Studies on Thrombopoiesis. 1. A Factor in Normal Human Plasma Required for Platelet Production; Chronic Thrombocytopenia Due to its Deficiency

By Irving Schulman, Mila Pierce, Abby Lukens and Zinet Curbimbho

THE DEMONSTRATION and characterization of a humoral erythropoietic factor raises the possibility that production of other cellular elements of the blood also may be regulated by circulating factors. The studies here to be presented indicate the presence of a factor in normal plasma which stimulates platelet production in a case of chronic thrombocytopenic purpura.

CASE REPORT

C. M., an 8 year old girl born in Germany, was noted to have a large ecchymotic area on the dorsum of one hand immediately after birth. No other hemorrhagic signs developed, and the infant seemed entirely well until the age of 1 year, when numerous petechiae were noted on the trunk and microscopic hematuria was detected. A urinary tract infection was diagnosed, and antibiotics were prescribed after which the petechial eruption and hematuria cleared. In 1953, at age 20 months, the patient developed severe epistaxis which required hospitalization. Blood and bone marrow examination led to a diagnosis of idiopathic thrombocytopenic purpura; the nose bleed ceased after transfusion of whole blood. Throughout the following year intermittent petechial eruptions were noted on the extremities, and in March, 1954 severe epistaxis again required hospitalization. On this occasion the child was given intramuscular injections of 5 ml. amounts of fresh whole blood obtained from the father, mother and grandmother and two whole blood transfusions of 250 ml. each. In May, 1954 the family prepared to move to the United States, and the parents were told that the child's platelet count was 60,000 cu.mm.

On May 13, 1954, three days after arrival in the United States, the child was admitted to The Children's Memorial Hospital. The family history was negative for any type of hemorrhagic diathesis. The patient's mother had a daughter by a previous marriage who was entirely well. There were no other children in the family.

Physical examination revealed a well developed, well nourished, slightly pale girl exhibiting numerous petechiae on the face, trunk and upper and lower extremities and scattered ecchymoses on the legs. There was no glandular enlargement, hepatosplenomegaly or jaundice. The remainder of the physical examination was normal.

Laboratory results were as follows: hemoglobin 10.0 Gm. 100 ml., RBC 3.0 million, cu. mm., WBC 10,000 cu.mm., reticulocytes 3.9 per cent, platelet count 80,000 cu.mm., bleed-
ing time over 15 minutes, clotting time 7 minutes. Bone marrow examination revealed increased numbers of megakaryocytes, predominantly of immature, nonplatelet-producing type. The marrow was otherwise normal.

During the two weeks following hospitalization the child was treated with cortisone and with 2 whole blood transfusions of 250 ml. each, as shown on figure 1. Because of the long history and the persistent thrombopenia, splenectomy was performed on May 28, 1954. The platelet count rose to 330,000/cu.mm. on the first postoperative day and then fell to previous thrombopenic levels within 1 week following surgery. During the next seven months, continued therapy with cortisone and ACTH failed to raise the platelet count, which ranged from 20,000 to 60,000/cu.mm. In this interval the patient constantly demonstrated numerous petechiae and ecchymoses, but serious hemorrhagic manifestations did not occur. Steroid therapy was discontinued in December, 1954 and two weeks later (January 4, 1955) a febrile episode associated with sore throat was followed by severe epistaxis which necessitated hospitalization. On admission mild icterus of the skin and sclerae were noted for the first time, and the blood findings suggested a hemolytic component in the anemia. The hemoglobin concentration was 7.7 Gm./100 ml., RBC 2.42 million/cu.mm., reticulocytes 9.4 per cent, WBC 13,050/cu.mm., platelets 19,000/cu.mm. Cortisone 100 mg. daily was resumed, and two transfusions of 250 ml. each of whole blood were given. Four days later, as shown in figure 2, the platelet count was 430,000/cu.mm. and 8 days after admission 670,000/cu.mm. Thereafter the platelet count fell, returning to previous thrombopenic levels within 23 days after transfusion. Since the dose of cortisone had been reduced from 100 to 50 mg. per day after the first six days of therapy, it was thought that the fall in platelet count resulted from the lowered dosage. Therefore cortisone was again raised to 100 mg. daily, but a rise in platelet count did not occur. During the next seven months maintenance doses of cortisone, 50 to 75 mg. daily were tolerated well, and although no severe hemorrhagic episodes occurred, petechiae and ecchymoses were present most of the time and minor fluctuations in hemoglobin concentration, a constant elevation of reticulocytes and the presence of Howell-Jolly bodies in the erythrocytes suggested that a chronic hemolytic process was active. Direct and indirect Coombs tests were negative. At the end of August, while the patient was receiving 75 mg. of cortisone daily, severe epistaxis recurred and required hospitalization. At this time the hemoglobin concentration was 6.9 Gm./100 ml., RBC 2.1 ml./cu.mm., platelets 59,000/cu.mm. Two transfusions of 400 ml. each of whole blood were given and ACTH, 40 U. intramuscularly daily, was started. Again a rapid rise in platelet count to 520,000/cu.mm. occurred within 10 days, followed by a sharp fall to 14,000/cu.mm. within 18 days after transfusion. Mild icterus was noted during the period of falling platelet count. On continued therapy with steroids the platelet count remained between 60,000 and 100,000/cu.mm., but six weeks later, during prednisone therapy, another exacerbation of purpura, epistaxis and icterus occurred. Two transfusions of 350 ml. each of whole blood were followed by an extraordinary rise in platelet count to a peak of 700,000/cu.mm. in eight days. Twenty days after transfusion the platelet count was again 20,000/cu.mm.

