Platelet Functional Abnormalities: A Report of Two Familial Defects in Interaction Between Collagen and Platelets and ADP Release

By A. G. Papayannis, E. J. Watson-Williams, and M. C. G. Israëls

Two families have been studied, members of which have a lifelong hemorrhagic diathesis. In both families, a platelet functional abnormality has been found, with the coagulation mechanism intact. Findings common to both families were a prolonged bleeding time, reduced platelet adhesiveness, absence of the second wave of aggregation with ADP and reduced ADP release from platelets. The clot retraction, platelet factor 3 availability and aggregation of platelets with ADP in a final concentration of 1.0 and 20.0 μM were normal. The differences between the families were the platelet morphology, the in vitro adherence of platelets to collagen fibrils, the collagen-induced aggregation, and the thrombin-induced aggregation. In one of them, the Windle family, the platelets were abnormally large in size and showed a delayed adherence to collagen fibrils. The aggregation with collagen was either absent or feeble, and with thrombin, showed an abnormal pattern. In the other, the Hughes family, the platelets were morphologically normal and adhered to collagen fibrils normally. The aggregation with collagen initiated normally but usually did not proceed to completion and the aggregation with thrombin was normal. It appears that in the Windle family the main abnormality concerns the reaction between platelet and collagen and the condition is associated with impaired ADP-release mechanism and abnormal thrombin-platelet reaction, and in the Hughes family the ADP-release mechanism of platelets is defective. On the basis of current information, including that from these families, a classification of the various platelet functional abnormalities and methods for characterizing them is proposed.

In the previous decade, two inherited platelet functional abnormalities were recognized, thrombocytopathia (thrombopathia or thrombopathy) and thrombocytasthenia (or thromboasthenia or Glanzmann's disease). Braunsteiner and Pakesh,1 in their classification of platelet functional abnormalities used the clot-retraction test (CR) to distinguish the two disorders. In the former the CR was normal, but in the latter it was abnormal, while in both syndromes, tests assessing the contribution of platelet to hemostasis were abnormal.

From the University Department of Clinical Hematology, Manchester Royal Infirmary, Manchester, England.

Submitted April 28, 1971; revised June 11, 1971; accepted July 1, 1971.

Requests for reprints should be made to: E. J. Watson-Williams, Methodist Hospital of Brooklyn, 506 6 Street, Brooklyn, N.Y. 11215.

Since the introduction of the aggregation method for the investigation of platelet function functional abnormalities of platelets have been reported by many investigators. Weiss, Hardisty and Hutton, Hirsh et al., O'Brien, and Sahud et al. collectively reported 23 individuals with similar findings namely; mild hemorrhagic diathesis generally prolonged bleeding time (BT) and reduced platelet adhesiveness (PAd), abnormal or absent collagen-induced aggregation but normal ADP-induced aggregation. Clot retraction was normal, and in all, except one with only 30% Factor XI coagulation factor deficiency was not found. Both Hardisty and Hutton and Weiss considered that the abnormality was due to a primary defect of ADP-release from platelets, whereas Hirsh et al. believed that in their case the basic abnormality was nonreaction between collagen and platelets. Caen et al. and Weiss et al. described several affected members in one family each with autosomal dominant type of inheritance.

In the study of the two families reported here, we give evidence that the platelet functional abnormalities in which the collagen induced aggregation and the ADP release are abnormal can be divided into at least two types. In one, the defect concerns the reaction between platelets and collagen and the condition is associated with impaired ADP-release mechanism, abnormal reaction between platelets and thrombin and morphologically abnormal platelets. In the other, the ADP-release mechanism is defective. On the basis of a group of platelet function studies we postulate six defined and sequential reactions of normal platelets that contribute to hemostasis.

**Materials and Methods**

Blood was collected into plastic syringes using 20-gauge needles (Becton-Dickinson) and 9 parts were mixed with 1 part 3.8% sodium citrate. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained by differential centrifugation. The PRP, adjusted to 300,000 platelets per μl with the patient’s own PPP, was kept at room temperature and tested within 4 hr. The PPP was kept at 4°C until tested. All the tubes and pipettes used for the PRP were siliconized. Full details for our methods of platelet adhesiveness, aggregation, ADP release, and platelet factor 3 availability (PF-3a) are given elsewhere. Major points only in technique are noted here.

