispose to this complication.⁽⁶⁾
The obvious inference in relation to therapy is that measures to reduce the leukocyte count promptly are important in patients with acute leukemia with white cell counts above 100,000 per cu mm.

Isolated coagulation factor deficiencies in leukemia are a rare occurrence. They have usually been attributed to hepatic disease from leukemic infiltration or drug toxicity. Factors II, V, VII, IX, X and fibrinogen have been found decreased in isolated patients by a number of investigators among which are Manai and Mandelli in Italy,⁽⁸⁾ Lewis et al⁽⁹⁾ and Gralnick and Henderson⁽¹⁰⁾ in the U.S., and others. In the cases described, the mechanism of hemorrhage has not always been clear because excessive utilization of clotting factors as in disseminated intravascular coagulation (D.I.C.) or accelerated destruction as in the case of activation of the fibrinolytic system, was not completely ruled out in all patients. This can be particularly difficult in some cases of atypical and chronic forms of D.I.C. or of activated fibrinolysis.

Investigation of the coagulation system in acute leukemia is particularly important because treatment of hemorrhage often depends on the precise diagnosis of its mechanism. In fact, the finding of hypofibrinogenemia may be due to 1) defective hepatic synthesis as in cases with liver damage. In this case it will require replacement therapy with fibrinogen, specific plasma fractions, fresh-frozen plasma or fresh blood. Fresh blood in addition to fibrinogen is more likely to control bleeding since the hemorrhagic diathesis seen in these patients is often caused by multiple deficiencies.⁽¹¹⁾ Hypofibrinogenemia may also be caused by:

2) excessive destruction, that is fibrinolysis due to activation of plasminogen or lack of antiplasmin, in which case treatment with a plasminogen inhibitor (ϵ -aminocaproic acid, EACA) is adopted. It may be caused by: 3) D.I.C. with excessive consumption of fibrinogen and clotting factors, in which case hemorrhage is paradoxically arrested by the use of heparin.

In recent years, several cases of severe hemorrhage due to hyperfibrinolysis have been described in leukemic patients. (12-18)

In most of these cases increased blood fibrinolysis was clearly demonstrated, in others it was only suspected. In a study of 131 leukemic patients, an overall increase in fibrinolytic activity was found in 33% of patients by Girolami and Cliffton (16) and was more frequent (36%) in patients with acute leukemia than in patients with chronic leukemia. Fever and chemotherapy did not appear to influence significantly the behavior of blood fibrinolysis. Brakman et al. (19) found evidence of fibrinolysis in several of 26 patients with untreated acute leukemia, and evidence of D.I.C. could not be obtained in any of the patients. In most patients, the increased fibrinolytic activity was probably secondary to increased activator activity. The origin of this activator is unknown. It has often been thought that liberation of activator from intact leukemic cells or from cells undergoing destruction (possibly from lysosomes) could be the cause. It has been known for some time that leukocytes have proteolytic activity, (20-22) but the importance and potency of leukocyte proteases in some cases of acute leukemia has probably not been fully realized. A significant case was recently studied by Talarico, at Boston University. (23) This was a 67 year old man with myelomonocytic leukemia presenting severe bleeding man

festations due to activated fibrinolysis and amegakaryocytic thrombocytopenia. The increased fibrinolytic activity was demonstrated by the presence of systemic plasmin most likely produced or activated by the leukocytes in the blood. There was no evidence of D.I.C. Smears of washed white blood cells suspended in human albumin were prepared. The dried smears were covered with a solution of thrombin and immediately thereafter, with a layer of fibrinogen.⁽²⁴⁾ After a uniform film of fibrin was obtained, the slides were incubated two hours at 37°C, then fixed for one hour in formalin. They were finally washed and stained with Wright and Giemsa stains. Surprisingly, a wide halo of light diffraction could be seen around the myelo- and monocytes of a diameter many times larger than the individual cells. It is, therefore clear, that in selected cases of acute leukemia myeloblasts, promyelocytes and monoblasts can demonstrate a pronounced fibrinolytic activity. It is, furthermore, known that bacteria may, at times, release kinases as well as proteases able to activate fibrinolysis and proteolysis.⁽²⁵⁾ The frequency of infection in leukemia may in part be responsible for increased fibrinolysis in some patients. Determination of the fibrinolytic activity of the blood is, therefore, mandatory in every leukemic patient with a pronounced bleeding tendency. The administration of epsilon aminocaproic acid has been effective in several patients.⁽¹⁸⁾