At this time it occurred to one of us (M. P.) that the rapid elevation in the platelet count following transfusions was due to the effect of plasma and that the sharp fall in the platelet level which was noted to appear at regular levels after the infusion of the blood might be due to the disappearance of the transfused plasma from the circulation. In order to test this hypothesis, steroid therapy was discontinued, and a series of transfusions using whole blood and fresh, banked or frozen plasma were used in therapeutic trials (see below).

Subsequent Clinical Course

During the period from 1955 through 1959 the patient's clinical status was influenced to a great extent by the investigations which were carried out. As demonstrated under Results, it was possible to induce repeated and predictable platelet responses by injections of plasma at regular intervals for as long as six months. During these periods the patient...
was free of major hemorrhages and demonstrated only slight cutaneous purpura and occasional mild epistaxis as the platelet counts returned to thrombocytopenic levels after an induced rise. During periods of sustained thrombocytopenia in the course of control studies and investigations designed to study the effects of corticosteroids, varying doses of plasma and other therapeutic measures, constant purpura was evident and severe epistaxis, requiring hospitalization for blood or plasma transfusion, occurred on seven further occa-
sions. In each instance transfusion was followed by a rise in platelet count and cessation of the hemorrhagic manifestation.

Two particular aspects of the patient's clinical course— infection and jaundice—require special mention. Repeated observations demonstrated that the occurrence of infection resulted in a precipitous fall in platelet count within 24 to 48 hours from the onset. The thrombopenic response appeared independent of the platelet count at the time of occurrence of infection and was noted with infections occurring 4, 9 and 16 days following administration of plasma. Likewise, the fall in platelet count seemed unrelated to the specific type of infection or offending organism and has been noted following tonsillitis, bronchitis and otitis media associated with isolation of beta hemolytic Streptococcus, coagulase positive Staphylococcus, Hemophilus influenzae, and Pneumococcus from nose and throat cultures.