**Platelet Adhesiveness**

Salzman’s method was used, slightly modified. The main difference between our technique and that of Salzman was that we used a standard length vinyl tubing (25.5 cm, I.D. 3 mm.) containing 2.7 ± 0.1 g glass beads (Jencons No. 8) instead of 1.3 g glass beads (Minnesota 3M 070) of the original Salzman’s method. One hundred and thirty-three normal persons gave between 30 and 66% reduction in platelet count (mean, 47.98; SD, 9.47).

**Platelet Aggregation**

A photometric technic based on those of Born and O’Brien was used. The Platelet Aggregation Meter (EEL Model 169) was used which provides continuous stirring of the sample and a thermostatic block to maintain the temperature at 37°C. The apparatus was connected with a recorder (desk type Servoscribe Potentiometric Recorder, Smith’s Industries). The following aggregation tests were performed: (1) Aggregation with ADP to a final concentration (f.c.) of 1.0 and 20.0 μM; also tests with incremental amounts of the agent from 1.5 to 4.0 (1.5, 2.0, 2.5, 3.0, 3.5, and 4.0) μM f.c. were performed. (2) Aggregation with collagen: 0.1 ml. human connective tissue extract prepared according to
Hovig\textsuperscript{13} was added to 1.0 ml PRP. (3) Aggregation with adrenalin 5 $\mu$M f.c. (4) Aggregation with thrombin, 1.0 U/ml f.c.

Our findings in more than 100 normal persons were as follows: With ADP (1) 1.0 $\mu$M f.c.—the addition of the agent was followed by an immediate narrowing of the amplitude of the tracing and by an aggregation curve that did not proceed to completion but within 60 sec was always followed by disaggregation; (2) 20.0 $\mu$M f.c.—this amount of the agent caused an immediate narrowing of the tracing and a steep aggregation which was complete (final aggregation) and never followed by disaggregation; (3) incremental amounts—with ADP ranging between 2.0 and 4.0 $\mu$M f.c., the second wave of aggregation was usually initiated;\textsuperscript{14} in a proportion of normal subjects the second wave of aggregation was not initiated with this agent.\textsuperscript{15} With collagen, the addition of the agent was followed by a delay period of about 40–50 sec, after which the amplitude of the tracing was narrowed; aggregation started and became final; disaggregation never occurred with this agent. With adrenalin, the response varied widely; the majority showed a final aggregation which was composed of two aggregation curves, some showed a partial aggregation in which only the first wave was present and some did not respond at all. With thrombin, the addition of the agent was followed by a delay period of about 10 sec, after which the tracing narrowed and a steep aggregation occurred not followed by disaggregation.

**ADP Release From Platelet**

This procedure has been modified from the method of Weiss.\textsuperscript{4} After reaction of the subject’s platelets with kaolin, collagen, or thrombin, a known volume (0.1–0.5 ml) of the supernatant was added to normal PRP. The amount required to produce aggregation was compared with the amount of ADP required to produce the same degree of aggregation. The supernatant was removed at exactly 10, 5, or 3 min after the start of reaction with kaolin, collagen, or thrombin, respectively. (3 $\times$ 10$^8$ platelets from normal persons treated in this way liberated into the supernatant aggregating activity equivalent to between 2.0 and 10.0 $\mu$g of ADP). Usually all the agents resulted in release of the same amount of aggregating activity from platelets of the same individual. This aggregating activity is probably the net result of release of ADP and of other substances, such as ATP and AMP, which have an inhibitory action.

**Platelet Factor-3 Availability Test (PF-3a)**

The method described by Weiss,\textsuperscript{4} slightly modified, was used.

**The Plasma ADP-Removing Mechanism**

Normal plasma has the ability to remove ADP probably by enzymatic mechanism.\textsuperscript{15,16} We tested the presence and effectiveness of this mechanism as follows: to 1.0 ml of the test PPP, incubated at 37°C, 0.1 ml of a solution of ADP in a concentration of 20.0 $\mu$M was added. Immediately after the addition of ADP and at 5 min intervals, 0.1 ml of the incubation mixture was transferred to normal PRP in the aggregation meter, and the aggregation produced gave a measure of the amount of ADP present in the test plasma. In normal subjects, the ADP in the PPP progressively diminished to half of the initial amount after 10 min and almost to zero after 20 min.