Disseminated intravascular coagulation (D.I.C.) has been reported most commonly in acute promyelocytic leukemia⁽²⁶⁻²⁹⁾ although a few patients with acute myeloblastic leukemia, myelomonocytic leukemia and acute stem cell leukemia have also been described.⁽³⁰⁻³⁴⁾

In many of these patients, evidence for systemic hyperfibrinolysis

could not be obtained and the low plasminogen values and the fibrin-fibrinogen degradation products returned to normal after the initiation of heparin therapy. From some of the recent studies⁽³⁵⁾ it would appear that D.I.C. in acute leukemia is a common event although often subclinical or compensated. In subclinical D.I.C. there are some isolated, positive laboratory findings (e.g. increased fibrinogen-¹²⁵I catabolism, increased plasma euglobulin fibrinolytic activity) and consumption of fibrinogen and other clotting factors occurs without evidence of hemorrhage. In compensated D.I.C. also there is no hemorrhage, but here laboratory findings are more clearcut. This condition can be considered a compensated state in which increased consumption is balanced by increased synthesis of coagulation factors. In this condition, rapid clearance of activated clotting factors and secondary fibrinolysis prevent fibrin deposition and organ impairment. In symptomatic D.I.C., many or all of the classic clinical signs are present with bleeding, oliguria, renal failure, thrombocytopenia, unresponsiveness to platelet transfusion. After heparin therapy, the coagulation factors and fibrinogen rise and bleeding is either reduced or stopped.

One must admit, however, that the three classic hemorrhagic syndromes in leukemia due to: 1) reduced hepatic synthesis of fibrinogen and clotting factors, 2) primary fibrinolysis, without evidence of increased consumption of fibrinogen and clotting factors, and 3) symptomatic D.I.C., are often seen in acute leukemia, but are confronted by at least an equal number of cases in which data are variable and interpretation is difficult, suggesting that hemorrhage in acute leukemia is often the result of a fortuitous

(11)
combination of hemostatic defects. In these patients, therapeutic trials associated with continuous testing and close observation are the only conduct that the physician can wisely follow. (19)

Marked elevation of blasts and of total white cell count in the peripheral blood (19,30,35) is often associated with major bleeding manifestations in leukemic patients. In these cases, hepatosplenomegaly and/or lymphadenopathy are usually pronounced reflecting widespread leukemic cell infiltration. Serum L.D.H. is often increased indicating rapid leukemic cell destruction. In many of these patients it can be demonstrated that D.I.C. plays an important role in the hemorrhagic diathesis. (35) But in some of these patients, D.I.C. (30) occurred when chemotherapy induced massive destruction of blasts. This suggests that leukemic blasts may contain procoagulant activity. (36a) similar to that of normal polymorphonuclear leukocytes. However, this has not yet been clearly documented. Whether hemorrhage is already present or not, markedly elevated white cell counts in acute leukemia demand immediate and intensive chemotherapy to lower the counts although this may intensify or, even, induce, at times, D.I.C. requiring prompt anticoagulation and platelet transfusions. It is clear, therefore, that patients with acute leukemia and white cell counts above 100,000 per cu mm must be routinely evaluated for coagulation abnormalities prior to and during chemotherapy. Fever when due to bacterial infections, has also been found to be related to bleeding in patients with acute leukemia. (13,36b) This may result from the anticoagulant effect of bacterial polysaccharides demonstrated in vitro (36c) or to the sweeping up of platelets caused by bacteremia, (36d) or to stimulated fibrinolysis by bacterial proteases (25) or to D.I.C. triggered by the severe infection.

