Roles of Prothrombin Activity, Heparin-Protamine Titer and Platelet Concentration in Bleeding of Leukemia

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Leukemia involves many tissues and may often become complicated by infection, bleeding, malnutrition and mechanical interference with organs. Studies have been made in this laboratory on the nature of the hemorrhagic diathesis as it occurs in leukemia. This report pertains chiefly to heparin-protamine titration, to prothrombin activity of plasma and to platelet concentration. Data on plasma fibrinogen in bleeding leukemic patients are also presented.

Howell's suggestion that the delayed clotting of blood in shock was the result of circulating heparin1 was substantiated by the quantitative isolation by Jaques and Waters of heparin from dogs that had been anaphylactically shocked.2 They indicated also that heparin existed in extremely low concentration. Later, Volkert and Hertel3 reported that a variable component of the antithrombic complex described by Astrup and Darling4 acted like heparin, and that this component was reflected in elevated antithrombin levels in some cases of leukemia. Their observations, however, were not related to bleeding in particular. Barnard5 claimed to have had similar experience, but data were not presented. Klein and Seegers6 explained the antithrombic effect of heparin on the basis of its interference with the thrombin-fibrinogen reaction, rather than by destruction of thrombin. Allen and his associates,7-8 using the method of Jaques and Waters2 initially reported an increase in heparin in dogs that had been treated with large doses of roentgen rays. They considered the associated bleeding to be caused by "heparinemia," and the concomitant thrombocytopenia to be of secondary importance.

The observation by Chargaff and Olson,9 that the affinity of protamine for heparin could be used to assay its anticoagulant effect in vivo, was applied by Allen and his associates to study the naturally-occurring concentration of "heparin-like substances" in idiopathic and leukemic thrombocytopenic purpura.10 Smith and Allen and their co-workers made a similar study of the concentration of these substances after nitrogen mustard poisoning.11 Other workers12,13 failed to verify the conclusions of Allen and his associates in regard to "heparinemia" in irradiated dogs, and feel that thrombocytopenia is the basic defect.

The obscurity of the significance of heparin-protamine titrations prompted a review of 420 tests performed at the Tumor Therapy Clinic of the Children's...
Medical Center.* Values obtained in two groups of tests carried out by the same technician under surveillance of different workers were mutually confirmatory. Therefore, all 358 tests made on leukemic children and the 62 made on normal controls (from which a base line had been established) were used in this study. The possible influence of deficient prothrombin activity was also considered.

On the other hand, the regularity with which thrombocytopenia does occur simultaneously with bleeding in leukemia prompted a reinvestigation of its role in the hemorrhagic diathesis.

In this report, data referring to leukemia is compared to similar data collected by Nygaard concerning other thrombocytopenic conditions14 and to the functional effect of thrombocytopenia upon the clotting mechanism investigated by Buckwalter, Blythe and Brinkhaus.15

METHODS

The technic used in this laboratory for the heparin-protamine titration is a modification of that used by Allen and his associates.19 All tests were carried out by the same technician over a period of eighteen months.

A solution of protamine sulfate (Lilly) was prepared every three weeks by dissolving 100 mg. in 100 ml. of distilled water. Each newly prepared solution was adjusted accurately by titration in normal blood to the same strength as the previous lot, and it was allowed to stand for twenty-four hours at 5 C. before it was used. The same lot of heparin sodium (Abbott) was used for all tests. Serologic tubes (1 by 8 cm.) were used for the titrations.

The following amounts of protamine sulfate solution were pipeted into a series of five tubes: 0.10, 0.12, 0.14, 0.16 and 0.18 ml., respectively. Therefore, the tubes contained 100, 120, 140, 160 and 180 y of protamine sulfate, respectively.

Exactly 0.05 ml. (0.5 mg.) of heparin solution was pipeted into the bottom of a conical graduated centrifuge tube.

Blood was obtained as follows: The skin was prepared with alcohol and allowed to dry. A tourniquet was applied. The plunger of the 20 ml. syringe was dipped into light mineral oil before being used, and a sharp 18 or 20 gauge needle was fitted into the Luer-lock. A neat, direct venipuncture was made, care being taken not to palpate or otherwise traumatize the vein. Approximately 15 ml. of blood was allowed to enter the syringe, largely by its own pressure.