The adhesion of platelets to collagen fibrils was investigated in vitro as follows: on a microscope slide 1 drop of connective tissue extract was mixed with 1 drop of the test PRP, and observed under the phase contrast microscope. Platelets from normal persons adhered to the collagen fibrils after a delay of 5–10 min.

**Other Methods**

The following blood coagulation tests were carried out routinely: prothrombin time (one stage), partial thromboplastin time, and factor VIII assay.

For the bleeding time, Ivy’s method was used and for the clot retraction that of Mac-
Farlane. The morphology of platelets and their tendency to form clumps were studied in peripheral blood smears; platelet counts were carried out using phase contrast microscopy. For the other tests, standard methods were used.

The patients were asked to avoid any kind of treatment for at least 15 days before testing with a special notice to avoid aspirin or any drug for pain relief.

CASE REPORTS

Family trees of the families studied are shown in Fig. 1.

The Windle Family

The proposita (II-1), a 49-yr-old female was referred for investigation of an unexplained familial bleeding tendency. In September 1967 she had had a hysterectomy for menorrhagia and bled vaginally for several days. The bleeding ceased after the transfusion of fresh blood. She has had frequent epistaxes from the age of 3 and has always bled for 1–2 days following dental extraction. Blood transfusion had been given after a cesarian section in 1947. Neither of her parents bled unusually.

The elder daughter (II-2), age 24, had always bruised easily but had normal periods. At age 7 she had hematuria and at age 22 a thyroidectomy was complicated by bleeding from suture holes. Dental extractions were uncomplicated, and she had a normal delivery of an apparently unaffected child.

The younger daughter (II-3), age 23, bruised easily and she had an occasional epistaxis. Menstrual loss was normal up to 16 yr of age, but is described as rather heavier since. At age 16 she had an appendectomy which was complicated by bleeding from the suture holes. She bled for 3 days following dental extraction.

The son (II-3), is 11 yr old. He has bruised easily and, at age 5, tonsillectomy was complicated by considerable bleeding. He bled for 2 days following dental extraction.

The Hughes Family

The proposita (III-4), a 15 yr-old female was referred because of menorrhagia. Menarche was 2 yr before and menstruation is described as heavy lasting 7 days and regular. In November 1968, bleeding persisted 14 days and she needed blood transfusion administered at home; a later operation showed normal curettages; bleeding ceased after 25 days under the influence of stilbestrol, 4 mg daily. In December she had a normal menstrual loss, but in January 1969 continuous severe bleeding necessitated further transfusion. The bleeding was again controlled with stilbestrol and since then, for 9 mo, she is continuing to take the drug. An attempt to stop the treatment in July 1969 was followed by a very heavy period. She has always bled a lot after dental extraction but not for more than 1 hr. Her mother stated that all three daughters from her second marriage had always bruised easily and that 10 yr ago the older two, the proposita (III-4) and the second daughter (III-5) now
PLATELET FUNCTIONAL ABNORMALITIES

Table 1.—Laboratory Tests on Members of the Windle Family

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Platelet Count (x 10^9/µl)</th>
<th>Bleeding Time (min)</th>
<th>Platelet Adhesiveness (%)</th>
<th>Tourniquet Test</th>
<th>Clot Retraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-1</td>
<td>15. 8.67</td>
<td>&gt;15</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16. 8.67</td>
<td>4</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11. 9.67</td>
<td>3</td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.12.67</td>
<td>141</td>
<td>5:9:12</td>
<td>Positive</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9. 1.68</td>
<td>96</td>
<td>2;4;6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. 1.69</td>
<td>240</td>
<td>10%;&gt;15;&gt;15</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20. 2.69</td>
<td>138</td>
<td>&gt;15;&gt;15;&gt;15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-1</td>
<td>15. 8.67</td>
<td>3</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.12.67</td>
<td>109</td>
<td>3;3;4</td>
<td>Negative</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. 1.69</td>
<td>256</td>
<td>7;7;8</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20. 2.69</td>
<td>180</td>
<td>7%;10%;10%</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-2</td>
<td>15. 8.67</td>
<td>3</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.12.67</td>
<td>188</td>
<td>7;7;8</td>
<td>Negative</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9. 1.68</td>
<td>149</td>
<td>%; %;2%</td>
<td>65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. 1.69</td>
<td>222</td>
<td>3%;6%;7</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20. 2.69</td>
<td>176</td>
<td>8%;9;9%</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-3</td>
<td>30. 8.67</td>
<td>&gt;15</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6. 9.67</td>
<td>&gt;15</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.12.67</td>
<td>225</td>
<td>7%;7%;8</td>
<td>Negative</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9. 1.68</td>
<td>183</td>
<td>1;2;5</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. 1.69</td>
<td>290</td>
<td>7%;11%;14</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20. 2.69</td>
<td>268</td>
<td>%;10%;&gt;15</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>150-400</td>
<td>&lt;6</td>
<td>30-65</td>
<td>Negative</td>
<td>38-70</td>
</tr>
</tbody>
</table>

