Thrombocytopathia Due to Abnormalities in Platelet Release Reaction—Studies on Six Unrelated Patients

By Harvey J. Weiss and John Rogers

The prolonged bleeding time in six unrelated patients ages 24–63, was attributed to impaired platelet aggregation; this could be accounted for by the decreased release of platelet adenosine diphosphate (ADP) that was obtained in all patients. As a consequence of this "platelet release abnormality," collagen-induced platelet aggregation was impaired, the second wave of epinephrine-induced aggregation was decreased (although not invariably absent), and the normal initial wave of ADP-induced aggregation was followed by rapid disaggregation at 37°C. In four patients, the abnormality in collagen-induced aggregation and ADP release appeared to be different than the defect produced by aspirin, whereas two patients appeared to have an "aspirinlike defect." The platelets of all patients adhered normally to connective tissue fibers but were poorly retained in glass bead filters. Other abnormalities included impaired kaolin-induced platelet factor 3 availability in four patients, large platelets (which aggregated normally with bovine fibrinogen) in two, and small platelets in one patient. The general term thrombocytopathia is suggested to describe abnormalities in platelet function; where a specific defect in the release reaction has been demonstrated, "platelet release abnormality" is suggested as an appropriate, specific term.

The primary arrest of bleeding has been attributed to a poorly defined vascular response and to the formation of platelet aggregates at the sites of vascular injury. Circulating blood platelets do not normally adhere to intact endothelial surfaces, but once the endothelium is broken they rapidly adhere to subendothelial substances, among which are collagen¹ and a newly described substance.² Following their adhesion, adenosine diphosphate (ADP), released from the platelets, produces platelet aggregation by a still disputed mechanism. ADP is also necessary for making the platelet clotting activity, platelet factor 3, available for its role in the intrinsic coagulation mechanism.³ The subject has been recently reviewed.⁴

In 1967 we described six patients with bleeding disorders in whom defects in platelet factor 3 availability and collagen-induced platelet aggregation were attributed to impaired release of platelet ADP. We present herein more extensive studies on two of these patients and our findings in four more recently discovered, unrelated patients with impaired ADP release. The results suggest at least two types of defect that may account for this abnormality.

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CASE REPORTS

S. K. is a 64-yr-old woman who first noticed easy bruising and excessive bleeding from superficial cuts in 1957 at age 51. Her most troublesome problem has been recurrent, painful ecchymoses on the trunk and chest. These are unrelated to trauma and are often preceded by premonitory feelings of being "washed out." The intradermal test of Gardner and Diamond for autoerythrocyte sensation was negative. Before the onset of her symptoms, she had undergone salpingoopherectomy, tonsillectomy, and appendectomy without untoward bleeding. There is no history of bleeding either in parents or six siblings.

L. C. is a 24-yr-old woman who observed the onset of easy bruising at age 17 and was told that she had von Willebrand’s disease. Bruises occurred mainly on her legs and occasionally on her arms. They were painless, unrelated to menses, and not associated with premonitory symptoms. She has never had any operations, and there is no history of menorrhagia, arthritis, or solar sensitivity. Her family history is negative with regard to bleeding.

E. P. is a 24-yr-old woman with a lifelong history of easy bruising. She bled excessively after a tonsillectomy at age 4 and has had persistent menorrhagia.

S. N., a 25-yr-old woman and F. A., a 57-yr-old woman, have had episodes of excessive bleeding all their lives as previously reported. D. C. is the propositus of a large, previously reported family whose bleeding disorder was found to be transmitted as an autosomal dominant trait. We think it useful to compare the findings in a typical member of that family with the five unrelated subjects reported herein.

Physical examinations in all patients were noncontributory, except for the presence of ecchymoses on some occasions. More specifically, there were no signs of a connective tissue disorder or albinism.

MATERIALS AND METHODS

Bleeding time (Ivy), prothrombin time, partial thromboplastin time (P.T.T.), clot retraction, antihemophilic factor (AHF, factor VIII) assay, and platelet counts (phase microscopy) were performed as previously described.

Blood for platelet studies was obtained by mixing nine parts of venous blood with one part of either 3.2% sodium citrate or a solution containing 1% sodium EDTA and 0.7% saline. The blood was centrifuged at 20°C to obtain platelet-rich plasma (PRP) and platelet-poor plasma (PPP) as previously described. EDTA-PRP was used to determine platelet size and the adhesion of platelets to connective tissue. Citrated PRP was used for all other studies.

Platelet size was kindly determined by Dr. Ralph Zalusky, using the previously described Coulter Counter technique, and the results were expressed as the mean window (W). With latex particles of 3.357 cu µ (Coulter Electronics), a mean window of 2.91 was obtained, so that the calibration factor for converting W to platelet volume is 1.16. Platelet adhesion to connective tissue fibers and the retention of platelets in directly sampled venous blood in glass bead filters (platelet adhesiveness to glass) were determined by previously reported methods. Platelet factor 3 availability (PF 3-a) was determined by a method in which the subject’s PRP is incubated with kaolin for 6 min, and a sample then added, with calcium, to normal plasma that had been previously incubated with kaolin to activate the contact system.

Platelet aggregation was determined by a turbidometric method on citrated PRP. The same connective tissue suspension, prepared as previously described, was used to test the platelets of the patients and of normal subjects. ADP-induced aggregation was tested at both room temperature and at 37°C, using a final ADP concentration of 2 µM. The degree of aggregation was determined from the initial optical density value (OD₁) and the value at maximal aggregation (OD₉) and was expressed as (1—OD₉/OD₁) × 100. Aggregation by epinephrine (5 µM) and bovine fibrinogen (Armour Pharmaceuticals, Kankakee, Ill., final concentration 50 mg/100 ml) was studied at 37°C. The effect of 1 mM aspirin, in vitro, on connective tissue-induced platelet aggregation was determined by previously reported methods. The amount of ADP released
THROMBOCYTOPATHIA

Table 1. Initial Studies on Patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Bleeding Time* (min)</th>
<th>Duration of Bleeding Sx.</th>
<th>P.T.T. (sec)</th>
<th>AHF (%x 10(^{-3}))</th>
<th>Platelets (per cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.N.</td>
<td>25</td>
<td>F</td>
<td>&gt;30, &gt;30, &gt;30</td>
<td>Lifelong</td>
<td>36</td>
<td>75</td>
<td>200</td>
</tr>
<tr>
<td>S.K.</td>
<td>64</td>
<td>F</td>
<td>19, &gt;30</td>
<td>Since age 51</td>
<td>45</td>
<td>80</td>
<td>130</td>
</tr>
<tr>
<td>L.G.</td>
<td>24</td>
<td>F</td>
<td>10,11</td>
<td>Since age 17</td>
<td>36</td>
<td>65</td>
<td>185</td>
</tr>
<tr>
<td>E.P.</td>
<td>25</td>
<td>F</td>
<td>20, &gt;25</td>
<td>Lifelong</td>
<td>35</td>
<td>100</td>
<td>215</td>
</tr>
<tr>
<td>D.C.</td>
<td>29</td>
<td>F</td>
<td>13,20</td>
<td>Lifelong</td>
<td>36</td>
<td>90</td>
<td>130</td>
</tr>
<tr>
<td>F.A.</td>
<td>57</td>
<td>F</td>
<td>18,15,8,3,11</td>
<td>Lifelong</td>
<td>37</td>
<td>120</td>
<td>410</td>
</tr>
</tbody>
</table>

Normal values

<table>
<thead>
<tr>
<th>Mean</th>
<th>3.4</th>
<th>39</th>
<th>97</th>
<th>187</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± 2 SD</td>
<td>1.2-5.6</td>
<td>35-45</td>
<td>49-194</td>
<td>187-395</td>
</tr>
<tr>
<td>Number studied</td>
<td>30</td>
<td>55</td>
<td>60</td>
<td>53</td>
</tr>
</tbody>
</table>

*Values shown obtained on different days.

from platelets in citrated PRP after incubation with either kaolin or a standard suspension of connective tissue was determined as either platelet-aggregating equivalent (P.A.E.)\(^5,7\) or enzymatically, using the Boehringer reagents.\(^7,8\)

All patients and normal subjects indicated that they had complied with a request to abstain from taking any medication for at least 2 wk before being studied.

RESULTS

Studies of Hemostasis

The bleeding time was increased in all patients, most strikingly in patients S.N., S.K., E.P., and D.C. (Table 1). Normal values were obtained for the prothrombin time, partial thromboplastin time, plasma fibrinogen, and anti-hemophilic factor (AHF). Platelet counts were normal in four patients, but moderately decreased to 130,000 in D.C. and S.K. Clot retraction was normal in all patients.

Hematology Studies

Hemoglobin values and white blood counts were within normal limits. Patient S.K. had a persistent monocytosis (8%–16%) during the 3 yr (1968–1970) in which she was studied. There were no inclusions in the white blood cells of any patient. Aside from the variations in size described below, the morphology of the platelets on Jenner-Giemsa stained smears of the peripheral blood was not remarkable.

Other Studies

L.E. tests were negative, and serum electrophoresis and BUN values were within normal limits in all patients.

Platelet Studies

Platelet Size: The platelets of patients S.K., S.A., and F.A. were of normal size (Table 2). The mean window (w) obtained for the platelets of patient D.C. indicated that her platelets were 22% smaller than the average normal plate-
<table>
<thead>
<tr>
<th>Patients</th>
<th>Platelet Size (Window)</th>
<th>PFA-1 (sec)</th>
<th>Adhesion to Connective Tissue (%)</th>
<th>ADP Release (µM/10^6 platelets)</th>
<th>P.A.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.N.</td>
<td>7.4</td>
<td>54.8452</td>
<td>19.13</td>
<td>17</td>
<td>0.5</td>
</tr>
<tr>
<td>S.K.</td>
<td>9.2</td>
<td>59.8073</td>
<td>0</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>L.G.</td>
<td>10.9</td>
<td>35.4040</td>
<td>6</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>E.P.</td>
<td>9.6</td>
<td>46.50</td>
<td>0</td>
<td>19.13</td>
<td>1</td>
</tr>
<tr>
<td>D.C.</td>
<td>6.2</td>
<td>68.63370</td>
<td>11.8</td>
<td>4</td>
<td>2.4, 1</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>8.0</td>
<td>34.51</td>
<td>23</td>
<td>12</td>
<td>0.25, 0.5</td>
</tr>
<tr>
<td>Mean ± 2 SD</td>
<td>6.4 ± 2</td>
<td>34.51 ± 2</td>
<td>56</td>
<td>16</td>
<td>4.28</td>
</tr>
<tr>
<td>Number studied</td>
<td>25</td>
<td>88</td>
<td>56</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

*ADP measured as either enzymatically (E) or as platelet-aggregating equivalent (P.A.E.).
*Calculated after log transformation of original values.
Platelet-size distribution. Particle-size plot distributions of platelets from patient L.G. (upper) and from a representative normal control (lower) were determined with Coulter Counter and recorded on the graph-out as shown. Excursions of the pen are proportional to number of platelets of increasing size, counted in each of successive windows.

Platelets; the platelets of E.P. and L.G. (Fig. 1), on the other hand, were 22% and 38% larger, respectively.

Platelet Adhesion: The retention of platelets in glass bead filters was studied by direct filtration of venous blood as described in Methods. Decreased values were obtained in all six patients. The adhesion of platelets to connective tissue was normal (Table 2).

Platelet Factor-3 Availability: PF3-a, assayed after incubating PRP with kaolin for 6 min, was consistently decreased in four patients (S.N., S.K., D.C., and F.A.), and, except in S.K., the activity was markedly increased by freezethawing the PRP before assay (Table 2). In some cases, when incubation of the PRP and kaolin was carried out for 20 min, the values obtained for patients were not significantly different than those for normal subjects. PF3-a in patients E.P. and L.G. was consistently normal.

Platelet Aggregation: Collagen-induced aggregation was studied by stirring 2.5 ml of PRP with varying amounts of connective tissue suspension. In four patients (S.N., S.K., E.P., and D.C.), the amount of aggregation produced by 0.05 and 0.10 ml of suspension was markedly decreased when compared with
### Table 3. Platelet Aggregation

<table>
<thead>
<tr>
<th>Subject</th>
<th>Room Temp.</th>
<th>Disaggregation, 37°C</th>
<th>Epinephrine 1st Wave</th>
<th>2nd Wave</th>
<th>Connective Tissue 0.05 ml</th>
<th>0.10 ml</th>
<th>0.20 ml</th>
<th>0.30 ml</th>
<th>0.50 ml</th>
<th>Effect of ASA</th>
<th>Bovine Fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.N.</td>
<td>64</td>
<td>—</td>
<td>2+</td>
<td>0-2+</td>
<td>0-5</td>
<td>20-25</td>
<td>—</td>
<td>—</td>
<td>51</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>S.K.</td>
<td>65</td>
<td>yes</td>
<td>0-2+</td>
<td>0</td>
<td>0-6</td>
<td>0</td>
<td>20-30</td>
<td>37</td>
<td>47</td>
<td>N.E.</td>
<td>—</td>
</tr>
<tr>
<td>L.G.</td>
<td>72</td>
<td>yes</td>
<td>2+</td>
<td>0-2+</td>
<td>15-34</td>
<td>59-69</td>
<td>—</td>
<td>—</td>
<td>34</td>
<td>1</td>
<td>3+</td>
</tr>
<tr>
<td>E.P.</td>
<td>69</td>
<td>yes</td>
<td>2+</td>
<td>0-2+</td>
<td>3-6</td>
<td>6-14</td>
<td>28</td>
<td>39</td>
<td>67</td>
<td>1</td>
<td>3+</td>
</tr>
<tr>
<td>D.C.</td>
<td>61</td>
<td>yes</td>
<td>2+</td>
<td>0</td>
<td>0-3</td>
<td>0-8</td>
<td>—</td>
<td>34</td>
<td>40-55</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>F.A.</td>
<td>69</td>
<td>yes</td>
<td>2+</td>
<td>0-3+</td>
<td>17,45,46</td>
<td>66-79</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>N.E.</td>
<td>—</td>
</tr>
</tbody>
</table>

Normal subjects

- Mean: 67 Variable: 1-3+ 2-4+ 73 76 — — — 2-4+
- Mean ± 2 SD: 53-81 — — — 57-89 64-90 — — —
- Number studied: 34 34 50 50 52 58 — — —

*Numerical values indicate amount of platelet aggregation, expressed as \((1 - \text{O.D.}_{490}/\text{O.D.}_{490}) \times 100.

†2.5 ml PRP + 0.05-0.50 ml of connective tissue suspension. Range of values obtained on three to six separate occasions are shown. Extensive studies on normal subjects were only obtained with 0.05 ml and 0.10 ml of suspension.

‡Aspirin, in final concentration of 1 mM, was added to PRP, and amount of connective tissue suspension that gave 40%-70% aggregation in buffer-treated PRP was added. In some patients, aggregation was decreased (1) by ASA, while in others it had no effect (N.E.).
values obtained in 42–48 normal subjects (Table 3). In patients L.G. and F.A.,
the amount of aggregation obtained with 0.05 ml of suspension was also de-
creased. However, the magnitude of the aggregation defect, as of the bleeding
time prolongation, was less in L.G. and F.A. than in the other four patients.
The defect in collagen-induced aggregation was not absolute, and the addition
of increasing amounts of connective tissue suspension produced significant
aggregation in all patients. In patients L.G. and F.A., aggregation values within
the range of normal values were obtained with 0.10 ml of suspension. The addi-
tion of 0.20–0.50 ml of suspension to PRP of the other four patients resulted
in increasingly greater amounts of aggregation, but since we did not study
many normal subjects with these larger amounts of connective tissue suspen-
sion, we are unable to say whether the degree of aggregation obtained in these
patients might still be decreased. In some cases, at least, the amount of aggre-
gation obtained with 0.20–0.50 ml of suspension was still less than obtained in
normal subjects tested with lesser amounts of the suspension. The possibility
that the optical density changes obtained with the larger amounts of suspen-
sion may have actually represented platelet-collagen adhesion, rather than
aggregation, appears to be eliminated by the findings that no such changes
were obtained in normal EDTA platelet-rich plasma.

The first wave of epinephrine-induced aggregation was normal in all pa-
tients except S.K., in whom no aggregation occurred on two of three occasions.
The second wave was always smaller than observed in normal subjects. It was
consistently absent in S.K. and D.C. and occasionally absent in the other
patients. In all but one of 50 normal subjects, the first wave of epinephrine-
induced aggregation was followed by a second and much larger wave of
aggregation.

ADP-induced aggregation at room temperature was normal in all subjects.
At 37°C, the initial wave of aggregation produced by 2 µM ADP was con-
sistently followed by disaggregation, but this sometimes occurred in normal
subjects.

Aggregation of platelets by bovine fibrinogen was normal in patients E.P.
and L.G., the patients with large platelets.

**Effect of Aspirin on Platelet Aggregation:** The amount of connective tissue
sufficient to produce 40%–70% aggregation when added to PRP at 37°C was
determined for each patient, and the possible inhibitory effect of 1 mM aspirin
was then determined. This concentration of aspirin markedly suppressed plate-
et aggregation in patients D.C., S.N., E.P., and L.G. but had little effect on
the platelets of F.A. and S.K. (Table 3).

**Release of Platelet ADP:** Connective tissue-induced release of ADP was de-
creased in all patients. Similar abnormalities in kaolin-induced ADP release
were also obtained (Table 2).

**DISCUSSION**

**Defects in Platelet Aggregation**

The prolonged bleeding time observed in these patients indicates a defect in
the primary arrest of bleeding, and this may be attributed to the impairment in
collagen-induced platelet aggregation that was demonstrated in all six patients. Since platelets must adhere to collagen before aggregation can occur, a defect in platelet-to-collagen adhesion might account for the abnormalities observed. No such defect was observed in any of these patients. The aggregation of platelets by collagen requires the release of intrinsic platelet ADP; therefore the impaired release of platelet ADP must be considered the basic defect that underlies the abnormalities in collagen-induced aggregation in these and previously reported patients. The release of ADP also accounts for the second wave of epinephrine and ADP-induced aggregation at 37°C and is necessary for optimal release of PF 3-α. and the abnormalities obtained in these tests on most occasions may be similarly attributed to a defect in ADP release. In contrast to the findings in Glanzmann’s thrombasthenia, the patients’ platelets underwent normal aggregation with exogenous ADP.

The process by which collagen, epinephrine, and ADP, among other substances, induce a release of platelet ADP, as well as ATP and serotonin, has been called the “release reaction” and has been tentatively divided into three phases: an initial induction phase, possibly involving a receptor site on the platelet membrane; the intracellular transmission of the message; and the extrusion from the platelet of a selective pool of metabolically inactive nucleotides (storage pool) present in the specialized “dense bodies.” A defect in any one or more stages in the sequence could, therefore, account for an impairment of collagen-induced aggregation. Previous studies on the family of patient D.C. have demonstrated a marked deficiency in the pool of metabolically inactive ADP that is extruded during the release reaction, and in studies to be reported separately, a similar deficiency was found in the three unrelated patients E.P., S.N., and L.G. Thus, a deficiency in the storage pool of adenine nucleotides, which we have called “storage pool disease,” is one type of defect that may account for abnormalities in platelet aggregation. Another type may be produced by aspirin, as well as other anti-inflammatory agents, phenothiazines, antihistamines, and tricyclic antidepressants. Aspirin, for example, by inhibiting ADP release markedly inhibits collagen-induced aggregation, although to a lesser degree than in thrombocytopenia, and completely blocks the second wave of epinephrine-induced aggregation. Since aspirin has no effect on the total amount of nucleotides or on the storage pool, it may inhibit the induction-transmission stage of the release reaction, so that with a relatively “weak” release inducer, such as epinephrine, little or no ADP is released even though the storage pool is present in normal amounts. By contrast, a small second wave of epinephrine-induced aggregation was sometimes seen in the patients with “storage pool disease.” Perhaps the induction-transmission stage is normal in these patients, and the release of the small amounts of storage ADP may be sufficient to induce some, if diminished, aggregation. The differences between the aspirin-induced defect and those in the four patients with “storage pool disease” are further underscored by the findings that the two defects were additive. Aspirin did not significantly increase the aggregation defects in S.K. and F.A., the patients with normal storage nucleotides, and this finding suggests a defect in the induction-transmission stage of the
release reaction in these two patients. This “in vitro aspirin tolerance test” may be useful in classifying patients with defects in platelet aggregation.

Nomenclature

The retention of platelets in glass bead filters was decreased in the patients reported herein, suggesting that the release of platelet ADP may play a role in this test. Although decreased platelet retention is also found in patients with von Willebrand’s disease, AHF is usually decreased in the latter disorder, and both collagen-induced platelet aggregation and ADP release are normal.15,16 The large platelets found in patients L.G. and E.P. are similar to the findings in the Bernard-Soulier syndrome.17-19 However, the platelets in the latter disorder appear to be considerably larger than in our patients and they do not aggregate with bovine fibrinogen20 in contrast to the normal aggregation obtained in L.G. and E.P. Except that patient S.K. developed acute myeloblastic leukemia 6 mo after completion of this study and 13 yr after the onset of her bleeding symptoms, there was nothing in the other patients described herein to suggest any of the other acquired21 or congenital22 disorders in which defects in platelet aggregation may also occur.

There is no nomenclature in current use that adequately describes the disorders of platelet function in any kind of rational manner, and a variety of names such as thrombopathia,5 Portsmouth syndrome,23 and “new platelet abnormality”24 have been used to describe patients with defects in platelet aggregation, PF-3 release, or both. The six patients described herein and previously5,7 all appear to have as their underlying platelet abnormality an impaired release of ADP, and a similar type of defect has been suggested in some patients with aggregation defects reported by other investigators.24,25 As reported elsewhere,13 some of these patients appear to lack the storage pool of adenine nucleotides that are specifically extruded during the release reaction. We found differences in PF-3 activity, platelet size, and platelet lipid composition26 among these patients with storage pool disease, suggesting that further subdivisions of this disorder may be possible. Perhaps until the nature of these disorders becomes more clearly established, the general term thrombocytopenia might be retained to describe abnormalities in platelet function, and where a specific defect in the release reaction has been demonstrated, “platelet release abnormality” might be an appropriate specific term.

Since platelet swelling may occur in the presence of EDTA,27 the platelet size determinations in patient L.G. and in five normal subjects were repeated using platelet-rich plasma obtained from blood which had been collected with 1/10 volume of 3.2% sodium citrate. The average \( \bar{w} \) in the normal subjects was 7.42 ± 0.64 (SD) while a value of 9.37 was obtained for patient L.G., again indicating that her platelets were abnormally large.

REFERENCES


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