During the five years of observation the patient has exhibited seven episodes of jaundice. One of these (August, 1956) seemed definitely to be due to hepatitis as indicated by a 3+ cephalin flocculation test, predominant elevation of the direct-reacting bilirubin, normal reticulocyte count, enlarged, tender liver, and light colored stools. The other six episodes (four before and two after the hepatitis) suggested a hemolytic pathogenesis and were associated with negative cephalin flocculation tests, elevation of the indirect-reacting bilirubin, a fall in hemoglobin concentration and a reticulocytosis ranging from 3.5 to 43 per cent. On four of these occasions severe epistaxis and hematemeses immediately preceded the onset of icterus, and in three of these instances an upper respiratory infection was also present. With the other two episodes of jaundice, anemia and reticulocytosis no preceding infection or hemorrhage was detected. Although the pathogenesis and significance of these episodes has not been fully clarified, repeated observations have disclosed the following facts of significance: (1) The syndrome of jaundice, anemia and reticulocytosis occurred only when the patient was thrombopenic. The first episode occurred after 7 months of sustained thrombopenia, and the second after 1 month of thrombopenia. The other four episodes occurred coincident with the thrombopenia immediately following a cycle of platelet response. On the other hand 41 other platelet cycles induced with blood or plasma were not followed by jaundice and anemia. (2) There was no relationship between the occurrence of jaundice and the administration of corticosteroids, three of the episodes occurring while the patient was receiving steroids, and three when she was not. (3) Coomb's tests were performed on seven occasions. The direct Coomb's test was consistently negative; the indirect Coomb's test was positive on one occasion during the fifth episode of jaundice and subsequently repeatedly negative. (4) Tests for platelet agglutinins were found to be positive on two and negative on four determinations. Negative results preceded and followed the two positive findings. In view of the multiple transfusions, the significance of the platelet agglutinins is questionable. (5) In five of the episodes of jaundice administration of blood or plasma was followed by a prompt rise in platelets, decrease in jaundice and fall in the reticulocyte count. In the other episode, one following epistaxis, transfusion was withheld. Jaundice persisted for one week, as did the hemoglobin concentration of 8.9 Gm. per cent, reticulocytosis of 23 per cent and platelet count of 40,000/cu.mm. A second epistaxis then necessitated hospitalization and whole blood transfusion, following which the platelets rose to 690,000/cu.mm. in 9 days and the jaundice cleared. The possible significance of the icteric episodes will be discussed further under Results.

METHODS AND MATERIALS

Platelet counts were performed by the method of Kristenson, as modified by Lempert. In all experimental studies platelets were counted in both counting chambers in each of two hemocytometers, and the results of the four separate counts averaged. Bone marrow aspirations were performed through a lumbar spinous process. The marrow was smeared on glass slides and stained with Wright's stain. Megakaryocytic differential counts were performed on 50 megakaryocytes on each of two slides. Whole blood was collected in glass bottles containing standard ACD anticoagulant. Fresh plasma was obtained from whole blood
centrifuged immediately after collection. Fresh-frozen plasma was prepared by freezing fresh plasma in 125 ml. amounts in glass bottles at —20 C. immediately after collection.

**EXPERIMENTAL RESULTS**

*The Effects of Transfusion of Whole Blood, Plasma and Fresh Frozen Plasma*

In 45 separate transfusion experiments the administration of fresh or stored whole blood, fresh or stored plasma or fresh-frozen plasma was followed by a striking rise in platelet count. Platelet counts reached maximum levels 9 to 11 days following transfusion and returned to baseline levels after 20 to 23 days. Whole blood which had been stored for as long as 10 days induced platelet responses equal to those obtained with equivalent amounts of freshly drawn blood.

The extraordinary reproducibility of platelet response is shown in figure 3, which demonstrates the changes in platelet count following eight successive injections of fresh-frozen plasma over a period of six months.

Figure 4 demonstrates the relationship between dosage of fresh-frozen plasma and peak platelet response in 22 experiments. It may be noted that as little as 1.9 ml./Kg. of fresh-frozen plasma was capable of inducing a platelet rise to 280,000/cu.mm. and that maximal responses ranging from 700,000 to 1,000,000 platelets per cu.mm. were achieved with approximately 7 ml. of plasma per Kg. The apparent wide range in platelet-stimulating capacity of individual plasmas is also indicated in figure 4. Thus at a dosage level of 6 ml./Kg., platelet peaks ranged from 540,000 to 690,000/cu.mm., while at 11 ml./Kg. platelet counts ranged from 700,000 to 1,000,000/cu.mm. Throughout the four years that studies have been carried out, no evidence of refractoriness to plasma has been noted.

*Mode of Action of The Platelet Stimulating Factor*

Serial marrow examinations during the platelet cycle following administration of plasma indicated that the plasma factor promotes megakaryocyte maturation and eventual platelet production. Table 1 demonstrates the megakaryocyte differential counts before and four and 11 days after the administration of 6 ml./Kg. of fresh frozen plasma. Figure 5 demonstrates serial platelet counts during this cycle. It will be noted that prior to the administration of plasma (platelet count 20,000) only 12 per cent of the megakaryocytes were seen to be producing platelets while 64 per cent of the megakaryocytes had granular cytoplasm but no evidence of platelet production. Four days after plasma, however, 61 per cent of the megakaryocytes exhibited extraordinary platelet production, with large masses of platelets surrounding the megakaryocyte (figs. 6, 6a). At the same time megakaryoblasts, promegakaryocytes, agranular megakaryocytes and particularly the granular, nonplatelet-forming megakaryocytes had decreased. Eleven days after the plasma administration, 84 per cent of the megakaryocytes were seen to be forming platelets while the immature megakaryocytes had decreased still further. Megakaryoblasts and promegakaryocytes constituted only 4 per cent of the total at this time as compared with 19 per cent before transfusion. The progressive megakaryocyte maturation with concomitant depletion of the immature precursors suggested that
Fig. 3 (at top).—Changes in platelet counts following eight successive transfusions of fresh-frozen plasma over a period of six months.