The mother (II-6) did not admit any unusual bleeding except easy bruising, especially when she was younger. Her three children from a previous marriage (III-1, 2, 3) have no history of bleeding, neither have her four grandchildren (IV-1, 2, 3, 4), but her father (I-1) had severe epistaxes throughout his life. Her mother, all her siblings, and both her husbands were described as normal.

RESULTS

The Windle Family

In Table 1, the platelet count, BT, PAd, tourniquet test, and CR of all affected members of the family tested on several occasions are indicated. The platelet count in the proposita (II-1) was found slightly below normal on three occasions and normal on another occasion. The other three members had normal counts. The BT was usually prolonged but an occasional normal test was encountered in all. The PAd was reduced. The tourniquet test was positive in the proposita (II-1) and negative in the others. The CR was normal. The platelets showed a morphological abnormality: 40-60% were large in size (over 3 µm) with many "giant" forms (over 5 µm); the clumping formation was normal. There was a delay of more than 15 min in the adherence of platelets to collagen fibrils observed with phase contrast microscopy. Tests of aggrega-
Aggregation of platelets of the Windle and Hughes families compared to normal. (Auxiliary curves in the same test indicate alternative aggression patterns.)

Fig. 2.

Aggregation (Fig. 2), ADP release (Fig. 3), and PF-3 a were performed twice for every member and gave consistent results.

The aggregation: (1) with ADP 1.0 and 20.0 nM f.c. was normal but incremental amounts of the agent did not initiate a second wave of aggregation; (2) with collagen there was almost no response at all; following the addition of the agent, the tracing did not show any change in the amplitude and the aggregation curve was either absent (II-1 and III-2) or feeble, starting after a delay of several minutes (III-1, 3); (3) with thrombin the pattern of aggregation was abnormal; the aggregation initiated normally but it was partial and always followed by disaggregation; (4) with adrenalin only the first wave was present. Mixture of the patient's PRP with normal PPP produced the same abnormal aggregation curves; the patient's PPP mixed with normal PRP did not alter the normal aggregation pattern.

Platelet ADP release was nil following aggregation with thrombin and also nil after the addition of kaolin in all affected members. There was also no ADP release after addition of collagen to the platelets of the two members who
showed no aggregation with this agent (II-1 and III-2), and markedly reduced release, 0.4 and 0.6 μg from 3 × 10⁸ platelets, from III-1 and III-3, respectively, whose platelets gave feeble aggregation with collagen. The plasma ADP-removing mechanism and the PF-3 a test were normal in all. The coagulation tests and blood counts were also normal in all.

The Hughes Family

This family can be divided into three groups: (1) history of abnormal bleeding and abnormal platelet function tests (III-4, 5, 6); (2) no history of abnormal bleeding but abnormal platelet function tests (II-6, III-3); (3) no history of bleeding and with normal platelet function tests (II-7, III-2, IV-2, 3, 4).

Detailed results for the first two groups are given in Table 2 and Figs. 2 and 3. Platelet count was within normal limits, as was clot retraction in all. The clinically affected members were found to have a prolonged BT on at least one occasion each and consistently reduced PAd; the tourniquet test was positive in two of them. The proposita’s mother (II-6) showed PAd reduced once and in lower normal range twice and also had a positive tourniquet test. Her son (III-3) gave normal results on the only occasion he was tested. Platelet function tests were performed at least twice for every member tested and gave consistent results except where indicated. Aggregation with ADP f.c., 1.0 μM and 20.0 μM, gave normal results in all. However incremental amounts consistently failed to initiate a second wave of aggregation. Collagen resulted in narrowing of the tracing after the normal 40–50 sec delay and aggregation was initiated normally but usually did not proceed to completion. However, on a few occasions with platelets from some members, aggregation did become complete. With adrenalin final aggregation never occurred but there was a varying pattern; III-3 had no response, II-6 and III-5 showed only the first wave, and the remainder, II-4 and 6, gave incomplete second
waves. The only abnormality noted with thrombin was occasional and weak disaggregation of platelets from II-6, III-3, 5, and 6. There was complete failure to release ADP with collagen, kaolin, and thrombin by the platelets from Group I members, and markedly reduced amounts of ADP (0.4 μg and 0.8 μg) were released from platelets from II-6 and III-3, respectively. In vitro adherence of platelets to collagen fibrils was normal in all members of this family. When normal PPP was added to PRP from Group I aggregation and ADP-release remained abnormal. When the Group I PPP was added to normal PRP aggregation with ADP was normal. The plasma ADP-removing mechanism was normal and so was the PF-3a test. The blood coagulation tests and the blood counts were normal in all of them.

