Platelet size and shape in hereditary giant platelet syndromes on blood smear and in suspension: Evidence for two types of abnormalities

JOHN G. MILTON, R. A. HUTTON, E. G. D. TUDDENHAM, and MONY M. FROJMOVIC

MONTREAL, CANADA, and LONDON, ENGLAND

Platelet size on blood smear is compared with platelet size and shape in suspension (i.e., whole blood and citrated platelet-rich plasma [PRP]) for normal donors and 16 patients with hereditary "giant" platelet syndromes (HGPS), including Bernard-Soulier syndrome (BSS) (seven patients), Montreal platelet syndrome (MPS) (three patients), May-Hegglin anomaly (one patient) and Rezael platelet defect (one patient). In whole blood platelet shape is normal for HGPS, but in PRP for 10 of 16 patients with HGPS there is a decrease in the proportion of smooth, discoid-shaped platelets (discocytes [D]). The platelets of all patients with HGPS had abnormally large mean volume ($V_d$) and increased size on peripheral blood smear. Furthermore, 12 of 16 patients with HGPS, including six of seven donors with BSS, had abnormally large discocytes. The measured size of HGPS shape-changed platelets was compared with the size predicted from the size of the D by assuming that the relationship between the size of shape-changed platelets and D was the same as observed for normal donors. In this manner it was shown that for all donors with BSS and MPS, the shape-changed platelets are disproportionately larger than the D. In contrast, in the remaining patients with HGPS the size of the shape-changed platelets was consistent with the size predicted from the D. Examination of $V_d$ for MPS as a function of time after addition of 10 μmol/L adenosine diphosphate to PRP revealed an abnormal time course, thereby pointing to an abnormality in the mechanisms that regulate platelet size during shape change. With the lone exceptions of BSS and MPS, the size of platelets on blood smear was well correlated with the total platelet plasma membrane surface area as measured by the osmotic spherocytosis method. Our observations point to two distinct abnormalities in platelet size in HGPS: (1) a disproportion between the size of D and "shape-changed" platelets, which may be related to an abnormal shape change and which is observed only for MPS and BSS, and (2) an abnormal increase in platelet size on blood smear, which appears to reflect the increased amount of platelet plasma membrane in other HGPS platelets. (J Lab Clin Med 106:326-335, 1985.)

Abbreviations: ADP = adenosine diphosphate; ATP = adenosine triphosphate; BSS = Bernard-Soulier syndrome; D = discocyte; $d_m$ = mean diameter of platelets on peripheral blood smear; DE = disco-echinocytes; $f_{de}$ = fractional disco-echinocytes; $f_{sd}$ = fractional spherocytosis; HGPS = hereditary giant platelet syndromes; HSC = hyperviscosity; MPS = Montreal platelet syndrome; PRP = platelet-rich plasma; SE = spherocytosis; $S_{m}$ = mean platelet plasma membrane surface area; $V_d$ = mean volume of platelets; $V_{de}$ = discocyte volume; $V_{se}$ = disco-echinocyte volume; $V_{sd}$ = spherocytosis volume.

From the Physiology Department, McGill University, Montreal, Canada, and the Katharine Dormandy Haemophilia Centre and Haemostasis Unit, Department of Haematology, Royal Free Hospital, London, England.

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Reprint requests: Dr. M. Frojmovic, Department of Physiology, McGill University, McIntyre Medical Sciences Building, 3655 Drummond St., Montreal, Quebec, Canada H3G 1Y6.
The appearance of abnormally large platelets on peripheral blood smears is characteristic of a number of hereditary bleeding disorders, collectively referred to as HGPS (for recent reviews see references 1 and 2). We have suggested that the increased platelet size on blood smear in two HGPS, namely BSS/3 and MPS, may reflect an abnormal increase in platelet size during platelet shape change. This suggestion was based on the observation that when platelet volume was examined as a function of shape, the shape-changed platelets were disproportionately larger than the smooth, discoid-shaped platelets. However, although the early changes in platelet shape that occur for platelets on a glass slide resemble shape change in suspension (compare references 1 and 2 with reference 8), there are the additional components of adhesion and spreading. Moreover, platelet size artifacts may be introduced during the preparation of the blood smear. Here we compare platelet size on blood smear to platelet size and shape in suspension for normal donors and donors with HGPS. The results demonstrate that abnormal platelet size in suspension and on a glass slide point to distinct abnormalities in the mechanisms that regulate platelet size.