Fig. 4 (at bottom).—Relationship between dosage of fresh frozen plasma and maximum platelet counts.

A refractory period should occur some time after the eleventh day, at which time additional plasma would be ineffectual in eliciting a rise in platelet count. Figure 7 indicates that this probably occurs. One hundred ml. of fresh frozen plasma (6.0 ml./Kg.) were given 23 days after a maximal stimulating amount of whole blood plus plasma. At this time the platelet count was 310,000. As will be noted from the figure, only minimal platelet production occurred, not sufficient to raise the platelet count but enough to alter slightly the descending slope in the cycle. Seven days later, however, when the platelet count was again 20,000/cu.mm., plasma infusion was followed by a complete re-
Table 1.—*Differential Counts of Megakaryocytes Before and After Administration of Fresh Frozen Plasma*

<table>
<thead>
<tr>
<th>Classification of megakaryocytes</th>
<th>Pre-plasma (%)</th>
<th>Post-plasma 4 days (%)</th>
<th>Post-plasma 11 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megakaryoblasts</td>
<td>8.0</td>
<td>4.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Promegakaryocytes</td>
<td>11.0</td>
<td>7.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Agranular; nonproducing</td>
<td>5.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Granular; nonproducing</td>
<td>64.0</td>
<td>28.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Granular; producing</td>
<td>12.0</td>
<td>61.0</td>
<td>84.0</td>
</tr>
</tbody>
</table>

Fig. 5.—Platelet counts during the cycle in which marrow aspirations listed in table 1 were performed.

...response. These observations, together with the serial marrow studies described above suggest the following sequence of events: (1) prior to plasma administration, megakaryocyte maturation is blocked at the level of the granular non-platelet-producing megakaryocyte. (2) Following a maximal stimulating dose of plasma, megakaryocyte maturation proceeds at all levels. (3) The megakaryocytes originally present degenerate after platelet production occurs, and the marrow is then refractory to further stimulation until a new population of megakaryocytes appears.

**Platelet Life Span**

Study of the platelet cycle in conjunction with simultaneous marrow studies permitted calculation of platelet life span. As will be seen on figure 5, there is a lag phase of approximately two days following which the platelet count rises in linear fashion for eight days. There then occurs a plateau of two to four days' duration, following which there is again a linear fall in platelet count. Since from the marrow studies it is obvious that platelet production is...
Fig. 6 (at top).—Granular, nonplatelet-forming megakaryocyte constituting 64 per cent of total megakaryocytes prior to plasma administration.

Fig. 6a (at bottom).—Platelet-producing megakaryocyte constituting 61 per cent of megakaryocytes four days after administration of fresh-frozen plasma.

still continuing at eight days, the development of the plateau can only mean that the platelet life span has been reached and platelet destruction has begun. The linear fall beginning at 14 days also proceeds for nine days until baseline levels are again reached. The calculated platelet life of eight to nine days is in very good agreement with results obtained by isotopic technic.3,7

Figure 8 demonstrates the variation in platelet cycles induced by equal
Fig. 7 (at top).—Effect of plasma transfusion given on the descending limb of a platelet cycle, 23 days after a maximum stimulating dose of blood plus plasma.

Fig. 8 (at bottom).—Variations in platelet cycles following transfusion of equal amounts of fresh-frozen plasma.

amounts of plasma from four different donors. It may be seen that despite the difference in maximum platelet count and in duration of the cycle, the rates of platelet production are virtually identical for the first eight to nine days. In the upper three curves a clear plateau is evident while in the fourth curve a sharp fall follows the peak at nine days. In all four curves analysis of the linear portions of the descending limbs indicates a platelet life span of eight to 10 days. Therefore, the height of the peak and the duration of the cycle would appear to depend upon the number of megakaryocytes stimulated and the duration of platelet production. In the upper three curves platelet
production apparently continues for 14 to 16 days after transfusion; in the fourth curve platelet production terminates after nine days.

The Role of the Spleen

As was mentioned in the case report, two transfusions of 250 ml. of whole blood were administered to the child in the week prior to splenectomy. Examination of the data in figure 1 suggests that a significant rise in platelet count may have been induced by the transfusions. Thus in the five days prior to transfusion the platelet count ranged from 20,000 to 40,000/cu.mm. Two days after the first transfusion, however, a platelet rise of 100,000 was noted, with a return to previous levels in four days. Six days after the second transfusion the platelet count had risen to 140,000. If these responses were indeed the result of the transfusions, they would imply that in the presence of the spleen the response to transfusion of the platelet stimulating factor is greatly attenuated since in post-splenectomy years equal amounts of blood induced rises in platelet counts to over 600,000/cu.mm. and complete cycles of 19 to 24 days' duration. Whether the spleen inhibits the action of the factor or exerts a direct effect on the megakaryocyte in the marrow cannot be stated at present. These observations may, however, bear on the known effects of splenectomy on platelet count.

Relationship of the Platelet-Stimulating Factor to Hemoglobin, Erythrocyte and Leukocyte Production

Throughout the entire period of observation in the patient, no constant relationship could be detected between changes in platelet count and those in hemoglobin concentration or reticulocyte, erythrocyte or leukocyte count. This was true during periods of protracted thrombopenia, as shown in figure 2, or during repeatedly induced platelet cycles, as shown in figure 3. The lack of relationship between the platelet levels and these of the other circulating cells is shown in table 2, in which it may be seen that the changes in platelet count induced by plasma transfusion are not accompanied by simultaneous changes in erythrocyte or leukocyte count. The patient has very frequently exhibited an elevated reticulocyte count, which, however, has not been related to platelet count or plasma administration. In many instances elevations of reticulocyte count could be attributed to spontaneous episodes of bleeding which occurred during periods of thrombopenia, most commonly at the end of an

<p>| Day after | Platelet | Hemoglobin | RBC | WBC | Reticulocytes |</p>
<table>
<thead>
<tr>
<th>plasma</th>
<th>(per cu.mm.)</th>
<th>(Gm. %)</th>
<th>(mill./cu.mm.)</th>
<th>(per cu.mm.)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5,000</td>
<td>12.7</td>
<td>4.2</td>
<td>12,800</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>33,000</td>
<td>14.2</td>
<td>3.8</td>
<td>7,800</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>125,000</td>
<td>13.7</td>
<td>3.5</td>
<td>5,600</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>824,000</td>
<td>13.7</td>
<td>3.6</td>
<td>11,700</td>
<td>5.6</td>
</tr>
<tr>
<td>14</td>
<td>586,000</td>
<td>14.1</td>
<td>4.3</td>
<td>8,600</td>
<td>—</td>
</tr>
<tr>
<td>21</td>
<td>131,000</td>
<td>13.0</td>
<td>3.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>17,000</td>
<td>12.8</td>
<td>4.1</td>
<td>15,100</td>
<td>3.4</td>
</tr>
</tbody>
</table>
induced platelet cycle. The episodes of jaundice, anemia and reticulocytosis referred to earlier were indicative of hemolytic anemia and suggested the possibility of a mechanism responsible for both platelet and red cell destruction. Numerous studies, however, failed to confirm this relationship. As mentioned in the Case Report, 41 separate platelet cycles were unaccompanied by parallel changes in hemoglobin concentration, white cell count or reticulocytosis. The direct Coomb's test was consistently negative, and platelet agglutinins were detectable in only two of six determinations over a period of years. Finally a red cell survival study of the patient's erythrocytes tagged with chromium-51 was carried out during a typical platelet cycle and was found to be entirely normal. This cycle, however, was not accompanied by jaundice or reticulocytosis. The facts that the episodes of jaundice invariably occurred in thrombopenic periods, and, on four occasions were preceded by epistaxis and hematemesis, make it difficult to evaluate the role of hemorrhage in the production of the icterus, anemia and reticulocytosis. Furthermore, the influence of antecedent infection remains to be clarified. Although the pathogenesis of the icteric episodes has not been elucidated, it should be emphasized that at each instance administration of blood or plasma was followed by a prompt rise in platelet count, decrease in reticulocytosis and fall in bilirubin concentration.