In the clinically affected members of the Hughes family the presence of aggregation with collagen, in spite of the absence of “ADP release” from platelets, is difficult to explain on the basis of the generally accepted assumption that the aggregation with collagen is caused by the ADP that is released from platelets following their reaction with this agent. A hyperactive ADP-removing mechanism in the patient's plasma would give these results but was shown not to be present in our in vitro tests. A more plausible hypothesis is

---

**Fig. 4.**—Findings of the in vitro tests used for the investigation of the “six stages of platelet function in hemostasis” in various platelet functional abnormalities compared with normal. For the investigation of Stage 1 indirect evidence was used as explained in the text. In Glanzmann's disease, the aggregation with collagen or thrombin are partial because in this disease the ability of platelets to aggregate to each other is impaired.
Platelet Functional Abnormalities

Table 2.—Laboratory Tests on Members of the Hughes Family

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Platelet Count (×10^9/µl)</th>
<th>Bleeding Time (min)</th>
<th>Platelet Adhesiveness (%)</th>
<th>Tourniquet Test</th>
<th>Clot Retraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-6</td>
<td>17. 2.69</td>
<td>275</td>
<td>4 : 4; 4 : 6</td>
<td>33</td>
<td></td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>8. 8.69</td>
<td>181</td>
<td>½: 2; ½: 6</td>
<td>37</td>
<td>Positive</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>19. 9.69</td>
<td>166</td>
<td>4 : 4</td>
<td>16</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>III-3</td>
<td>8. 8.69</td>
<td>234</td>
<td>1 : 1</td>
<td>70</td>
<td>Negative</td>
<td>38</td>
</tr>
<tr>
<td>III-4</td>
<td>28. 1.69</td>
<td>238</td>
<td>&gt;15: &gt;15: &gt;15</td>
<td>0</td>
<td></td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>12. 9.69</td>
<td>376</td>
<td>½: 5: 5</td>
<td>25</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>III-5</td>
<td>17. 2.69</td>
<td>322</td>
<td>13½: &gt;15: &gt;15</td>
<td>7</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12. 9.69</td>
<td>280</td>
<td>2: 5: 8</td>
<td>22</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>III-6</td>
<td>17. 2.69</td>
<td>351</td>
<td>11½: &gt;15: &gt;15</td>
<td>15</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12. 9.69</td>
<td>282</td>
<td>14: &gt;15: &gt;15</td>
<td>11</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>150–400</td>
<td>&lt;6</td>
<td>30–65</td>
<td>Negative</td>
<td>38–70</td>
<td></td>
</tr>
</tbody>
</table>

that collagen has an aggregating effect on platelets, unrelated to their ADP release. (In agreement is the finding of Hugues 19 that specific antagonists of ADP, such as adenosine or AMP, have only slight inhibitory effect on collagen-induced platelet aggregation.) To substantiate this suggestion we performed the following experiment several times on platelets from five normal subjects.

To 1.0 ml of PRP increasing amounts of ADP were added every 120 sec, resulting in progressively weaker aggregation until finally two consecutive additions of 0.1 ml of ADP (f.c. 20 µM) did not produce further aggregation. Sixty seconds later, 0.1 ml of collagen solution was followed, after 50 sec, by aggregation. We interpret this experiment as indicating that collagen has a direct aggregating effect on platelets that have become completely unresponsive even to great amounts of ADP.

Discussion

In both families, the clinically affected members have a lifelong bleeding tendency characteristic of platelet abnormality: easy bruising, bleeding from mucous membranes, menorrhagia, and delayed hemostasis following surgery.