METHODS

Collection and processing of blood samples. Venous blood was collected through a 19-gauge butterfly infusion set with a two-syringe technique, discarding the first 5 ml, and processed as follows: (1) 1 ml blood was immediately mixed with 4 ml warm (37°C) 1% vol/vol glutaraldehyde buffered with either Mg++, Ca++-free Tyrod’s solution, or 0.1 mol/L cacodylate, pH 7.4, and, left to fix and settle at room temperature (20° to 24°C) until sufficient supernatant formed for drawing one drop with a pipette and transferring it to a glass slide (typically 30 minutes); and (2) 4.5 ml blood was mixed with 0.5 ml 3.8% trisodium citrate in a polypyrrole tube, and PRP prepared at either 37° C or room temperature by centrifugation at 150 x g for 12 minutes. The PRP samples were further incubated at their respective temperatures for 30 minutes before fixation of 0.1 ml with 4 vol 1% buffered glutaraldehyde.

Platelet shape was classified as described previously. Under phase-contrast microscopy, edge-on D appear ellipsoid with a clear center, whereas face on they appear circular with a dark center (for example of D, DE, and SE see references 1 and 2 and Fig. 1). Platelets that appear circular with a white center in all orientations are referred to as SE. Platelet shapes that do not satisfy either of these criteria are put into a third group referred to as DE.

Determination of platelet size parameters was carried out according to Milton and Frojmovic and co-workers. In brief, the freely rotating platelets in samples prepared as described above were filmed under phase-contrast microscopy, and platelet size analyzed from projected images. With previously established criteria, the diameter (d) and thickness (t) of individual platelets were determined and the axial ratio, that is, t/d, calculated. V_d, V_de, and V_se were calculated from the formula V = π/6 d³t, where t = d for SE, and were corrected for pseudopodia as described previously. The platelet mean volume was determined from the relation V = f_dV_d + f_deV_de + f_seV_se, where V_d, V_de, and V_se are the mean volumes and f_d, f_de, and f_se are the fractions of D, DE, and SE, respectively. Platelet size and shape were examined as a function of time after addition of 10 μmol/L ADP as described previously. For one donor with HGPS, namely BS (see Table I), photomicrograph and electronic size measurements were performed on the same sample of PRP. V_d was 19.1 μm³, and the mean electrical size was 23.0 μm³. For each platelet measured from photomicrographs it was possible to calculate the electronic size. The mean electronic size as calculated in this manner was 22.3 μm³, that is, within 3% of that measured.
Table I. Comparison of $t_0$ and $V_i$ in whole blood and PRP for normal donors and donors with HGPS

<table>
<thead>
<tr>
<th>Donor</th>
<th>($t_0$)WB</th>
<th>($t_0$)WB - ($t_0$)PRP (37°C C)</th>
<th>($t_0$)WB - ($t_0$)PRP (20-25°C C)</th>
<th>($V_i$)WB (µm³)</th>
<th>($V_i$)PRP (37°C)/($V_i$)WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal*</td>
<td>0.63 ± 0.22</td>
<td>0</td>
<td>0.53 ± 0.20†</td>
<td>6.0 (4.0-7.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>BSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bt</td>
<td>0.78</td>
<td>0.50</td>
<td></td>
<td>9.3</td>
<td>1.8</td>
</tr>
<tr>
<td>P§</td>
<td>0.70</td>
<td>—</td>
<td>—</td>
<td>12.7</td>
<td>—</td>
</tr>
<tr>
<td>Vl</td>
<td>0.72</td>
<td>0.60</td>
<td>0.60</td>
<td>17.1</td>
<td>1.4</td>
</tr>
<tr>
<td>BS</td>
<td>0.66</td>
<td>0.20</td>
<td>—</td>
<td>12.9</td>
<td>1.5</td>
</tr>
<tr>
<td>BSS 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI-1</td>
<td>0.53</td>
<td>0.12</td>
<td>—</td>
<td>22.3</td>
<td>0.9</td>
</tr>
<tr>
<td>VI-2</td>
<td>0.65</td>
<td>0.23</td>
<td>—</td>
<td>15.4</td>
<td>1.0</td>
</tr>
<tr>
<td>BSS 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-1</td>
<td>0.76</td>
<td>0.47</td>
<td>—</td>
<td>12.2</td>
<td>1.4</td>
</tr>
<tr>
<td>MPSII</td>
<td>0.72 ± 0.38</td>
<td>0.72</td>
<td>—</td>
<td>7.1-8.6</td>
<td>1.0-1.4</td>
</tr>
<tr>
<td>MHA I-1</td>
<td>0.47</td>
<td>0.25</td>
<td>0.42</td>
<td>16.8</td>
<td>1.1</td>
</tr>
<tr>
<td>RPS V-7</td>
<td>0.64</td>
<td>0.01</td>
<td>0.43</td>
<td>12.5</td>
<td>0.8</td>
</tr>
<tr>
<td>L-M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-1</td>
<td>0.50</td>
<td>0.19</td>
<td>0.43</td>
<td>14.4</td>
<td>1.2</td>
</tr>
<tr>
<td>IV-2</td>
<td>0.52</td>
<td>0.27</td>
<td>0.46</td>
<td>12.8</td>
<td>1.1</td>
</tr>
<tr>
<td>LID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-1</td>
<td>0.60</td>
<td>0.41</td>
<td>—</td>
<td>9.6</td>
<td>1.0</td>
</tr>
<tr>
<td>II-2</td>
<td>0.80</td>
<td>0.71</td>
<td>—</td>
<td>13.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

WB = whole blood.

*86 donors; values are expressed as mean ± 2 SD.
†Seven donors; values are expressed as mean ± 2 SD.
‡Published data for platelet morphology of donor B.5,4
§Published data for platelet morphology of donor P.3
¶Published data for platelet morphology of donor V.5
¶¶Three donors; preliminary data for platelet morphology of MPS donors was published;9 but data presented here are more extensive, with each donor having been examined on a minimum of two occasions for total of 10 observations for PRP and three for WB. Values are expressed as mean ± 2 SD.

Size of platelets on peripheral blood smears was determined by filming platelets on blood smear under light microscopy and measuring outside diameters of 100 projected images. The $d^2$ thus obtained for normal donors is the same as that for SE, suggesting that platelet volume can be calculated as $\pi \cdot \frac{d^2}{6}$.

Platelet plasma membrane measurements were made after osmotically stressing the platelets in the unfixed PRP sample prepared at 37°C by the stepwise addition of 4 vol distilled water over a 20 to 30 second period.7,18 Measurements of platelet diameter and volume were made 12 minutes after addition of distilled water, and S, calculated as $\pi \cdot \frac{d^2}{6}$.

Platelet aggregation for donors with HGPS was monitored by aggregometry as previously described19 and was compared with the response to normal PRP that had been diluted with platelet-poor plasma to give a similar platelet count. Aggregating agents used were ristocetin (final concentration 1.5 mg/ml), ADP (2 µmol/L and 10 µmol/L), epinephrine (2 µmol/L and 10 µmol/L), and collagen (2 µg/ml). Aggregation responses were graded as normal, weak, or absent. In the range of platelet counts from 40,000 to 100,000/µl, platelets from control subjects showed slower and less intense aggregation than with counts > 200,000/µl.

Bleeding times22 were determined by use of the template method with the Simplate II device (General Diagnostics, Morris Plains, N.J.), and platelet counts in PRP and whole blood were determined by light microscopy by the method of Brecher and Cronkite.31

Platelet adenine nucleotides were assayed by the firefly luciferase method as previously described.22

Patients. These experiments were performed according to the principles of the Helsinki declaration, and informed consent was obtained. Healthy donors were chosen from students and academic staff. Sixteen patients with HGPS were examined, and family pedigrees of previously unreported HGPS are shown in Fig. 2. On the basis of platelet aggregation studies it was possible to divide these patients into five distinct groups: two groups with abnormal ristocetin-induced platelet aggregation (BSS and Rafael platelet defect) and three groups with normal ristocetin-induced aggregation (MPS May-Hegglin anomaly, and a miscellaneous group).

BSS, (families BSS 1 and BSS 2). Four of the patients with BSS included in this study have been described previously in detail and have documented glycoprotein Ib deficiencies (namely B,23 V,24 P,25,26 and BS,27,28 where the notation is the same as in the original descriptions).

In family BSS 1, the proband was a 14-year-old Iraqi girl. (VI-1: generation-offspring) with a lifelong bleeding tendency. A great-uncle (III-5) had died of bleeding in the mouth, and her younger sister (VI-2) also had epistaxis and bruising. Both sisters had thrombocytopenia (55,000 to 135,000/µl), abnormal bleeding time (> 30 minutes, normal = 5 minutes), absent ristocetin-induced aggregation, and normal plasma
concentration of von Willebrand factor. Moreover, their platelets failed to react to a monoclonal antibody specific for glycoprotein Ib. Unaffected members of the family (IV-3, V-1, V-2, and VI-3) had normal platelet parameters.

In family BSS II, the proband (IV-1) was an Iranian boy aged 6 years, who was the only member of his kindred with a bleeding tendency. He was a healthy active child with no clinical abnormality other than scattered ecchymoses up to 8 cm mainly on the lower limb. Platelet parameters were consistent with a diagnosis of BSS (i.e., platelet count ~70,000/μl; bleeding time >30 minutes; absent ristocetin-induced aggregation). No attempt was made to identify a glycoprotein abnormality.

**Rafael platelet defect (family RPS).** A 58-year-old woman (V-7) was referred for investigation of a lifelong tendency toward easy bruising and excessive bleeding. Results of a clinical examination were normal except for multiple large ecchymoses. Bone marrow was normal. Serological findings included antinuclear factor normal, antinuclear antibody normal, and Rose-Waaler 1/64. Antiplatelet antibodies were normal by the technique of Von dem Borne et al. There was thrombocytopenia (39,000/μl), an abnormal bleeding time (>30 minutes), and weak ristocetin-induced aggregation. Collagen-, ADP-, and epinephrine-induced platelet aggregation was weak, suggesting a platelet-release defect. Platelet adenine nucleotide content was not reduced (see Table II). The mother (IV-10) and two daughters (VI-6 and VI-7) of the propositus had normal platelet counts and no history of bruising, but several observers detected a small population of excessively larger platelets on their blood smears. Unaffected family members (IV-10, V-8, VI-6, and VI-7) had normal platelet parameters.

**MPS.** The clinical histories and laboratory investigation of these donors have been published in detail elsewhere. In brief, there was thrombocytopenia, an abnormal bleeding time, spontaneous platelet aggregation, normal ristocetin-
Table II. Comparison of nucleotide content, ATP/ADP ratio, and calculated nucleotide concentration for normal and HGPS platelets

<table>
<thead>
<tr>
<th>Donor*</th>
<th>Total adenine nucleotides (nmol/10^8 platelets)</th>
<th>ATP/ADP Ratio</th>
<th>Nucleotide concentration†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Whole blood (nmol/10^9 μm^3)</td>
</tr>
<tr>
<td>Normal</td>
<td>5-12</td>
<td>1.0-2.2</td>
<td>8-20</td>
</tr>
<tr>
<td>BSS 1</td>
<td>49.5</td>
<td>1.1</td>
<td>22</td>
</tr>
<tr>
<td>VI-1</td>
<td>26.5</td>
<td>1.6</td>
<td>17</td>
</tr>
<tr>
<td>VI-2</td>
<td>12.4</td>
<td>1.5</td>
<td>10</td>
</tr>
<tr>
<td>MPS</td>
<td>5.7</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>RPS</td>
<td>22.2</td>
<td>1.9</td>
<td>18</td>
</tr>
<tr>
<td>MHA</td>
<td>26.6</td>
<td>2.0</td>
<td>16</td>
</tr>
<tr>
<td>L-M</td>
<td>22.6</td>
<td>1.3</td>
<td>—</td>
</tr>
<tr>
<td>III-2</td>
<td>68.1</td>
<td>1.4</td>
<td>—</td>
</tr>
<tr>
<td>IV-1</td>
<td>39.8</td>
<td>1.2</td>
<td>28</td>
</tr>
<tr>
<td>IV-2</td>
<td>35.2</td>
<td>1.6</td>
<td>28</td>
</tr>
<tr>
<td>IV-3</td>
<td>59.4</td>
<td>1.6</td>
<td>—</td>
</tr>
<tr>
<td>LID</td>
<td>13.8</td>
<td>1.2</td>
<td>14</td>
</tr>
<tr>
<td>I-1</td>
<td>13.8</td>
<td>2.4</td>
<td>10</td>
</tr>
<tr>
<td>II-2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Donors with HGPS are referred to by notation introduced in Fig. 2 and Table I.
†Calculated for whole blood and PRP by use of respective values for Vt (see Table I).

ADP-, and collagen-induced aggregation, and a reduced sensitivity to thrombin-induced aggregation. Glycoprotein Ib levels ranged from normal to reduced in the affected family members.11

May-Hegglin anomaly (family MHA). A Jamaican woman of West African descent (I-1) was investigated for a bleeding tendency when she presented with erythema nodosum and was found to have the typical peripheral blood changes of May-Hegglin anomaly, that is, Döhle bodies in granulocytes. One son (II-2) had similar peripheral blood changes. Both the proband and her affected son had suffered from frequent and prolonged epistaxis. There was thrombocytopenia (38,000 to 86,000/μl), and although the bleeding time was prolonged (9 to 16 minutes), it was consistent with that predicted for a normal donor with the same platelet count by the method of Harker and Slichter20 (8 to 21 minutes). Aggregation to ristocetin and collagen was normal, but weak to ADP and epinephrine.

Miscellaneous group (families L-M and LID). In family L-M, the proband (IV-1) was a man aged 18 years who had lymphadenopathy, purpura, and epistaxis, with clinical investigations consistent with autoimmune thrombocytopenia secondary to infectious mononucleosis. Persistent macrothrombocytopenia (34,000/μl) was noted on peripheral blood smear, and subsequent investigations revealed macrothrombocytopenia in his two brothers (IV-2 and IV-3) and his father (III-2) (platelet count ~20,000 to 38,000/μl). His mother's blood film was normal, as was that of his great grandmother (I-2). However, the paternal grandmother (II-2) had mild thrombocytopenia. His father (III-2) had nerve deafness with marked loss of upper range in both ears. However, the three affected brothers all had normal audiogram results. Furthermore, blood urea and creatinine values were normal in all of the individuals with macrothrombocytopenia. These observations suggest that this condition is not the same as that described by Eckstein et al.15 and Epstein et al.19 The only abnormal bleeding in the family apart from the presenting episode in the proband was that III-2 bled for three days after dental extraction and II-1 died of a cerebral hemorrhage at the age of 11. Generation I came from Russia to the United Kingdom, and there is no Mediterranean ancestry known. Platelet aggregation was normal, and the bleeding time, although prolonged (8.5 to 12 minutes), was actually shorter than predicted from the reduced platelet count.30 (21 to 25 minutes).

In family LID, the proband (I-1) was referred for investigation of thrombocytopenia, discovered on routine monitoring after gold therapy for rheumatoid arthritis, which did not return to normal levels during 3 years after cessation of gold therapy (platelet count ~98,000/μl). She gave no history of abnormal bleeding. Clinical examination revealed only scattered ecchymoses on arms and legs and moderate rheumatoid arthropathy. Bone marrow obtained by trephine showed normal histologic conditions with no excess of reticulin and normal numbers of megakaryocytes. Her daughter (II-1) was observed and found to have macrothrombocytopenia (72,000/μl), but was otherwise healthy. Subsequently the daughter developed marked spontaneous bruising related to aspirin
ingestion that had been prescribed for symptomatic treatment of viral laryngitis. Whereas the proband’s platelets exhibited weak collagen-induced aggregation, the daughter’s platelets exhibited weak epinephrine-induced aggregation. Bleeding time was only mildly elevated (9 minutes) and was similar to that predicted on the basis of the platelet count (5 to 12 minutes), in contrast to family L-M.

RESULTS

Platelet shape and mean volume in whole blood and PRP. The f₀ in freshly fixed whole blood for each of the donors with HGPS is in the range observed for normal donors (see Table I). For normal donors, there is no difference between f₀ in whole blood and PRP prepared at 37°C, but a large decrease in f₀ is seen when PRP is prepared at room temperature (~20°C to 25°C). In contrast, for 10 of 16 donors with HGPS there is a decrease in f₀ on preparing PRP at 37°C, and a further decrease in f₀ is seen on preparing PRP at room temperature for four of five donors with HGPS. Coincident with the decrease in f₀, there is an increase in the fraction of echinocytes (f₁D ~1.1 to 2.2 times normal; f₃B ~3 to 50 times normal), indicating that mean platelet shape in PRP is more spherical than normal for many donors with HGPS.¹⁴,³⁴

For all donors with HGPS, Vᵣ is larger than normal in whole blood. For MPS and four of six donors with BSS, there is an increase in Vᵣ in PRP compared with WB. In contrast, for the patient with May-Hegglin anomaly, family L-M, and family LID there is no significant change between Vᵣ in whole blood and PRP despite changes in f₀ of similar magnitude. Inasmuch as platelet size in PRP is either the same as or larger than that in whole blood, the observed decrease in f₀ cannot arise from a selective loss of larger D during centrifugation, but presumably reflects shape change occurring during PRP preparation.

Platelet volume as a function of shape. Fig. 1 compares the mean cross-sectional surface areas of D, DE, and SE for normal donors and selected donors with HGPS, and Table III gives the measured mean volumes. There are three observations that support our earlier conclusion that in MPS and BSS there is a disproportion in the size between D and the nondiscoid shapes, DE and SE: Vᵣ is relatively smaller than Vᵣ. For all of the donors with MPS, Vᵣ is in low normal range, but Vᵣ is from 1.2 to 1.4 times normal. For one BSS donor, donor B, Vᵣ is normal, but Vᵣ is from 1.6 to 3.1 times normal. Even for the other donors with BSS, although Vᵣ is larger than normal it is certainly not large enough to account for the observed Vᵣ, particularly in PRP. (2) The measured mean cross-sectional surface area of DE and SE is larger than the largest cross-sectional surface area predicted from the size of the D, assuming that normal shape change occurred (see figure legend for details of calculation). (3) The ratio of the volume of DE and SE compared with D is larger than normal. In particular, whereas for normal donors VₛD/Vᵣ > 1, for both donors with MPS and those with BSS VₛD/Vᵣ > 1. It is easy to see from these observations why platelet shape change on preparing PRP results in the increase in Vᵣ noted for donors with BSS in Table I.

A different trend is observed for the remaining donors with HGPS. In these cases, comparing the cross-sectional surface areas and volumes of D, DE, and SE leads to the same trends as observed for normal donors. In other words, although these platelets are larger than normal, there is a normal proportionality between the relative sizes of D, DE, and SE. A minor exception to this generalization occurs in the family L-M, donor IV-2, in whom the DE are larger than anticipated, yet VₛD/Vᵣ < 1.

Time dependence of platelet mean volume and axial ratio after activation. Fig. 3 compares platelet Vᵣ,
as a function of time after addition of 10 μmol/L ADP to PRP for normal donors and those with MPS. For normal donors there is a rapid and transient increase in \( V_T \) (complete in 1 second) followed by a decrease such that by 6 seconds \( V_T \) becomes 20% less than observed before addition of ADP.\(^{13}\) There are no differences in the time course for the appearances of DE and SE between normal donors and those with MPS.\(^7\) However, the time course for \( V_T \) for MPS platelets differs from normal in three aspects: (1) the early maximum in \( V_T \) occurs at a later time (3 seconds), (2) the subsequent decrease in \( V_T \) is complete at a later time (10 seconds), and (3) \( V_T \) does not become less than observed at zero time. Moreover, at 100 seconds after ADP addition for normal donors, \( V_T \) typically becomes less than the initial \( \bar{V}_D \), whereas for the donor with MPS \( V_T \) was 1.5 times the initial \( \bar{V}_D \) (for the donor with MPS \( f_D = 0.52 \) at zero time and 0 at 100 seconds). Similar studies have not been done with BSS platelets because of the difficulty in obtaining PRP wherein platelets had not already undergone significant shape change (see Table I).

**Platelet size on peripheral blood smears for normal donors and donors with HGPS.** The size of platelets in whole blood and on peripheral blood smears is compared for normal donors and those with HGPS in Table IV. In contrast to the abnormality in the interplay between platelet size and shape seen only for donors with MPS and BSS, all donors with HGPS have abnormally large platelets on blood smear, as indicated by both the increased mean diameter and the increased fraction of platelets with diameter >2.5 μm. Estimation of HGPS platelet mean volume in whole blood from the diameters of platelets on blood smear overestimates the volume determined for platelets in suspension by 40% to 180%. In contrast, the size of normal platelets is slightly underestimated from blood smears. A similar trend can be seen in the data of Holme et al.\(^{14}\) (see Fig. 3 in reference 14 excluding points for acquired platelet disorders).

**Comparison of \( S_5 \) and size of platelets on blood smear.** Fig. 4 compares platelet mean diameter on blood smear with \( S_5 \) for normal donors and those with HGPS. With the exception of MPS and BSS, there is a good correlation between platelet size on blood smear and \( S_5 \).

**Comparison of platelet adenine nucleotide content for normal and HGPS platelets.** Table II compares platelet total adenine nucleotide content for donors with HGPS and normal donors. Platelets for donors with HGPS have elevated total adenine nucleotide levels (two to six times normal) with normal ATP/ADP ratios. On the other hand, nucleotide concentrations, that is, nucleotide content per unit volume, is normal except in the case of family L-M and one of three donors with BSS, in whom it is only slightly elevated.

**DISCUSSION**

Two methods are commonly used to detect the presence of abnormally large platelets in HGPS: (1) diameter of platelets on peripheral blood smear,\(^9\) and (2)
platelet sizing in suspension either by electronic or photomicroscopic methods. Here we have shown that by comparing these methods in different HGPS, it becomes necessary to define two types of abnormal increases in platelet size. First, there can be a disproportion in the size between D and echinocytes (DE and SE) in suspension. We term this a type 1 HSC to emphasize both the abnormal increase in volume of the echinocytes relative to D and the fact that the DE and SE typically arise from the D via shape change. Second, there is the apparent increase in HGPS platelet volume suggested from the diameter of platelets on blood smear. This is an apparent increase in volume, because there is no rigorous way to determine platelet volume from blood smear. We term this phenomenon a type 2 HSC. In our original discussions of platelet size and shape in MPS and BSS, emphasis was placed on the observations that although V and d were abnormally large, the majority of the platelets in whole blood were D and normal sized. It was suggested that a type 1 HSC lay at the basis of both these observations in MPS and BSS and possibly other HGPS as well. In view of the data presented here, it is necessary to modify these suggestions. First, f for these donors is in the normal range, and hence, as for normal donors, up to 60% of the platelets in whole blood can be echinocytes (typically predominantly DE). Second, for six of seven donors with BSS the D are abnormally large. However, even in these cases the echinocytes are relatively larger. Clearly large D do not exclude the possibility that a type 1 HSC occurs. Third, a type 1 HSC with both DE and SE relatively larger than D appears to be specific for MPS and BSS. Although HGPS may exist in which only one type of echinocyte, that is, DE or SE, is abnormally large, for example, in family L-M, our studies suggest that for most HGPS the relative sizes of D, DE, and SE are no different than are seen for normal donors. The observation that BSS platelets have a type 1 HSC but those of May-Hegglin anomaly do not may be related to micropipette elastometry measurements that indicate that increased platelet membrane deformability is seen for BSS but not May-Hegglin anomaly. Finally, for most HGPS, increased d appears to reflect increased S, rather than a type 1 HSC. Indeed only for MPS can it be argued that a type 1 HSC accounts for the increased d, because in this case S is normal. For BSS, d may actually be smaller than predicted from S (see Fig. 4). These observations emphasize that the size of HGPS platelets on blood smear in general reflects the interplay between shape change and S.

The mechanisms underlying a type 1 HSC are unknown. Although it is possible that the glycoprotein Ib abnormality is related to this phenomenon in BSS, it is clear that there must be other factors involved, inasmuch as platelets from two of these donors with MPS have normal amounts of glycoprotein Ib. Measurements of MPS V as a function of time after addition of ADP suggest that the initial rapid increase in V is normal, but that the subsequent contraction of V is abnormal. In principle this abnormality could
be related to ionic fluxes or to membrane or contractile protein abnormalities.

In the case of the type 2 HSC, the increased plasma membrane implies the existence of an abnormality of platelet production, that is, there is either an abnormal demarcation of megakaryocyte membrane or an abnormal disintegration of megakaryocyte fragments ("pro-platelets"**1,3,4**). These suggestions are supported by the observed normal adenine nucleotide concentrations per unit volume of HGPS platelets.

Our measurements are based on a morphometric analysis of populations of glutaraldehyde-fixed platelets. As such, they do not necessarily describe the shape change of individual platelets. At best all that can be said is that there is at least a subpopulation of BSS and MPS platelets that undergoes a type 1 HSC. It is anticipated that the development of methods to measure dynamically the shape change of single platelets will greatly clarify the nature of the shape change abnormality suggested by our studies.

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