It is without doubt that thrombocytopenia is the most frequent cause of hemorrhage in leukemia. Qualitative platelet defects important enough to cause bleeding in the presence of a normal platelet count are rare. ^(36e, 36f) Thrombocytopenia is the major cause of death in leukemia. ⁽¹⁾ The availability of an adequate supply of platelets for transfusion, either for prophylaxis or for replacement purposes, is most important during the periods of intensive chemotherapy. Prior to the use of repetitive platelet transfusions, the percentage of hemorrhagic deaths in a five year period (1954 to 1959) was 22% in the Acute Leukemia Service of the National Institutes of Health, and declined markedly to about 14% in the 1960 to 1963 period, when repetitive platelet transfusions were introduced into the therapy program. Similar striking declines in the frequency of hemorrhage have been noted in other clinics in which major bleeding as the cause of death declined from 63% to only 15% in platelet treated patients. ^(36g) These results have more recently improved further with the more expanded application of platelet transfusion therapy. However, we must recognize that during the past decade chemotherapeutic methods have also improved and more rapid and prolonged remissions have been obtained with reduction in hemorrhagic tendency.

There is a quantitative relationship between platelet count and clinical hemorrhage in patients with acute leukemia, ⁽³⁷⁾ but there is no secure "threshold level". In general, gross bleeding is rare with counts above 50,000 platelets per cu mm, it is likely with counts below 30,000, but it is very common with platelet counts below 5,000 per cu mm. ⁽⁶⁾ Simone in Memphis has recently shown that only a small number of platelet transfusions are needed to prevent and/or

arrest hemorrhage during the period of remission induction of acute leukemia.⁽³⁸⁾ More transfusions are usually needed in adults than in children and, even more, during the periods of relapse. The number of platelet transfusions to be given to a bleeding patient is the number necessary to arrest hemorrhage. At times, this may require 6 to 12 units of platelet concentrates in the adult patient, at times even more. When platelet transfusions are given for preventive purposes, the rule has varied and we have obtained good results with the infusion of two to three platelet concentrates at alternate days. Others⁽³⁹⁾ have regulated the number of platelet concentrates with the daily platelet count and maintained it above 30,000 per cu mm during intensive chemotherapy.

Assessment of platelet transfusion therapy is still empirical, but it is usually based on two important principles: 1) that there must be an increment in platelet count in the patient's circulation following platelet infusion, and 2) that hemorrhage when present, must be reduced or arrested. For an average child of 1 square meter of body surface, transfusion of platelets from 4 units of blood (4×10^{11} platelets) will give increments of 40,000 to 60,000 platelets per cu mm, and treatment twice weekly will maintain the platelets above 20,000 per cu mm for most of the time.⁽⁴⁰⁾ Institution of such program⁽¹⁾ has demonstrated a sharp reduction in the frequency of hemorrhage in patients under therapy for acute leukemia. The development of polyvinylchloride plastic blood packs and of double and triple pack systems has made blood collection and platelet separation and transfusion a relatively safe and easy procedure, and has favored expansion of platelet transfusion therapy. The prepara-

tion of platelet concentrates by the American National Red Cross (41) has increased from 79,000 units in 1969 to 286,000 units in 1972. Most of these preparations were used in the treatment of leukemia.

It has been clearly demonstrated that to be effective on hemostasis platelets must be viable, that is capable of circulating and surviving upon infusion. (42) Platelets which cannot circulate and survive because of damage during collection and separation, because of the presence of antiplatelet antibodies in the circulation of the recipient or because of prolonged storage before reinfusion, have no effect in arresting hemorrhage due to thrombocytopenia.

It is now known that ACD is the anticoagulant of choice for the collection and preparation of platelet concentrates and that EDTA is deleterious to platelets. (43) Platelets survive better in the patients if they are ABO blood group compatible, (44) but there is no apparent need for compatibility of the rhesus antigen. (45) However, we demonstrated in 1961 and 1962 that platelets have a complex antigenic structure and that they contain histocompatibility antigens. In one series of experiments (46) we measured the survival of isogeneic platelets in the circulation of rabbits previously sensitized by one to three skin homografts. After three successive skin homografts, survival of platelets collected from the skin donor and infused in the homografted animal was consistently reduced. In a second series of experiments, (47) the rejection time of skin homografts was measured in rabbits previously sensitized by one intradermal injection of isogeneic blood platelets. The skin homografts were rejected significantly earlier in the platelet sensitized rabbits than in non-sensitized animals. These results demonstrated that skin tissue and blood platelets share antigens in common and that blood platelets contain

histocompatibility antigens. Further studies in human volunteers⁽⁴⁸⁾ published by us in 1962 showed that two to eight infusions of donor ABO blood group compatible platelets, equally spaced in time, caused platelet isosensitization in all the recipients as demonstrated by reduction in the life span of ⁵¹Cr-labeled donor platelets infused in the volunteers. A pair of "non-identical" twins was also studied. They had "identical" red cell antigens (28 antigens tested). After six infusions of platelets from the donor twin, the recipient brother showed marked reduction in viability of the infused "fraternal" platelets (Fig. 1). These results clearly indicated that reduction in survival and, therefore, reduction in hemostatic effectiveness of transfused platelets would be an unavoidable event in multitransfused patients. This conclusion was later confirmed by the characterization of multiple platelet specific isoantigens by Shulman⁽⁴⁹⁾ and by the demonstration by Yankee et al⁽⁵⁰⁾ of refractoriness to multiple platelet transfusions in patients with aplastic anemia and by the effectiveness in these patients of transfusions with platelets collected from family donors or donors selected by lymphocyte HL-A matching. Although patients with acute leukemia receive chemotherapeutic agents which are also immunosuppressive, and are therefore less prone to developing isoimmunization to platelets, they can still develop it after repetitive infusions for prolonged periods of time. We have seen this on a number of occasions and conclude, therefore, that donors selected from closely related family members and, preferably, by the method of lymphocyte HL-A matching should be utilized in the future. Proper selection of donors has recently indicated that platelet transfusions can be effectively utilized for

months if the recipient is compatible by HL-A matching. (50)

The continuing expansion of platelet transfusion therapy and the indication for selected donors have created important limitations to the availability of platelets for transfusion. These limitations may probably be resolved in the future by improved and more widely used HL-A typing, by the use of efficient cell separators which can allow repeated withdrawals of 4 to 6 platelet units with one venesection from a single donor every week or, ~~even,~~ biweekly, and by the availability of improved methods of platelet preservation.

Presently, platelets are used as fresh preparations within a few hours from collection. Platelet storage at non-freezing temperatures is presently inefficient because at 4°C platelets lose viability to significant degrees in a few hours to one day. (51) There is no method available as yet which can prevent the platelet damage caused by chilling. At room temperature, platelet viability is well preserved for one or two days, but platelet function is partially lost (52, 53) and the capacity to increase the platelet count in thrombocytopenic recipients is considerably reduced. (54)

During the past two years we (55,56) and others (57) have demonstrated that human platelets stored after controlled freezing with 5% dimethylsulfoxide (DMSO) in polyolefine plastic containers maintain a nearly normal viability as assessed by their in vivo survival after labeling with ^{51}Cr . In more recent studies, (58) we have demonstrated a nearly normal hemostatic effectiveness of frozen human platelets (Fig. 2). The hemostatic effectiveness was studied by measuring the bleeding time by the template method devised by Mielke (59) et al before and at regular times after infusion of four isogeneic

platelet concentrates (63×10^9 platelets per concentrate, on the average) which had been preserved for one to four days by freezing with 5% DMSO. The 11 patients studied had severe and stable amegakaryocytic thrombocytopenia due either to idiopathic aplastic anemia or to chemotherapy for acute leukemia. The platelet count in the patients varied between 16,000 and 40,000 per cu mm before infusion of the frozen platelets. Nine of the eleven patients studied had a bleeding time longer than 25 or 30 min and the other two had a bleeding time of 19 and 21 min, respectively (normal 4 to 8 min). Fifteen min after infusion of the frozen platelets there was a remarkable shortening of the bleeding time to an average value of 14 min. The bleeding time remained above 30 min in only one patient. It was surprising to see, however, that in all patients the bleeding time became shorter three hours post-infusion with an average value of 10 min closely approaching the normal range. After 24 hours the bleeding time was still shorter than before infusion. It had a mean value of 19 min in eight patients while it was longer than 25 min in one and longer than 30 min in two patients. Increases in the circulating platelet counts occurred in all patients. The mean platelet increase was maximal 15 min after infusion and was of 14,700 platelets per cu mm. It decreased thereafter becoming 12,000 per cu mm 3 hours, and 5,500 per cu mm 24 hours post-infusion. These values were similar to those observed with the infusion of freshly prepared platelet concentrates. There was a discrepancy between effect on hemostasis which was more pronounced 3 hours than 15 min post-infusion, and the platelet count which showed its highest increase 15 min post-infusion. This time discrepancy between maximal increase in platelet count and

maximal effect on hemostasis was not seen with freshly prepared platelets and clearly indicated that the frozen platelets carried a functional lesion which impaired more their hemostatic effectiveness than their capacity to survive in the circulation. The frozen platelets however, could rapidly recover from this functional impairment reaching maximal hemostatic effect 3 hours post-infusion. In subsequent experiments, we could demonstrate also in vitro the functional impairment of the frozen platelets which showed reduction in aggregation with ADP and collagen, reduction in the in vitro reaction to hypotonic shock and in the in vitro serotonin uptake.

The results so far obtained are of practical significance and indicate that by freezing with DMSO human platelets can be preserved viable probably for unlimited periods of time and can usefully be utilized for large scale application in thrombocytopenic patients. So far, however, the uncontrolled use of these platelet preparations in patients has been prevented in the U.S. by the fact that after thawing and washing with platelet-poor plasma, the platelet concentrates still contain trace amounts of DMSO (21 to 78 mg per unit). Although there is no evidence that these small quantities of DMSO can cause any deleterious effect in human beings or animals, the Federal Drug Administration has not yet lifted the ban against DMSO because a few years ago, this chemical was shown to be toxic when administered to animals in large doses. We expect, however, that this regulation will be changed in the near future and that the wide-spread use of frozen human platelets will reduce the present shortage of platelets for transfusion purposes and aid in the treatment of leukemia and other malignancies.

Fig. 1 A non-identical twin was transfused six times with one platelet concentrate collected from his twin brother. Although the red cells of the two twins had identical antigens (28 tested), platelets were not identical. Survival of platelets from the donor-twin in the recipient brother was normal (EDTA method) with the first infusion, but became reduced to minimal values after six infusions (left). Isoimmunity was rather specific since platelets from a new, unrelated donor, given with the seventh infusion, had normal survival in the sensitized twin (right). (Baldini, M.G. et al., Proc. Europ. Soc. Hematol., Vienna 1961, Karger, New York, 1962, p. 378).

Fig. 2 Hemostatic effectiveness of human platelets previously frozen with 5% DMSO in plasma and infused in 11 thrombocytopenic patients. Bleeding times (Mielke's Technique) measured before and three times after platelet infusion are reported in the upper portion of the chart, while increments in platelet counts are in the lower portion. Before infusion, most of the patients had bleeding times longer than 25 to 30 minutes. After infusion of the frozen platelets, the bleeding time became greatly shortened in all patients. (Baldini, M.G. and Ebbe, S. "Platelets: Production, Function, Transfusion, and Storage." Grune and Stratton, Inc., New York, 1974 (in press).

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