The bleeding time was prolonged and the platelet adhesiveness reduced on at least one occasion in all clinically affected members of both families. Although not consistently abnormal, it is notable that there was a correlation between these tests; the lower the platelet adhesiveness, the longer the bleeding time. The tourniquet test was positive in some affected members and negative in others. The tests of aggregation and ADP release revealed that in both families the platelets were functionally abnormal.

Although the present families showed several common findings (prolonged bleeding time, reduced platelet adhesiveness, absence of the second wave of aggregation with incremental amounts of ADP, and abnormal ADP release from platelets), they differed in the morphology of platelets and in the interaction between thrombin and platelets and between collagen and platelets.

In the Windle family, all affected members had a high proportion of
“giant” platelets. A bleeding disorder characterized by large and functionally abnormal platelets was first described by Bernard and Soulier in 1948. Two years later, Hirsh et al. reported a patient with thrombocytopenia and large and functionally abnormal platelets. Since then similar cases with or without thrombocytopenia have been reported by many investigators. However, none of these cases was tested with the newly developed methods for investigation of platelet function and thus it is not known whether they had an abnormal pattern of aggregation similar to that present in the Windle family.

In the Windle family, the aggregation with thrombin was partial and was followed by disaggregation. In the Hughes family, the aggregation was always final. In the clinically affected members of both families, thrombin did not produce any ADP release from platelets. Obviously, the absence of ADP release alone cannot explain the abnormal pattern of aggregation induced by thrombin in the Windle family since absence of ADP release in the Hughes family was associated with normal thrombin-induced aggregation.

In the Windle family, the aggregation with collagen was not initiated at all (or it was very feeble and long delayed), which, in association with the finding of delayed in vitro adherence of platelets to collagen fibrils, suggests abnormal reaction between collagen and platelets. In the Hughes family, the aggregation with collagen was initiated normally and was either normal or partial, but never delayed, and the in vitro adherence of platelets to collagen fibrils was normal. From our results showing collagen-induced aggregation of normal platelets that have become resistant to ADP, we conclude that collagen by itself has a direct aggregating effect, absent with platelets from the Windle family. Following interaction between collagen and platelets there is normally a release of ADP which completes the aggregation and this does not occur in platelets from the Hughes family, although they do aggregate with collagen.

We believe therefore that our two families have distinct platelet functional abnormalities. Both show deficient Platelet ADP release, but, in the Windle family, this is a direct result of failure of the collagen-platelet interaction, whereas, in the Hughes family, that process is normal, but there is a primary defect of ADP release.

Comparing our families with the previously reported cases of platelet functional deficiency cited in the introduction suggests that they also can be divided into two groups. Thus, Hirsh et al. noted defective platelet adhesion to collagen but normal ADP release (measured by an enzymatic method) from platelets exposed to connective tissue. In spite of some dissimilarities, explicable by differences in methods, it is probable that this case had the same defect as found in the Windle family, whereas the cases reported by Weiss showed markedly reduced ADP release from platelets with decreased but not absent aggregation of platelets with collagen. Very recently, Holmsen and Weiss have demonstrated that, in their family, the platelet storage pool of ADP is deficient. This may very well be the defect in our Hughes family.

Classification of Platelet Function Abnormalities

A classification of platelet functional abnormalities that is derived from present knowledge must be incomplete, as it is likely that the contribution of
platelets to hemostasis is a complex phenomenon not easily divisible into separate stages. However, a working hypothesis, such as one which follows, has the advantage of allowing a practical interpretation of the investigational methods available.

There is evidence for the existence of a plasma factor that interferes in the process of the formation of the platelet aggregate, possibly by facilitating the adherence of the platelets to the exposed "foreign" surface and that this factor is deficient in von Willebrand's disease. In this condition, transfusion of normal plasma to the patient corrects the abnormal bleeding time and platelet adhesiveness test, and the abnormal platelet adhesiveness is corrected by in vitro mixture with normal plasma or by covering the glass beads with normal plasma. Including this factor the nature of which is unknown, we can distinguish six stages of platelet participation in hemostasis: Stage 1: A plasma factor facilitates the adherence of platelets to the damaged blood vessel wall. Stage 2: Platelets react with the collagen fibrils and/or other substances (basement membrane) of the damaged surface. Stage 3: The platelets that have become adherent to collagen release ADP and other substances (ATP, AMP, serotonin, etc.) and the PF-3 becomes available. This phenomenon called "release reaction" probably continues through stages 4 and 5. Stage 4: Other platelets aggregate onto those already adherent to the damaged surface — by means of the direct aggregating effect of the collagen and of the released ADP — and so the chain of reaction begins that builds a platelet plug. Stage 5: Thrombin, which is produced during the blood coagulation process, reacts with the platelets (further aggregation and ADP release) and firms the platelet aggregate. Coagulation consolidates the platelet plug. Stage 6: Finally the platelets participate in clot retraction. (The significance of this function in hemostasis is not known.)