**Family Studies**

The early onset of purpura in this child, plus the apparent deficiency of a factor present in normal plasma, suggested the possibility of a genetically determined defect. To investigate this the patient was transfused with plasma from her father and mother on two occasions for each. At least two cycles with normal plasma, in equivalent amounts, preceded each injection of parent's plasma. Figure 9 demonstrates the maximum platelet responses attained with the mother's and father's plasmas, as compared with equivalent quantities of 15 normal plasmas. It will be seen that the mother's plasma on each of two injections induced platelet responses entirely comparable with the normals. The father's plasma, on the other hand, induced maximum platelet responses, on each occasion, which were lower than those attained with any normal or with the mother's plasma. The father, who has shown no evidence of bleeding, maintains a platelet count of 180,000 to 220,000/cu.mm. on repeated determinations. The mother's platelet count ranges from 190,000 to 350,000/cu.mm. The above studies suggest the possibility that the defect in the patient may have a familial basis.

**Discussion**

The data in this study suggest that the chronic thrombocytopenia in the patient results from deficiency of a factor, present in normal plasma, which is required for megakaryocyte maturation and platelet production. Alternatively, the data might be interpreted as indicating the presence of an inhibitor of megakaryocyte maturation which is neutralized by a factor in normal plasma. However, the striking rise in platelet count following administration of small amounts of plasma tends to militate against this concept.
Fig. 9.—Maximum platelet counts following transfusions of mother's plasma and father's plasma as compared with responses to equivalent amounts of 15 normal plasmas.

The wide experience with transfusion in treatment of thrombocytopenic purpura would suggest that the mechanism implicated in the present case must be involved very rarely in the pathogenesis of other thrombocytopenic states. On the other hand, the data on the comparative effects of transfusion in the pre- and post-splenectomy periods offer the possibility that the intact spleen may minimize the response to the administered plasma factor, thus making recognition of similar patients difficult. Moreover, there exist in the literature certain indications that transfusion may induce remissions in some patients with thrombocytopenia. Thus in 1933 Jones and Tocantins stated that "transfusion, properly administered, intravenously, in small amounts and frequently, controls the bleeding time, retards the production of hemorrhagic phenomena and brings about a cure." In the latter report, benefit is reported in 45 per cent of patients. However, the age distribution of the patients is not given nor are serial platelet counts in individual patients recorded. Thus it is difficult to determine the probable frequency of spontaneous remission. Most provocative in regard to the effect of transfusions are the more recent observations of Dameshek on the effects of transfusion of polycythemic blood as follows: "One of us (W. D.) has repeatedly observed that direct transfusions of polycythemic blood are not infrequently followed by a sustained or permanent remission." In the paper of Stefanini et al. from which the quotation is taken, two examples of such responses are given, in one of which changes in the megakaryocytes are illustrated by photomicrographs which are almost identical with those pictured in figures 6 and 6a of this report. The authors state further: "The relative frequency . . . of remissions following transfusion of polycythemic
blood indicates that some factors in polycythemic blood may be responsible for them. The typical marrow findings in ITP are quite similar to those found in the present patient and reveal normal or increased numbers of megakaryocytes without evidence of platelet formation. In view of the observations of Dameshek, one wonders whether administration of excess megakaryocyte-stimulating factor, either in polycythemic blood or by repeated injections of normal plasma, may result in megakaryocyte maturation and subsequent remission, even if actual deficiency of the factor may not be primarily responsible for the thrombocytopenia. Such studies are currently in progress. In addition, the effects of plasmas from patients with thrombocytopenia of varying etiology and from patients with polycythemia vera are being studied in the patient reported herein. These results will be presented separately.

In view of the many extensive investigations on erythropoietin, surprisingly little information is available on the responses in platelet count following injections of plasmas or plasma extracts with red cell stimulating properties. Erslev\(^\text{10}\) studied two normal rabbits that were given 50 ml. of plasma from anemic rabbit donors and reported that “there was a definite rise in red cell count, hematocrit and per cent nucleated red cells in the marrow, but no significant change in platelet count or granulocyte count.” However, scrutiny of the published figure indicates that an effect on platelet count may actually have been induced. Thus one of the rabbits with a platelet count of about 300,000/cu.mm. in the control period demonstrated a rise to about 700,000/cu.mm. after two daily injections of 50 ml. of anemic plasma. The platelet counts then remained above baseline levels throughout the study period. In the second rabbit there is also a suggestive rise on the third day of plasma administration; unfortunately, platelet counts in rabbits given normal plasma are not presented. In a recent study, Linman et al.\(^\text{11}\) administered to rats an ether extract of boiled plasma from patients with polycythemia vera. The extract, given in a dose of 2 per cent of recipient rat body weight per day for 10 days, induced a rise in reticulocyte count, red cell count and erythropoietic precursors in the marrow. In addition, all rats demonstrated significant thrombocytosis throughout the term of the experiment, with return to baseline levels immediately thereafter. Extracts of normal plasma induced insignificant changes in platelet count. Steinberg et al.\(^\text{12}\) studied the effects of fractions obtained from normal human serum on hematopoiesis in the rabbit. Doses for the various fractions corresponded to 10 ml. of original plasma per kilogram body weight of the recipient animal. One fraction (C-1) was found to increase the megakaryocytes in the marrow by 20 to 35 per cent and the erythroid cells by 40 to 55 per cent. Changes in myeloid elements were variable. There was no change in any of the peripheral blood elements, however. A combination of two other fractions (C-3 and C-4) produced an increase in marrow megakaryocytes by 20 to 30 per cent in most animals, suppression of maturation of myeloid elements, but no change in erythroid cells. In the peripheral blood thrombocytes were increased by 10 to 25 per cent in 14 of 56 animals, granulocytes disappeared, and a questionable, slight anemia developed.

Several clinical situations now known to be associated with increased levels of erythropoietin such as acute hemorrhage,\(^\text{13}\) hemolysis\(^\text{14}\) and polycythemia
SCHULMAN, PIERCE, LUKENS AND CURRIMBHONY

vera are also characterized by elevated platelet counts. Thus a relationship between the erythropoietic factor and the stimulation of thrombopoiesis might be suggested. The results of the present study indicate, however, that the thrombopoietic principle is independent of erythropoietic and myelopoietic factors. Such a distinction may also be noted in patients with cyanotic congenital heart disease and in individuals exposed to high altitudes. In both situations polycythemia develops and is accompanied by elevated levels of erythropoietin. Platelet counts, on the other hand, are not elevated. It seems likely, however, that some stimuli, such as acute anemia, are capable of inducing release of both erythropoietic and thrombopoietic factors.

Studies on the isolation and chemical characterization of the platelet-stimulating factor, on the role of infection in the production of thrombopenia, and on the implication of the plasma factor in thrombopenias of varying etiology are now in progress.

SUMMARY

1. A case of chronic thrombocytopenic purpura has been presented in which the pathogenesis appears to be due to congenital deficiency of a platelet-stimulating factor.
2. The factor exists in normal plasma and is stable on storage under normal blood banking conditions and on freezing.
3. The factor appears to act by stimulating megakaryocyte maturation and platelet production in an orderly and sequential manner.

REFERENCES

STUDIES ON THROMBOPOIESIS. I.


Sheep erythropoietic factor prepared by Borsook's method has been examined. Testing of erythropoietic activity was done by reticulocyte and erythrocyte counts. Chromatography revealed in the erythropoietic preparations the same free amino acids as in normal plasma, but with some quantitative changes: the former contained more glutamic acid, glutamine, glycine, lysine and arginine; there was no correlation between the erythropoietic effects of the preparations and their tyrosine level. Sheep may be used as donors for large amounts of erythropoietic plasma, which may be useful in the production of erythropoietin on a major scale.—E. K.


Case report of a 68 year old man with spindle cell thymoma, marrow hypoplasia and severe pancytopenia: The pancytopenia and the tumor were discovered simultaneously, and the patient died before thymectomy was performed. Sixteen previously reported cases of thymoma associated with hemopoietic insufficiency are tabulated, and it is concluded that this association is more than fortuitous.—A. J. E.
Studies on Thrombopoiesis. I. A Factor in Normal Human Plasma Required for Platelet Production; Chronic Thrombocytopenia Due to its Deficiency

IRVING SCHULMAN, MILA PIERCE, ABBY LUKENS and ZINET CURRIMBHOY