Blood was added carefully to the heparin in the centrifuge tube until the 5.5 ml. mark was reached, and the tube was sealed with a clean rubber stopper. The blood-heparin mixture was inverted gently fifteen times. The heparinized blood was then taken up in a 1 ml. pipet, and exactly 1 ml. was added carefully to each tube containing protamine sulfate. The tubes were shaken vigorously, one by one, fifteen times, after which the rack of tubes was allowed to stand undisturbed at room temperature. Readings were made at the end of 1, 3, and 24 hours.

The end-point was defined as the protamine content of the tube containing the least amount of protamine in which a solid clot formed. Under conditions in this laboratory, the sharpest end-point was observed at the end of three hours, and that reading was used exclusively in this study. Calculating an end-point on the basis of three temporal readings gave essentially the same results.

At the same time, hematocrit readings were made by the Wintrobe method16 on portions of specimens, and these readings were considered in conjunction with the protamine titers.

Protamine titrations were carried out on 420 specimens, representing 134 subjects, including normal controls. Of these, 228 were taken from patients during episodes of clinically

* The authors are indebted to Dr. Robert Mercer, who originally established the technic and the base line for normals in these laboratories, and to Dr. W. Jacobson for having made available their data on the earlier group of determinations that are included in this series.

† Developed by Dr. Robert Mercer.
active bleeding, and 130 were obtained from nonbleeding leukemic patients. All but 14 of the specimens from bleeding individuals were from children with acute leukemia. Fourteen were obtained from patients who had neuroblastoma involving the bone marrow. Tests were carried out in a number of instances on the same patients during nonbleeding periods. The control group consisted of 23 normal young technicians, nurses, and doctors, and provided 62 specimens. From these, the normal control value of protamine that permitted clotting in three hours had been established. There proved to be a well defined end-point for the controls in the tube containing 120γ. A few control specimens clotted in the tubes containing 100 and 140γ. The average hematoctrit reading for the same group was 0.42 ml. per milliliter of blood, or, reciprocally, 0.58 ml. of plasma per milliliter of whole blood.

In view of the diversity of hematoctrit values among leukemic subjects and the probability that "anticoagulants" would exist in solution in plasma rather than within the formed elements of blood, protamine values were recalculated according to unit plasma volume. Hematoctrit readings were subtracted from 1.00 ml. to give plasma volumes. In each case, the protamine value obtained by assay was multiplied by the proportion of the normal plasma volume (0.58 ml.) to the specimen's volume. A second factor to be considered was the standard excess of protamine used in all tubes to neutralize the excess heparin introduced into the test to prevent coagulation. A common excess of 80γ of protamine was used in the calculation rather than 100γ because tubes contained increments of 20γ and represented maximal values in each case. For example, the lowest reading (100γ) represented the range between 81 and 100γ. The common excess of 80γ was subtracted from all values prior to calculation for plasma volume, and then added again so that final figures would be comparable to uncorrected values. Accordingly, the following formula was adopted:

\[(γ \text{ of protamine} - 80γ) × \frac{0.58 \text{ ml. plasma per ml. of blood}}{\text{corrected for hematoctrit}} + 80γ = \text{Protamine titer}}

Prothrombin activity and protamine titers were determined simultaneously in 51 bleeding subjects. Prothrombin time was measured by Quick's one-stage method,17 using fresh thromboplastin (Difco), and was translated into prothrombin activity by the dilution method of Rosenfield and Tuft.18 The average prothrombin time of normal plasma in this clinic was 134 seconds, with a range between 12 and 15 seconds. Prothrombin activity of 50 per cent or more was considered arbitrarily to be well above the level for clinical bleeding.19, 20 Prothrombin tests were divided into two groups, those with activity within 50 per cent of normal and those with less, for the purpose of comparing results with protamine titers among leukemic subjects having active bleeding.

Plasma fibrinogen concentrations had been determined on 161 specimens, including 76 normal controls. Of the remaining 85, 63 were from bleeding and 22 from nonbleeding leukemic children. Fibrinogen was determined as fibrin by coagulating decalcified plasma with beef thrombin (commercial). The clot was washed, hydrolyzed and nesslerized. Nitrogen was determined in a Klett colorimeter with a no. 540 filter. Fibrinogen levels among bleeding and nonbleeding individuals were compared.

Platelet counts were made weekly in a large series of children with leukemia and in some with disseminated neuroblastoma, over a period of eighteen months. In 351 instances, heparin-protamine titrations and platelet counts were carried out simultaneously. The 351 samples represented 134 subjects so that in several instances the same subjects' platelets were counted during bleeding episodes as well as at other times during the disease. All platelet determinations were made by the direct method on capillary, finger-prick blood, using 3.8 per cent sodium citrate solution as the diluent.21 Three technicians who had had mutually consistent results made the counts. Of the 351 tests, 228 were from children with evidence of active bleeding, and 123 from those who were not bleeding. The type of bleeding varied. Spontaneous petechiae and ecchymoses in the skin and mucous membranes were considered positive as well as frank hemorrhage from viscera and mucous membranes. Normal young adults who had served as controls for protamine-heparin assays were included among the nonbleeding subjects. The relationship between circulating thrombo-
cyte levels and heparin-protamine assay values (based on whole blood volume) in bleeding and nonbleeding individuals was recorded, and the results appear in figure 1.

Using a modified Lee-White coagulation method for whole blood, only occasional prolongation of the clotting time in bleeding of leukemia had been detected in this laboratory. Therefore the clotting time of oxalated plasma, measured by a technic modified from that of Gram, was used because it proved to be a more sensitive test for rate of coagulation in a series of leukemic plasma specimens having varying concentrations of platelets. The recalcification time of oxalated plasma was determined as follows:

Precautions were taken to minimize trauma to the tissues while blood was drawn from a vein into a clean, dry syringe. Immediately, 4.5 ml. of blood were transferred to a 15 ml. centrifuge tube containing 0.5 ml. of tenth molar potassium oxalate solution. The contents were mixed gently, but adequately, to prevent coagulation. The oxalated blood was placed immediately in an ice bath. Then it was centrifuged at 1,000 r.p.m. for eight minutes at 5 C. The supernatant plasma containing the platelets was transferred quickly to a clean tube in the ice bath and kept cold until ready for use. Tests were carried out within two hours after the blood was drawn. Next, 0.1 ml. of plasma was pipetted into each of two pyrex test tubes (10 by 75 mm.) held in a rack in a water bath at 37.5 C. Five minutes were allowed for the cold plasma to come to temperature equilibrium. Then, 0.2 ml. of an aqueous 0.025 molar solution of calcium chloride at 37.5 C, was added quickly to each of the tubes, and the contents were mixed by flicking the bottoms of the tubes sharply three times. Simultaneously with the addition of calcium chloride, two stop watches were started, one for each tube. At the end of one minute, the tubes were examined for clotting by gently inclining them for evidence of forming coagulum. Subsequently, this was repeated every 15 seconds. The end-point was the time at the end of the 15 second period during which the meniscus failed to shift with gravity upon inclining the tube. Duplicates seldom varied more than 15 seconds. When they did, the test was repeated and the results averaged.

Recalcification time was carried out on 28 specimens from 16 normal subjects in order to

* A No. 2 International Centrifuge with a No. 240 head was used.
establish a base line. Likewise, 55 specimens obtained from 30 leukemic subjects were studied. The results are recorded in figure 2, together with platelet counts made simultaneously on capillary blood. The clinical status with respect to bleeding is indicated in each instance.

Data were compiled from Nygaard's monograph\textsuperscript{11} with respect to recalcification time studies and platelet counts made on 110 specimens from 27 patients with essential thrombocytopenic purpura (before and after splenectomy) and from 11 patients representing a variety of eight other conditions attended by thrombocytopenia. Only 2 had leukemia. Co-ordinates were plotted from this data (fig. 3) in a fashion similar to that used for data from leukemic patients in figure 2.

**Fig. 2.—**Distribution of 55 specimens from bleeding and non-bleeding leukemic patients according to platelet counts on capillary blood and recalcification times of oxalated plasma. The control zone represents 28 specimens. (Ordinates are plotted on logarithmic scale.)

It is well to note that platelet concentrations refer to undiluted whole blood or plasma. Determinations of recalcification time were made after a 1:2 dilution of plasma in aqueous calcium chloride so that both platelets and other elements of the coagulation mechanism were diluted proportionately except for calcium ion.

In order to view recalcification time as a function of coagulation, median curves made from both sets of data were compared to a similar curve adapted from the data of Buckwalter, Blythe and Brinkhous.\textsuperscript{14} Using a two-stage technic, Brinkhous and his co-workers\textsuperscript{24} demonstrated dependence of prothrombin utilization on plasma platelet concentration. This method avoided the exaggerated effect of fully evolved accelerator of serum that is operative in the serum prothrombin time technic.\textsuperscript{25} Their data were converted from per cent of platelets to absolute numbers based on their published normal averages of 549,000 per cu. mm. in venous plasma and 285,000 in venous blood. The resulting curve was continued downward to the average platelet concentration observed empirically in clinical thrombocytopenia.
cytopenic disease, thus conforming to the values in figures 2 and 3. Their data on prothrombin utilization were obtained by varying the platelet concentrations experimentally in normal plasma. The portions of the curves representing normal recalcification time in figures 2 and 3 were superimposed on the limb of normal prothrombin utilization so that the relative effects of diminishing platelet concentrations would become apparent (fig. 4).

FIG. 3.—Distribution of 110 specimens from patients having thrombocytopenic disease (mainly essential thrombocytopenic purpura) according to platelet counts on venous blood and recalcification times of oxalated plasma. (Ordinates are plotted on logarithmic scale.) Data from Nygaard.14

RESULTS

In table 1 it is apparent that the proportion of elevated to normal heparin-protamine titers varies, increasing from the normal group to the nonbleeding leukemic group, and then to the bleeding leukemic group. The last can be considered, generally, to be in the most advanced stage of disease. The preponderance of normal titers (81 per cent) among the nonbleeding leukemic patients and the high incidence of normal titers (45 per cent) among the bleeding group indicates, however, that elevated protamine titers are not characteristic of leukemia. In regard to the phenomenon of bleeding, per se, the pertinent figures are the 103 normal titers (45 per cent) and the 125 elevated protamine titers (55 per cent) among bleeding leukemic individuals. The majority of bleeding patients, therefore, have elevated titers, but the degree of difference is not great.

Protamine titers shown in table 1 were obtained directly on whole blood. Titers for 301 specimens plotted against hematocrit determinations are arranged graphi-
Fig. 4.—Superimposition of median curves from figures 2 and 3 on a curve representing rate of prothrombin utilization in normal plasma having varying concentrations of platelets. Plasma platelet concentrations recalculated from data of Buckwalter, Blythe and Brinkhous to make them comparable to those for whole venous blood, and plotted on logarithmic scale.

Table 1.—Distribution of Normal and Elevated Protamine Titers in Whole Blood with Reference to Leukemia and to Bleeding

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Protamine titers</th>
<th></th>
<th></th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120 γ or less</td>
<td></td>
<td>140 γ or more</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>Per cent</td>
<td>Number</td>
<td>Per cent</td>
</tr>
<tr>
<td>Normal*</td>
<td>54</td>
<td>87</td>
<td>8*</td>
<td>13</td>
</tr>
<tr>
<td>Leukemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not bleeding</td>
<td>105</td>
<td>81</td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td>Bleeding†</td>
<td>103</td>
<td>45</td>
<td>125</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>58</td>
<td>150</td>
<td>42</td>
</tr>
<tr>
<td>Grand total</td>
<td>262</td>
<td>158</td>
<td>420</td>
<td></td>
</tr>
</tbody>
</table>

* Normal controls, some of whom were menstruating.
† A few specimens from children with metastatic neuroblastoma involving marrow were included.
‡ Of these, only 109 had simultaneous hematocrit readings.

cally in figure 5. This illustrates the general tendency for protamine titers to rise as the hematocrit values fall, and also the fact that specimens from bleeding
subjects usually have hematocrit readings consistent with anemia. The inverse relationship between protamine titers and hematocrit values may be viewed simply as a direct one between titers and plasma volumes. To a large extent, the normal controls make up the grouping centered at the coordinates of 120γ and a hematocrit of 0.42 ml.

Elimination of the variable of plasma volume from the titration, by adjusting protamine titers for unit plasma volume (using the formula), results in the values seen in table 2. In figure 6, the 301 recalculated titers are grouped according to the presence or absence of active bleeding. The proportion of bleeding

![Graph showing distribution of protamine titers and hematocrit values.](image)

**Fig. 5.**—Distribution of protamine titers and hematocrit values for all subjects, leukemic and nonleukemic. Protamine values are based on volume of whole blood.

leukemic patients having elevated protamine titers, based on unit plasma volume, is 51 per cent of 109 tests. This is similar to 55 per cent of 228 tests made on the basis of whole blood volume, without consideration of plasma volume. However, of the 130 nonbleeding leukemic patients, 55 per cent have elevated titers after correction for plasma volume as compared to 19 per cent prior to recalculation. The redistribution of protamine titers in the two groups, shown in figure 6, results in an identity of median values at approximately 120γ, for both bleeding and nonbleeding subjects. The two main peaks of the distribution for nonbleeding individuals include 62 normal controls. Elimination of the normal controls (so that only leukemic subjects remain) does not change the median for that group. The distribution, however, becomes similar to that for the bleeding group. Therefore, from available data, no positive correlation can be made between elevated heparin-protamine titers and the phenomenon of bleeding in leukemia.
The results of studies on prothrombin activity are given in table 3 in conjunction with protamine titers based on whole blood volume. Eleven of 18 bleeding

**Table 2.** Distribution of Normal and Elevated Protamine Titers after Correction for Hematocrit with Reference to Leukemia and to Bleeding

<table>
<thead>
<tr>
<th></th>
<th>Protamine titers</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120 γ or less</td>
<td>More than 120 γ</td>
<td>Total number</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>Per cent</td>
<td>Number</td>
</tr>
<tr>
<td>Normal*</td>
<td>39</td>
<td>63</td>
<td>23</td>
</tr>
<tr>
<td>Leukemic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not bleeding</td>
<td>50</td>
<td>45</td>
<td>71</td>
</tr>
<tr>
<td>Bleeding</td>
<td>53</td>
<td>49</td>
<td>56</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>47</td>
<td>127</td>
</tr>
<tr>
<td>Grand total</td>
<td>151</td>
<td>150</td>
<td>301</td>
</tr>
</tbody>
</table>

* Normal controls, some of whom were menstruating.

Fig. 6. Distribution of protamine titers for bleeding and nonbleeding groups of subjects. Protamine values are based on unit plasma volume by calculation from original titers obtained in whole blood.

Individuals (61 per cent) having normal protamine titers also had at least 50 per cent of normal prothrombin activity. Among the 33 bleeding individuals having elevated protamine titers, 20 (61 per cent) had prothrombin activity of less than 50 per cent of normal plasma, and 13 (39 per cent) had adequate prothrombin activity. Therefore, bleeding subjects who had normal heparin-
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Protamine titers seemed likely to have adequate or normal prothrombin activity also, whereas deficient prothrombin activity frequently was associated with elevated protamine titers. It is particularly noteworthy, however, that 39 per cent of the bleeding patients with elevated protamine titers had 50 to 100 per cent of normal prothrombin activity. The data presented in table 3 indicate that bleeding that is characteristic of the hemorrhagic diathesis occurred in leukemic patients having both normal heparin-protamine titers and normal prothrombin activity.

Plasma fibrinogen concentrations among leukemic children generally were increased over the normal average of 310 mg. per 100 ml. of plasma (table 4).

**Table 3.—Distribution of Prothrombin Activity Among Specimens from Bleeding Leukemic Children Having either Normal or Elevated Heparin-protamine Titers**

<table>
<thead>
<tr>
<th>Prothrombin activity</th>
<th>Heparin-protamine titer*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>50 to 100 per cent of normal</td>
<td>11</td>
</tr>
<tr>
<td>Less than 50 per cent of normal</td>
<td>7</td>
</tr>
</tbody>
</table>

* On whole blood (uncorrected).

**Table 4.—Plasma Fibrinogen Concentrations in Normal and in Bleeding and Nonbleeding Leukemic Patients**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Fibrinogen, mg. per 100 ml. plasma</th>
<th>Number of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>Normal individuals</td>
<td>310</td>
<td>195–528</td>
</tr>
<tr>
<td>Leukemic patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Bleeding</td>
<td>380</td>
<td>151–845</td>
</tr>
<tr>
<td>Bleeding</td>
<td>440</td>
<td>38–796</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Twenty-two nonbleeding leukemic patients had an average concentration of 350 mg. per 100 ml., and had a wide range of values. Among the 63 specimens from bleeding patients, the average was 440 mg. per 100 ml. In only 5 of these was the plasma fibrinogen concentration less than 200 mg. per 100 ml. of plasma; in 2 of these 5 it was less than 100 mg. Seldom was a deficiency of fibrinogen associated with clinical bleeding in leukemia.

Among the 351 specimens shown in figure 1, it can be seen that there was no clinical evidence of bleeding in individuals having 55,000 or more circulating platelets per cu. mm., whereas bleeding was the rule, but not invariable, among those having fewer platelets. Failure to bleed among critically thrombocytopenic subjects was not uncommon, there being 36 instances among 257 that fell within the critically thrombocytopenic range. Thus it appears that platelet deficiency may be an essential factor, but not a completely adequate cause of bleeding.
The average normal recalcification time proved to be approximately 120 seconds by the technic used in this laboratory (fig. 2). The coagulation time tended to become prolonged when the capillary platelet concentrations were in the range of about 40,000 to 50,000 per cu. mm., or 15 to 20 per cent of normal. This tendency became further exaggerated until a concentration of approximately 20,000 per cu. mm. was reached, at which point coagulation was often considerably delayed. It is of special interest to note that a few bleeding subjects with critically reduced platelet levels fell within the normal range of coagulation time (fig. 2). In the particular group studied, the lowest count was 6,000 platelets per cu. mm.

A similar graph made from data accumulated by Nygaard is shown in figure 3. Information with regard to the presence or absence of bleeding was not available. The distribution of coordinates and the resulting median curve resemble figure 2 closely. Nygaard used a photoelectric technic for determining an endpoint for coagulation of plasma and obtained an average normal of 170 seconds (equivalent to 120 seconds by the method used in this laboratory). His platelet counts were made on venous blood after sedimentation of red cells. This probably accounts for the difference in platelet concentrations at which the two median curves begin to deviate from the normal (fig. 2). This is in accordance with the findings of Tocantins that venous blood has a higher concentration of platelets than does capillary blood.

The observations of Buckwalter and his associates concerning the manner in which the concentration of platelets affects the rate of prothrombin utilization are demonstrated in figure 4. Utilization remains at a normal rate, having an index of 1.00, until the platelet level falls to approximately 70,000 to 75,000 per cu. mm. in venous blood. Under experimental conditions, utilization diminished rapidly below that concentration, reaching a theoretical index of zero when the measurable platelet deficiency was absolute. By superimposition of the three curves, an interesting relationship among platelet concentration (regardless of type of disease), recalcification time, and prothrombin utilization in normal plasma can be seen (fig. 4). It appears that diminishing rates of both recalcification time and prothrombin utilization are related to platelet concentration in a strikingly similar way. Apparently there is no disturbance in rate of coagulation in plasma of blood having at least 70,000 platelets per cu. mm., and little or none in plasma of blood having between 40,000 and 70,000 platelets per cubic millimeter.

**DISCUSSION**

The ultimate concern of an investigation into the nature of the hemorrhagic diathesis in leukemia is the loss of vascular integrity and the extravasation of blood. It was the particular purpose of this study to evaluate some particular factors that have been thought to influence the hemorrhagic diathesis either through deficiencies of coagulative factors or through excess anticoagulant.

The disturbance of prothrombin activity often seen in bleeding leukemic patients is confirmed here, and can be anticipated readily in view of the frequent involvement of the liver and the partial avitaminosis that generally attends states of malnutrition and infection in advanced disease. However, the frequency
of bleeding without evidence of defective prothrombin activity is of greater interest and is more pertinent to the basic issues inherent in the hemorrhagic diathesis.

In view of the reports of measurable, circulating anticoagulant in various hemorrhagic conditions, and in leukemia in particular, the method of heparin-protein titration became of interest. The titration, as carried out, is an assay with dependence of the end-point upon the rate of the complicated biologic process of coagulation. The test is complicated further by the back titration necessary to overcome the effects of heparin that is added to the blood. The major problem, however, proves to be the variability in plasma volume among specimens in which quantitative titrations are carried out. Subsequent recalculation of protamine titers designed to refer titers to unit plasma volume (in order to eliminate the red cell mass from the coagulation system) revealed a lack of distinction between the bleeding and nonbleeding groups of leukemic patients. This shift in titers after recalculation compensates for the severity of anemia to be expected in bleeding leukemic subjects in comparison with nonbleeding ones and normal controls. Besides the lack of distinction, the possible association of elevated protamine titers with bleeding, per se, is further minimized by the presence of a large proportion (49 per cent) of bleeding individuals with normal titers.

The tendency for bleeding leukemic children with normal protamine titers to have normal prothrombin activity also, is strong evidence that the hemorrhagic diathesis of leukemia is not basically dependent on coagulation defects measurable either by prothrombin time or by heparin-protein titration. The possibility exists also that protamine titer might be affected by defective prothrombin activity.

According to observations in this laboratory, plasma fibrinogen generally exists in an abnormally high concentration in bleeding leukemic patients, although rarely it may be low enough to be considered causative of bleeding. Apparently, fibrinogenopenia is not an important etiologic factor in the hemorrhagic diathesis of leukemia, but it may be a factor in severe liver disease or in the presence of excessive, recent hemorrhage.

The uncertainty that an increase in circulating “heparin-like” substance has been found in association with bleeding in leukemia accentuates the relative importance of thrombocytopenia in that regard. In this series, the association has been almost invariable. That is to say, clinical bleeding rarely occurred unless the circulating platelet count was below the critical level of approximately 50,000 to 60,000 per cu. mm. of capillary blood. Thrombocytopenia, per se, however, appears not to be a completely adequate cause of bleeding. This view is supported by the presence of 36 instances of critical thrombocytopenia in which there was no evidence of bleeding. Obviously, a permeable vascular tree is prerequisite to clinically detectable bleeding, regardless of the physiologic status of its circulating contents. In view of the probability that weakening or rupture of vascular tissue may have several causes, either obvious or obscure, this discussion is limited to the spontaneous type of bleeding that is characteristic of leukemia and other conditions regularly attended by thrombocytopenia. It is to be expected that hemorrhage from trauma or from erosion of vessels secondary
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to inflammatory, neoplastic, and other disease processes, in spite of adequate circulating platelets, may occur in leukemia also. These accessory causes of bleeding, fortuitously, were not encountered apart from the typical hemorrhagic diathesis in this series. It appears that the changes in the blood vessels in leukemia, whatever their basic nature may be, are likely to result in a degree of porosity that is compatible with extravasation of blood whenever fewer than approximately 50,000 to 60,000 circulating platelets per cubic millimeter are available between source and depository.

The laboratory demonstrations by Eagle, Quick and his associates, and Buckwalter, Blythe and Brinkhous, have drawn attention to a coagulative defect in thrombocytopenic states. This is in contrast to the classical teaching that the clotting time in thrombocytopenic purpura is normal. The commonplace usage of the whole blood method probably is responsible for the continuous inference that coagulation is not disturbed in thrombocytopenic states.

Of particular interest is the critical concentration of circulating platelets that is common both for the tendency toward bleeding and for the retardation of clot formation and retraction. This is in conformity with the idea that platelets may have a function beyond that related to the thromboplastic system and to retraction of fibrin. Obviously, the process of coagulation is not invoked without prior insult to the vessel. It would appear, therefore, that the type of vascular damage associated with thrombocytopenic purpura either may fail to appear in the presence of more than 50,000 to 60,000 platelets per cu. mm., or may be neutralized too rapidly to become clinically manifest. A continent vascular bed in the presence of severe thrombocytopenia suggests that there may be other factors that provoke vascular damage. Such factors may be operative in the thrombocytopenic state without affecting the recalcification time (fig. 2). The frequency with which striking platelet depressions have followed clinical infection in leukemia, in the experience at this clinic, suggested studies from the point of view that infectious agents or the effects of their presence may contribute toward the activation of bleeding.

SUMMARY

A test for circulating "heparin-like" anticoagulants neutralizable by protamine has been studied in children with acute leukemia. Bleeding occurred with equal frequency among those with normal and those with elevated heparin-protamine titers. When the protamine titers are calculated on the basis of unit plasma volume, similar distributions of titers for both bleeding and nonbleeding leukemic individuals and identical medians for both groups are observed. The hemorrhagic diathesis in acute leukemia does not necessarily appear to be dependent on coagulation defects demonstrable by prothrombin time, although such defects often are present.

The regular association of circulating platelet levels below 50,000 to 60,000 per cu. mm. with bleeding in acute leukemia has been reaffirmed. Below this critical level, the rate of plasma coagulation is often, but not always, prolonged. Although critical thrombocytopenia appears to be essential to activation of the
hemorrhagic diathesis, it seems not to be causative by itself. The relationships among bleeding, thrombocytopenia, and coagulation are discussed.

REFERENCES

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Roles of Prothrombin Activity, Heparin-Protamine Titer and Platelet Concentration in Bleeding of Leukemia

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