Hemorrhagic diatheses with clinical characteristics of platelet function deficiency may be divisible into defects at one of these stages by employing a combination of in vitro and in vivo tests, thus: Stage 1: There is no satisfactory method to test this stage; the finding of prolonged bleeding time and reduced platelet adhesiveness in the absence of abnormality of the tests of aggregation, ADP-release, PF-3a, and CR, and the correction of both abnormal tests by transfusion of plasma, are indirect evidence of abnormality of this stage. Stage 2: (1) Defective platelet aggregation following addition of collagen. (2) Deficient in vitro adherence of platelets to collagen fibrils. Stage 3: Deficient ADP-release from platelets after normal aggregation by collagen. Stage 4: Defective platelet aggregation by ADP. Stage 5: Defective platelet aggregation by thrombin. Stage 6: Deficient clot retraction.

The response to these tests in five distinguishable clinical syndromes and the usually clinically silent Glanzmann's trait is shown and contrasted with normal response in Fig. 4. It will be seen that some of the syndromes have abnormalities at more than one stage, for instance, the Windle defect shows abnormalities at Stages 2, 3, and 5, the Sprowson anomaly at Stages 3, 4, and 5, and Glanzmann's disease at Stages 4 and 6.

It is very likely that other familial syndromes will be discovered, since the hemostatic process has proved to be notably complex. Also, a better under-
standing of the biochemical basis of the platelet function would alter the present scheme. If a generic term is required for this group of diseases (except von Willebrand's disease), we suggest “thrombocytopenia,” the term “thrombopathy” or “thrombopathia” and “thromboaesthesia” are incorrectly derived since the Greek word “thrombos” means “clot” but it is the platelets, the thrombocytes, that are primarily involved.

ADDENDUM

Since we finished this study we have investigated four additional cases, from three families, with a lifelong hemorrhagic diathesis. They all showed in vitro findings as those of the Hughes family. The main constant abnormality was absent or markedly reduced ADP release from platelets ($3 \times 10^8$ platelets released $0.1 \mu g$ of ADP); at some time the BT was prolonged and the PAd reduced (however, occasionally both these tests were normal); in the aggregation tests: (1) with ADP $1.0 \mu M$ f.c., the response was normal, but incremental amounts of the agent did not initiate the second wave of aggregation; with ADP $20.0 \mu M$ f.c., a final irreversible aggregation was achieved in all (except that, in one of them, on one occasion the aggregation was partial and followed by a rapid disaggregation); (2) at some time, with collagen, the aggregation curve was partial in all (but in three of them it was occasionally normal); (3) with adrenalin, the response was variable—in one subject the second wave of aggregation was present; (4) with thrombin, a normal pattern was always present. The PF-3a test was normal in two, slightly abnormal in one and in the fourth subject on one occasion it was normal and on another slightly abnormal. The CR, platelet count and morphology, and the coagulation tests were normal.

Two other persons from these three families had definite histories of bleeding, but at the time we examined them they did not show any abnormality in the in vitro tests. It is notable that both subjects had been free from bleeding for the last few years.

ACKNOWLEDGMENT

We are grateful to Mr. Ellis Jones of Chester and to Dr. J. Rusius of Burnley for referring these patients to us. We are also grateful to Miss M. Shorrock (AIMLT) for technical assistance, to the Audiovisual Department of Methodist Hospital of Brooklyn, and to Miss J. Wolen for her secretarial assistance.

REFERENCES

Platelet Functional Abnormalities: A Report of Two Familial Defects in Interaction Between Collagen and Platelets and ADP Release

A. G. PAPAYANNIS, E. J. WATSON-WILLIAMS and M. C. G. ISRAËLS

Updated information and services can be found at:
http://www.bloodjournal.org/content/38/6/745.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml