Factor V Deficiency in Philadelphia-Positive Chronic Myelogenous Leukemia

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Factor V deficiency has been identified in 8 of 8 patients 7-20 yr of age, with Philadelphia-positive (Ph1+) chronic myelogenous leukemia (CML). In these 8 patients, factor V deficiency was not due to hepatic dysfunction, factor V inhibitors, or disseminated intravascular coagulation. In 3 patients, factor V activity rose 10%-12% (0.10-0.12 U/ml) after the infusion of 28-31 ml/kg body weight of fresh frozen plasma (FFP). The rise persisted less than 14 hr. The mean measured postinfusion rise in factor V was 18% of the expected rise calculated from the volume of FFP infused in the patients' plasma volume. In 4 patients, a small transient rise in factor V activity occurred after splenectomy or plateletpheresis. Factor V deficiency was completely corrected after a marked reduction in bone marrow cellularity in 2 patients with Ph1+ CML treated with extensive chemotherapy, total body irradiation, and bone marrow transplantation. Factor V deficiency was retrospectively observed in 6 of 20 patients, ages 20-80 yr, with Ph1+ CML and 3 of 6 patients with other myeloproliferative disorders. The factor V deficiency appears to be associated with the large myeloid-megakaryocytic cell mass characteristic of CML and other myeloproliferative disorders.

MATERIALS AND METHODS

Clinical

Studies of 8 Younger Patients with Ph1+ CML During Chronic Phase and Blast Crisis

From June 1976 to June 1979, 8 patients were evaluated during chronic phase, blast crisis, or both (Table I). Cytogenetic studies on peripheral blood and bone marrow specimens demonstrated the presence of the Ph1 chromosome in all patients. All patients had elevated serum vitamin B12 and vitamin B12 binding capacity and decreased leukocyte alkaline phosphatase values. Leukocyte counts (WBC) and platelet counts were measured using standard laboratory methods.

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Bone Marrow and Hepatic Function Studies in CML

The course of the disease and response to therapy in all patients were evaluated by bone marrow aspirates and trephine biopsy specimens, which were stained and prepared as described previously. Hepatic function was tested in all patients by serum albumin, bilirubin, transaminase, and alkaline phosphatase determinations using standard laboratory methods. In two patients, liver biopsies were performed at the time of splenectomy.

Therapy of CML in Chronic Phase and Blast Crisis

All patients were treated with hydroxyurea during chronic phase. Three patients (S.B., G.S., and D.G.) also received short courses of uracil mustard, and one patient (T.B.) was treated with busulfan. The therapy in patient G.S. prior to this study was previously described. Blast crisis and the response to therapy were defined by the criteria of Peterson et al. The four patients studied in blast crisis received various chemotherapeutic regimens.

Correlation of Clinical and Plasma Coagulation Studies in CML

Plasma coagulation studies with particular emphasis on factor V assays were correlated with each patient's WBC, platelet count, bone marrow studies, hepatic function, and spleen size during chronic phase and/or blast crisis. Because the ages and sizes of the patients varied widely, estimation of spleen size on physical examination was done as follows: 0 = not palpable; 1+ = palpable at left costal margin; 2+ = palpable between the left costal margin and umbilicus; 3+ = palpable at the level of the umbilicus; and 4+ = palpable below the umbilicus and/or crossing the midplane of the abdomen.

Retrospective Studies in Older Patients With Ph1 CML and Other Myeloproliferative Disorders

Plasma coagulation and hepatic function studies were retrospectively reviewed from 1974 to June 1979 on 20 randomly selected patients with Ph1 CML, aged 20–80 yr. Plasma coagulation studies were similarly reviewed on 3 patients with polycythemia vera, 3 patients with essential thrombocythemia, and 1 patient (age 12 yr) with Ph1-negative CML during blast crisis.

Laboratory Methods

Platelet Function Studies

The platelet function studies on 8 patients with Ph1 CML (except C.M.) were previously described by Gerrard et al.

Plasma Coagulation Studies

Plasma collection. Nine volumes of venous blood were collected into plastic tubes containing 1 vol of citrate anticoagulant (3 parts 0.1 M sodium citrate and 2 parts 0.1 M citric acid). Specimens for platelet-poor plasma (PPP) were centrifuged at 16,000 g for 10 min at 4°C. PPP was transferred to siliconized glass tubes in 1-ml aliquots by plastic pipettes, and the tubes were stopped by corks wrapped in Parafilm-M. PPP was tested either within 1 hr of collection or within 3 days on specimens that had been quickly frozen at -70°C, stored at -45°C, and thawed at 37°C just before use. Specimens for platelet-rich plasma (PRP) studies were centrifuged at 30 g for 10 min at 25°C. Tests on PRP were performed within 1 hr of collection. Except as described below, all tests were done on PPP.

Standard coagulation studies. The one-stage prothrombin time (PT), kaolin partial thromboplastin time (PTT), and the plasma thrombin time (TT) were measured as previously described. Factors II, VII, IX, X, XI, and XII were measured by standard one-stage assays, except that both patient and control plasmas were tested at four different plasma dilutions.

Laboratory evaluation for DIC. Factor VIII activity was measured by a one-stage assay. Fibrinogen was measured by the method of Jacobsson, and in some specimens, also by the method of Claus. Serum FDP were determined either by the tanned red cell hemagglutination-inhibition test or the latex agglutination immunosay (Thrombo-Wellcotest, Burroughs Wellcome).

Factor V assays. Factor V assays were done using a modified one-stage prothrombin time system. Factor-V-deficient substrate was prepared by mixing 9 vol of blood with 1 vol of 0.1 M sodium oxalate, and the plasma was separated by centrifugation. The plasma was incubated at 37°C (final pH 7.35) until the PT was greater than 120 sec. This substrate had normal factor II, VII, IX, and X activities. Two-milliliter aliquots of substrate were quickly frozen at -70°C in siliconized glass tubes and stored at -45°C until used. Substrate plasma, pooled normal control plasma, and the patient plasma were thawed at 37°C immediately prior to use. The clotting time system contained 0.1 ml of substrate plasma, 0.1 ml of liquid rabbit brain thromboplastin (Activated Thromboplastin, Dade Diagnostics, Inc.), and 0.1 ml of patient or control plasma dilutions. After warming this mixture at 37°C for 2 min, 0.1 ml of 0.033 M CaCl2 was added, and the clotting time was visually observed. Both patient and control plasmas were tested at 1:10, 1:20, 1:40, and 1:80 dilutions in duplicate. Diluting fluids were those previously used.
described. The mean clotting times for the patient and control plasma dilutions were plotted on log-log graph paper, and the factor V activity of the patient plasma determined. The control plasma consisted of quickly frozen plasma from at least 5 normal donors. Factor V assays on the control plasma pool showed no appreciable fall in factor V activity over 21 days when stored as described above. Factor V activity of the control plasma pool had previously been determined by comparison to a primary pool of 50 normal plasmas as the mean of a minimum of 25 factor V assays. Factor V assays on frozen specimens from the primary pool showed no appreciable fall in factor V activity when compared to fresh primary pool plasma specimens.

Special Studies

In vitro factor V inhibitor studies. CML blood was prepared to yield PPP and PRP. Factor V assays, PT, PTT, and TT were done on CML PPP, CML PRP, normal plasma, and a mixture of equal parts of normal and CML PPP immediately after preparation of plasma samples and after incubation at 37°C for 2 hr. The decay of factor V activity with time was compared between normal and CML PPP by measuring factor V activities on normal plasma and CML PPP at 0, 2, 4, and 6 hr of incubation in a 37°C waterbath. Test plasmas were kept in siliconized glass tubes that were tightly stoppered between tests. A plastic sheet occluded the waterbath opening, and a CO₂ source was introduced into this space. This system had previously been shown to maintain the plasma pH at 7.30-7.40 for 6 hr or more.

In vitro factor V studies on hydroxyurea-treated normal plasma. Factor V assays were performed on normal plasma (9 vol) mixed with 1 vol of either buffered saline or hydroxyurea (Hydrea, Squibb) diluted in buffered saline at 0, 1, and 2 hr of incubation. The buffer was sodium diethyl barbiturate, final pH 7.35. The final concentrations of hydroxyurea in the test plasmas were 0.015, 0.15, and 1.5 mg/ml of plasma. These concentrations were selected for comparison to the maximum serum hydroxyurea levels of 0.266 mg/ml observed by Rosner et al.²³

In vitro incubation studies of normal and CML platelets with normal plasma on factor V activity. Normal and CML PRP were prepared as described above. Platelets from both normal and CML PRP were obtained by the albumin density gradient method of Walsh.²⁴ Both normal and CML PRP were centrifuged at 1200 g for 15 min at 25°C. The platelet-rich layer was removed by plastic pipettes and gently resuspended in 3 ml of pooled normal PPP in siliconized glass tubes that were tightly stoppered. Platelet counts by phase microscopy were performed on the platelet-normal plasma suspension. In 3 experiments with normal platelets, the platelet count on the suspension was adjusted to 400-500 × 10⁹/liter by further addition of normal plasma. In 4 experiments from 3 patients with Ph¹ + CML (G.S., L.F., and D.G.) and in 1 normal control, the platelet count on the platelet-normal plasma suspensions was not adjusted. Both normal and CML platelet-normal plasma suspensions were incubated in a 37°C waterbath. The platelets were maintained in suspension by gentle agitation with a plastic pipette every 60 min. Factor V assays were performed at 0, 2, and 4 hr on aliquots of normal platelet-normal plasma suspensions and at 0, 2, 4, and 6 hr on all CML platelet-normal plasma and 1 normal platelet-normal plasma suspension. As an additional control, six sets of pooled normal PPP were incubated under identical conditions, and factor V assays were performed at 0, 2, 4, and 6 hr of incubation.

In vivo factor V recovery studies. Three patients with CML (Table 1) were transfused with 28-31 ml fresh frozen plasma (FFP)/kg body weight over 12 hr. As a control, a severe congenital factor-V deficient patient (R.G.) was transfused with 30 ml FFP/kg body weight over 18 hr. Factor V activity, PT, PTT, TT, and fibrinogen were measured immediately before, during, and up to 118 hr following the infusion of FFP. Several samples of FFP were assayed for factor V activity immediately after individual units were infused. The recovery of transfused factor V was calculated according to the method of Rush and Ellis.²⁵ The corrected plasma volume following FFP was estimated according to the method of Biggs and Denson.²⁶ The calculated recovery of factor V was based on the difference between the observed factor V assays immediately prior to and the peak postinfusion factor V activity. Factor V half-life in the patient with congenital factor V deficiency was determined by plotting the log factor V activity against time in hours. Factor X activity was serially measured in the two patients who had low factor X levels prior to the FFP infusion.

Factor V studies with platelethpheresis and splenectomy. Two patients with severe thrombocytosis were treated with platelethpheresis on a continuous-flow cell separator (Table 1). Factor V assays and platelet counts were done within 8 hr before and after platelethpheresis. Three patients were treated with splenectomy (Table 1). Factor V activity was measured prior to and following splenectomy. Factor V studies with bone marrow transplantation (BMT). Patient L.F. remained in chronic phase for 28 mo. Allogeneic BMT was done 3 mo after myeloblastic transformation. In patient G.S., syngeneic BMT was performed during chronic phase 113 mo after diagnosis. Splenectomy was performed 34 and 69 days prior to BMT in L.F. and G.S., respectively. Preparation for BMT was as described by Fefer et al.²⁷ with minor modifications. Twenty-four hours after the dose of dimethyl busulfan, both patients received 4 consecutive days of cyclophosphamide, 50 mg/kg body weight/day. Twenty-four hours after the last dose of cyclophosphamide, both patients were treated with 750 rad of total-body irradiation by means of a linear accelerator at 26 rad/min. Two days thereafter, normocellular Ph¹-negative bone marrow obtained from siblings with normal factor V activities was infused into the patients. PT, PTT, TT, and factor V assays were done sequentially from the time of splenectomy, during therapy, and after BMT. The response to BMT was evaluated at various times by trephine bone marrow biopsies and marrow aspirates for cytology and chromosome analysis.

Consents

Prior to participation, the patients and/or their parents gave informed consent. Consent forms and protocols were approved by the Committee on the Use of Human Subjects in Research, University of Minnesota.

RESULTS

Factor V Activity in Chronic Phase

Figure 1 shows the results of 57 factor V assays and the median factor V activity for 7 patients studied during chronic phase. The median factor V activity was 45%, with a range of 24%–73%. At diagnosis and prior to any therapy, the factor V activity was 73%, 51%, 42%, and 39% in patients D.V.H., C.M., D.G., and L.F., respectively. In our laboratory, the mean factor V activity of 37 individual normal persons was 99%, and the observed range was 70%–129%. The
range for ±2 standard deviations from the mean was 64%–133%. Thus, 55 of 57 factor V assays on CML plasma were below our observed normal range, and 51 of 56 were more than 2 standard deviations below the mean.

Platelet count and WBC determinations and changes in their values were compared to the observed factor V activity. All patients studied during chronic phase frequently had elevated platelet counts and WBC, with moderate to marked variation in the absolute values of both. Neither an increase nor a decrease in the platelet count or WBC correlated with factor V levels. Clinical estimation of spleen size showed a variable degree of splenomegaly (0 to 4+) in each patient. Changes in spleen size did not correlate with the observed factor V levels.

In all patients studied, the factor V levels did not appreciably change with respect to the dosage, duration, or types of chemotherapy used during chronic phase. At diagnosis and when studied, all bone marrow biopsies were markedly hypercellular. Significant myelofibrosis was not observed in any patient.

**Factor V Activity in Blast Crisis**

The results of 45 factor V assays and the median factor V activity of 4 patients studied in blast crisis are also shown in Fig. 1. The median factor V activity was 32%, with a range of 10%–58%. At diagnosis of blast crisis, the factor V activity was 42%, 44%, 29%, and 14% in patients D.G., L.F., T.B., and C.F., respectively. In patients D.G., L.F., and T.B., no significant difference in factor V levels was observed before or during blast crisis nor was it observed in a fourth patient (C.F.) in blast crisis during chemotherapy.

In all 4 patients, blast crisis was documented by bone marrow studies that showed myeloblastic transformation and markedly increased bone marrow cellularity. All patients had massive (3+ to 4+) splenomegaly during blast crisis. None responded to chemotherapy, and all patients with blast crisis died within 6 mo.

**Factor V Activity in Older Patients With CML and Other Myeloproliferative Disorders**

A total of 120 factor V assays were done on the 20 patients, ages 20–80, with Ph' + CML who were studied retrospectively. In 6 of these patients, on whom a total of 50 factor V assays were done, the mean factor V activity was 39%, 45%, 47%, 51%, 55%, and 62%, respectively, and the range was 23%–73%. There was no laboratory evidence of liver dysfunction or DIC in these 6 patients. Comparably low mean factor V activities were observed in 6 of the remaining 14 patients who did have liver dysfunction or DIC. The other 8 patients either had a mean factor V activity within the normal range or could not be evaluated for hepatic disease and DIC. During blast crisis, the patient with Ph'-negative CML had 7 factor V assays ranging between 33% and 43%; no evidence for hepatic dysfunction or DIC was found.

One of 3 patients with polycythemia vera and 2 of 3 patients with essential thrombocythemia had factor V activities between 49% and 56%.

**Evaluation for DIC in the Eight Younger Patients With Ph' + CML**

The results of the laboratory evaluation for DIC in the 8 younger patients with Ph' + CML are shown in Table 2. The range of 12 factor VIII assays was 73%–196%. The range of 75 fibrinogen assays was 0.17–0.52 g/dl (normal range, 0.17–0.35 g/dl). No patient had factor VIII or fibrinogen levels below the established normal range in our laboratory. Thirteen of 16 serum FDP determinations were normal (less than 10 μg/ml). Three patients had FDP values of 10...
FACTOR V DEFICIENCY IN CML

Table 2. Plasma Coagulation Studies on 8 Patients With Ph' + CML (Underlined Values Denote Abnormalities)

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>PT (sec)</th>
<th>PPIT (sec)</th>
<th>TT (sec)</th>
<th>II</th>
<th>VII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
<th>V</th>
<th>VIII</th>
<th>Fibrinogen (g/dl)</th>
<th>FDP (µg/ml)</th>
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<tr>
<td></td>
<td>10.0-13.0</td>
<td>35.0-45.0</td>
<td>&lt;20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.17-0.35</td>
<td>&lt;10</td>
</tr>
<tr>
<td>S.B.</td>
<td>12.7</td>
<td>46.8</td>
<td>16.1</td>
<td>78</td>
<td>84</td>
<td>49</td>
<td>59</td>
<td>74</td>
<td>79</td>
<td>54</td>
<td>59</td>
<td>0.36</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D.V.H.</td>
<td>11.2</td>
<td>38.4</td>
<td>12.9</td>
<td>78</td>
<td>114</td>
<td>106</td>
<td>95</td>
<td>62</td>
<td>87</td>
<td>66</td>
<td>140</td>
<td>0.24</td>
<td>&lt;10</td>
</tr>
<tr>
<td>G.S.</td>
<td>12.2</td>
<td>46.3</td>
<td>13.7</td>
<td>59</td>
<td>45</td>
<td>95</td>
<td>82</td>
<td>38</td>
<td>82</td>
<td>47</td>
<td>150</td>
<td>0.26</td>
<td>10</td>
</tr>
<tr>
<td>C.M.</td>
<td>10.7</td>
<td>38.5</td>
<td>15.6</td>
<td>79</td>
<td>78</td>
<td>164</td>
<td>116</td>
<td>118</td>
<td>100</td>
<td>49</td>
<td>196</td>
<td>0.41</td>
<td>&lt;10</td>
</tr>
<tr>
<td>D.G.</td>
<td>11.3</td>
<td>48.5</td>
<td>14.6</td>
<td>80</td>
<td>126</td>
<td>83</td>
<td>68</td>
<td>58</td>
<td>46</td>
<td>58</td>
<td>97</td>
<td>0.25</td>
<td>&lt;10</td>
</tr>
<tr>
<td>L.F.</td>
<td>12.6</td>
<td>74.3</td>
<td>13.6</td>
<td>47</td>
<td>78</td>
<td>100</td>
<td>58</td>
<td>32</td>
<td>22</td>
<td>40</td>
<td>82</td>
<td>0.49</td>
<td>10</td>
</tr>
<tr>
<td>T.B.</td>
<td>12.7</td>
<td>45.5</td>
<td>15.0</td>
<td>75</td>
<td>100</td>
<td>94</td>
<td>76</td>
<td>73</td>
<td>71</td>
<td>37</td>
<td>128</td>
<td>0.32</td>
<td>&lt;10</td>
</tr>
<tr>
<td>C.F.*</td>
<td>13.5</td>
<td>36.6</td>
<td>16.9</td>
<td>76</td>
<td>55</td>
<td>90</td>
<td>123</td>
<td>78</td>
<td>68</td>
<td>23</td>
<td>196</td>
<td>0.34</td>
<td>&lt;10</td>
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</tbody>
</table>

*During blast crisis.
†Normal range of factors II, VII, IX, X, XI, V, and VIII is 70%-130% and 60%-200%.

μg/ml on one occasion each, which were all less than 10 μg/ml on subsequent testing.

Plasma Coagulation Tests in Eight Younger Patients With Ph' + CML

Table 2 gives a plasma coagulation test profile for all eight patients. Except patient C.F., who was studied only in blast crisis, the values are at a single time during chronic phase when they were clinically stable and at least 1 mo before or 1 mo after splenectomy, platelethapheresis, or BMT. Profiles on three patients who entered blast crisis remained similar to those observed during chronic phase. In the eight patients, one had factor XII deficiency, two had factor XI deficiency, and two had deficiencies of both factors XI and XII.

Figure 2 shows the results of all the assays for factors II, VII, IX, and X performed on the eight patients with Ph' + CML. Four patients initially had low factor II levels, and three rose into the normal range after vitamin K. Three patients initially had low factor X levels, and all rose into the normal range after vitamin K. No patients initially had low factor IX levels, but the level dropped from 100% to 58% in a single patient. Factor VII responded variably to vitamin K.

Hepatic Function in CML

All patients had serum albumin, bilirubin, transaminase, and alkaline phosphatase determinations on three or more separate occasions. Serum albumin and bilirubin levels were consistently normal during chronic phase and blast crisis in over 30 determinations of each. All patients had consistently normal serum transaminase and alkaline phosphatase values in over 40 sets of measurements. The liver biopsies at splenectomy in 2 patients showed normal hepatocellular histology with evidence of extramedullary hematopoiesis.

In Vitro Factor V Inhibitor Studies

In Table 3, factor V assays on CML PPP, CML PRP, normal pooled PPP, and equal mixtures of normal and CML PPP are shown. The measured factor V for five patients immediately after mixing CML PPP with normal PPP was essentially the same as the mean values for the plasmas assayed separately. Over a 2-hr incubation, the drop in factor V in the mixtures was small and similar to the drop in factor V when normal PPP was incubated alone. Inhibition of
Fig. 3. The effect of 37°C incubation of normal platelets in normal plasma on factor V activity. The solid circles represent the mean factor V activity of three incubation studies with normal platelets and normal plasma. The final platelet counts were between 480 and 520 x 10^9/liter. For comparison, the open circles represent the mean factor V activity of 6 experiments in which pooled normal platelet-poor plasma was incubated at 37°C for 6 hr. The vertical lines represent the observed range in factor V activity. The shaded portions depict the range of factor V activity on normal pooled platelet-poor plasma.

Table 3. In Vitro Factor V Inhibitor Studies

<table>
<thead>
<tr>
<th>Column No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
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<tr>
<td>Normal Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>0 hr</td>
<td>+2 hr</td>
<td>Calculated Mean of Columns 1 and 6</td>
<td>Normal + Patient PPP (1:1)</td>
<td>Patient PPP</td>
<td>Patient PRP</td>
<td>Peripheral Platelet Count (x 10^9/liter)</td>
<td></td>
</tr>
<tr>
<td>S.B.</td>
<td>101</td>
<td>97</td>
<td>74</td>
<td>74</td>
<td>71</td>
<td>48</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>D.V.M.</td>
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<td>97</td>
<td>84</td>
<td>83</td>
<td>76</td>
<td>63</td>
<td>63</td>
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</tr>
<tr>
<td>L.F.</td>
<td>102</td>
<td>ND*</td>
<td>69</td>
<td>75</td>
<td>73</td>
<td>36</td>
<td>ND</td>
<td>36</td>
</tr>
<tr>
<td>C.M.</td>
<td>101</td>
<td>97</td>
<td>73</td>
<td>77</td>
<td>72</td>
<td>45</td>
<td>47</td>
<td>—</td>
</tr>
<tr>
<td>G.S.</td>
<td>104</td>
<td>97</td>
<td>73</td>
<td>70</td>
<td>64</td>
<td>41</td>
<td>42</td>
<td>—</td>
</tr>
</tbody>
</table>

The factor V activity of an equal mixture (1:1) of normal and CML platelet-poor plasma (PPP) is shown immediately after mixing (0 hr) and at 2 hr of incubation. The factor V activities of normal plasma and CML PPP are shown immediately (0 hr) after specimen collection and at 2 hr of incubation. The factor V activities on CML PPP and platelet-rich plasma (PRP) are compared in 2 patients. The values for normal plasma were obtained at the time of each study.

*ND, not done.

factor V activity of normal plasma was thus not seen either immediately or after 2-hr incubation. In the two patients tested, factor V levels on PPP and PRP were the same in one and differed by only 1% in the other.

When normal and CML PPP from 2 different patients were simultaneously incubated for 6 hr little if any decay in factor V activity was observed. For example, PPP from D.G. had factor V assays of 62%, 62%, 66%, and 62% at 0, 2, 4, and 6 hr of incubation, respectively. Normal plasma had factor V assays of 85%, 92%, 84%, and 80% at 0, 2, 4, and 6 hr respectively.

In Fig. 4, additional experiments involving 3 CML patients and 1 normal are shown. In G.S., the final platelet count in the incubation mixture was 300 x 10^9/liter, and the factor V rose slightly between 4 and 6 hr incubation. In L.F., the incubation mixture platelet counts were only 120 x 10^9/liter (the patient was thrombocytopenic) before BMT and 33 days after BMT after engraftment had occurred. There was a

The Effect of Hydroxyurea on Factor V Activity in Normal Plasma

Factor V assays on mixtures of normal plasma and buffered saline were 92%, 87%, and 70%, at 0, 1, and 2 hr of incubation, respectively. The factor V activities of a mixture with a final concentration of 1.5 mg hydroxyurea/ml plasma were 82%, 74%, and 72% at 0, 1, and 2 hr of incubation, respectively. Similar factor V assays were observed in mixtures containing a final concentration of either 0.15 or 0.015 mg hydroxyurea/ml plasma incubated for 2 hr. The addition of hydroxyurea to normal plasma, therefore, did not appreciably alter the factor V activity in this test system.

The Effect of Incubating Normal and CML Platelets With Normal Plasma on Factor V Activity

In Fig. 3, the effect of incubating normal platelets with normal plasma in 3 separate experiments is shown by the solid circles. No significant change in factor V activity was observed. The open circles represent the mean of 6 experiments in which pooled normal PPP was incubated for 6 hr at 37°C. Little change in factor V activity was observed.
Fig. 4. Factor V activity on suspensions of CML platelets and normal plasma incubated at 37°C. Factor V assays were performed at 0, 2, 4, and 6 hr of incubation on mixtures of normal plasma and CML platelets from 3 patients (G.S., L.F., and D.G.). The final platelet counts (+10^9/liter) in each experiment are shown in parentheses. Patient L.F. was studied 14 days before (pre) and 33 days after (post) bone marrow transplantation. Patient G.S. and D.G. were studied during chronic phase. For comparison, the open circles represent the factor V activity of normal platelets (control) incubated with normal plasma in which the final platelet count was 900 x 10^9/liter. The shaded portions depict the range of pooled normal plasma factor V activity.

A slight downward trend in factor V activity in both cases. The normal control in Fig. 4 had a platelet count of 900 x 10^9/liter, and there was little change in factor V activity. In contrast, D.G., while still in chronic phase, had a platelet count of 1000 x 10^9/liter in the incubation mixture and showed a striking drop in factor V activity from 65% to 15% over 6 hr incubation.

In Vivo Factor V Recovery Studies

The effects of FFP infusions in three patients with Ph^1 + CML and in the patient with severe congenital factor V deficiency (R.G.) are shown in Fig. 5. The rise in factor V in the CML patients was so transient that half-lives could not be calculated, while in R.G., the graph of factor V on a logarithmic scale against time on a linear scale was a straight line from which a half-life of 25 hr could be estimated. The estimated recoveries of factor V following infusion of FFP in the three CML patients and in R.G. are shown in Table 4. The calculated recovery of factor V was based on the difference between the observed preinfusion and the Table 4. The Recovery of Factor V After Fresh Frozen Plasma (FFP) Transfusions in 3 Patients With Ph^1 + CML (C.M., G.S., L.F.) and Severe Congenital Factor V Deficiency (R.G.)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Weight (kg)</th>
<th>Plasma Volume (ml)</th>
<th>FFP Transfused (ml)</th>
<th>Corrected Plasma Volume* (ml)</th>
<th>Pre-FFP</th>
<th>Observed Rise After FFP (%)</th>
<th>Expected Rise After FFP (%)</th>
<th>Observed/Rise After FFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.G.</td>
<td>70</td>
<td>2870</td>
<td>2025</td>
<td>3440</td>
<td>&lt;1</td>
<td>32</td>
<td>32</td>
<td>59</td>
</tr>
<tr>
<td>C.M.</td>
<td>84</td>
<td>3440</td>
<td>2400</td>
<td>4110</td>
<td>52</td>
<td>63</td>
<td>11</td>
<td>58</td>
</tr>
<tr>
<td>G.S.</td>
<td>59</td>
<td>2420</td>
<td>1775</td>
<td>2920</td>
<td>31</td>
<td>43</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td>L.F.</td>
<td>48</td>
<td>1970</td>
<td>1500</td>
<td>2390</td>
<td>42</td>
<td>52</td>
<td>10</td>
<td>63</td>
</tr>
</tbody>
</table>

Plasma volume was estimated to be 41 ml/kg body weight. Factor V assays were done on samples of transfused FFP immediately after the infusion of individual units. Transfused FFP had factor V assays of greater than 85%. FFP was assumed to have 100% factor V activity in the calculations. The pre-FFP factor V activities were measured immediately before the FFP infusion (see Fig. 5). The peak factor V activity, which was observed immediately after the FFP infusion, was used in the calculation of the observed rise in factor V activity. Factor V recovery is represented by the ratio of the observed/expected rise in factor V activity based on the corrected plasma volume.

*According to the method of Biggs and Denson.31
peak postinfusion factor V assays (see Fig. 5). The expected rise in factor V activity for these 3 CML patients was 58%, 60%, and 63%, respectively. The peak rises in factor V activity were 11%, 12%, and 10%, which occurred immediately after the FFP infusion. The calculated recoveries of factor V were 19%, 20%, and 16%, respectively. In contrast, the expected rise in R.G. was 59%, and the observed rise was 32% for a recovery of 54%.

**The Effect of FFP Infusions on Factor XI Activity in CML**

Two CML patients who received FFP infusions both had factor XI activities of 38% before the start of the infusion (Fig. 5, insert). Immediately after completion of the FFP infusion, the factor XI activities were 83%, and 76%, and the initial factor XI recoveries were calculated to be 75% and 63% in patients G.S. and L.F., respectively. Factor XI activities 1.5 times the preinfusion level or greater were found for 12 hr or more after completion of the FFP infusion.

**Effect of the FFP Infusion on Plasma Fibrinogen in CML**

In C.M., the preinfusion fibrinogen was 0.41 g/dl, and the value 8 hr after the end of the infusion was 0.57 g/dl. In G.S., the fibrinogen was 0.24 g/dl prior to FFP and 0.38/dl 18 and 57 hr after the end of the FFP infusion. In L.F., the postinfusion fibrinogen was not measured. Immediately prior to and following the administration of FFP, the serum FDP were <10 µg/ml in each of the 3 patients infused.

**Effect of Plateletpheresis and Splenectomy on Factor V Activity**

In patient D.G. (Fig. 6), the factor V was 45% 14 days before plateletpheresis and 50% immediately before plateletpheresis. The factor V values immediately postplateletpheresis, 36 hr, 60 hr (not shown on graph), and 19 days later were 68%, 58%, 65%, and 58%, respectively. There was thus a slight but definite rise in factor V after plateletpheresis in this patient. In G.S. (Fig. 7), the factor V activity was between 39% and 41% during the 3 days prior to splenectomy and 72% and 70% on the third and fourth postoperative days, respectively. The preoperative platelet count of 118 x 10^9/liter rapidly rose to 1900 x 10^9/liter 4 days after splenectomy. On the fifth postoperative day, the platelet count was 2700 x 10^9/liter, and the factor V was 47%. Plateletpheresis was carried out, and the following day the platelet count was 1100 x 10^9/liter and the factor V was 64%. A second plateletpheresis
was done 2 days after the first, accompanied by no change in the factor V activity.

In L.F., splenectomy was done during blast crisis (Fig. 7). Factor V activity was 33% 3 days before splenectomy and 38% immediately after the operation. At 1, 2, 4, and 5 days postoperatively, the factor V activities were 30%, 57%, 47%, and 41%, respectively. In S.B., splenectomy was done during chronic phase. Factor V activity was 62% 2 days before splenectomy and 56% immediately after surgery. One week later, factor V was 84%. In both patients L.F. and S.B., post-splenectomy rises in platelet counts occurred that did not correlate either directly or inversely with the observed changes in factor V activity.

### Effect of Bone Marrow Transplantation (BMT) on Factor V Activity

Figure 7 shows the effects of chemotherapy, total-body irradiation, and BMT on factor V activity in two patients with Ph1+ CML. Both patients had low factor V assays, thrombocytosis, and marked bone marrow hypercellularity after splenectomy and prior to cytotherapy. Both patients developed pancytopenia following the 6 days of therapy prior to BMT.

In patient L.F., normal factor V activity was first observed during cytotherapy when an abrupt increase in factor V from 50% to 100% was noted. Normal to greater than normal factor V activity was consistently observed during the post-BMT period of bone marrow hypoplasia. Bone marrow studies 21 and 72 days after BMT showed hypocellularity and Ph-negative marrow elements. Repeat bone marrow studies 84 days after BMT showed hypocellularity, myeloblasts, and the return of Ph1+ marrow cells. A normal factor V activity of 88% was observed 94 days after BMT when therapy was discontinued. Death occurred 2 mo later, and no additional studies were obtained.

In patient G.S., an abrupt increase in factor V activity from 62% to 100% occurred during chemotherapy and total-body irradiation. On the day of BMT, factor V activity was 122%. Normocellular Ph1-negative bone marrow studies were obtained 21, 35, 70, and 168 days after BMT. During this time after BMT, all factor V assays were either normal or greater than normal. Bone marrow relapse was apparent 291 days after BMT when a normocellular bone marrow specimen showed that 5 of 20 cells tested were Ph1+. A normal WBC and a platelet count of 478 x 10^9/liter were noted. Normal PT, PTT, TT, fibrinogen, and serum FDP determinations were observed. A fall in factor V activity to 68% was subsequently observed. Repeat studies 392 days after BMT showed a markedly hypercellular bone marrow with increased megakaryocytes and 5% myeloblasts. Bone marrow cytogenetic studies showed that all marrow cells tested were Ph1+. The WBC and platelet count were 30 and 1218 x 10^9/liter, respectively. Normal serum hepatic enzymes, serum bilirubin, PT, PTT, TT, and fibrinogen levels were observed. The factor V activity was 62%. When last studied 410 days after BMT, G.S. remained in the chronic phase of CML with an observed factor V activity of 63%.

### DISCUSSION

During a 3-yr prospective study, factor V deficiency was observed in 8 of 8 patients, 7 to 20 yr old, with Ph1+ CML. Factor V deficiency was noted in 3 of 4 patients at diagnosis of chronic phase and before any therapy. The median factor V activities did not appreciably change in 3 patients following transition from chronic phase to blast crisis.

We initially supposed that the low factor V levels would be due to one of the previously described causes of factor V deficiency. Laboratory tests to exclude DIC and liver disease were repeatedly normal in these eight patients.

In three patients, a very low recovery and transient rise in factor V was observed in each case following the infusion of 28–31 ml/kg body weight of FFP, suggesting the presence of a factor V inhibitor. Our in vitro studies excluded factor V inhibitors as a cause of either the low factor V levels or the poor response to FFP infusion. Hydroxyurea, an agent used to treat all 8 patients studied, did not inhibit the factor V of normal plasma in vitro either at once or over a 2-hr incubation.

In the 8 prospectively studied patients, low levels of factor XI or XII were present either singly or in combination in 5 patients. In 2 patients with factor XI deficiency, factor XI levels rose strikingly with FFP infusion and remained 1.5 times or more the preinfusion values for 12 hr or more. The half-life of factor XI infused in FFP has been reported to be 60–70 hr. This response was very different from that observed for factor V. L.F. had a factor XI of 32% and a factor XII of 22%. The patient’s mother had a factor XI of 118% and a factor XII of 34%, suggesting that at least the factor XII deficiency in L.F. was on a hereditary basis. Other family studies were not done, but it is unlikely that all low levels of factor XI and XII were inherited. Deficiencies of one or more vitamin-K-dependent factors were found in 5 of 8 patients. As shown in Fig. 2, all 3 abnormally low factor X levels were normal after oral administration of vitamin K-1, and 3 of 4 initially low factor II levels became normal.
The response of factor VII to vitamin K was more variable. Since low levels of factors II, VII, IX, X, XI, and XII were mild and found in only some of the 8 patients studied, we did not investigate the mechanisms further. In contrast, factor V deficiency was the most striking coagulation abnormality and the only plasma coagulation factor deficiency common to all 8 patients.

We investigated possible causes of factor V deficiency in the eight patients with Ph' + CML with both in vitro and in vivo studies. In vitro studies in five patients clearly showed that they did not have inhibitors to factor V. Infusions of FFP caused only a transient rise in factor V in three patients. Since inhibitors to factor V had already been excluded as a cause of factor V deficiency, the rapid in vivo disappearance of infused factor V had to be due to some other cause. The in vivo change in factor V following plateletpheresis was transient and small. The changes in factor V incident to splenectomy could be explained by nonspecific in vivo activation of coagulation following surgery. The changes in factor V after both plateletpheresis and splenectomy were thus inconclusive.

In contrast, cytoreduction for BMT produced a striking rise in factor V. In two patients prior to BMT, we observed persistently low factor V levels and marked bone marrow hypercellularity. Following cytoreduction and BMT, factor V levels were persistently normal or elevated at a time when bone marrow hypoplasia was observed. In one of these patients, the reappearance of Ph' + marrow elements and bone marrow hypercellularity was associated with a drop in factor V to abnormally low levels. While the data are not conclusive, the response to cytoreduction and BMT strongly suggests that the low factor V was associated with the large myeloid-megakaryocytic cell mass present in these patients prior to cytoreduction. The response to FFP infusion suggests that if the low factor V in these patients is associated with a large myeloid-megakaryocytic cell mass, the factor V may be rapidly adsorbed to some or all of the neoplastic cells, a situation somewhat analogous to the rapid disappearance of infused factor X and diffuse binding of factor X in systemic amyloidosis. In one of four in vitro experiments we observed a very striking decrease of factor V of normal plasma incubated with CML platelets in high concentration. These preliminary data suggest that the mechanism of factor V deficiency may be the adsorption of plasma factor V on CML platelets, at least in that patient. It should be pointed out that adsorption of factor V by myeloid elements other than platelets has not been excluded.

Our retrospective studies showed that 6 of 20 patients with Ph' + CML had factor V deficiency not due to liver disease or DIC. There was a slight age overlap between the prospectively and retrospectively studied patients, but the latter patients were almost all older. The group of 8 younger prospectively studied patients all had factor V deficiency, while this was less common in the older CML patients. Among normal subjects, factor V levels have been reported to increase with age at a rate approximating 0.6% per annum, which could explain why factor V deficiency was less common in older CML patients. Whether this distribution of factor V deficiency in younger and older CML patients would be true in a larger series is unknown.

Our retrospective studies have shown that factor V deficiency is not limited to Ph' + CML, but may occur in other myeloproliferative disorders. Factor V deficiency was observed in one patient with Ph' -negative CML, one of three patients with polycythemia vera, and in two of three patients with essential thrombocytopenia. The mechanisms of factor V deficiency in myeloproliferative disorders deserve further investigation; they may not be the same in all patients.

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Factor V deficiency in Philadelphia-positive chronic myelogenous leukemia

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