Platelets from “giant platelet syndrome (BSS)” are
discocytes and normal sized

M. M. FROJMOVIC, JOHN G. MILTON, J. P. CAEN, and G. TOBELEM
Montreal, Que., Canada, and Paris, France

A comparison is made between the shape of human platelets obtained from nine normal donors and two BSS donors. Sizes are evaluated from a cinematographic analysis of freely rotating, unfixed, and glutaraldehyde-hardened platelets in citrated PRP and of platelets on blood smear. On blood smear, the mean diameters of BSS platelets are 1.7 to 1.8 times larger than those of normal platelets, with a major fraction having a diameter greater than 2.5 μm. As for normal donors, 80% to 90% BSS platelets in PRP are in the disc form (discocyte). In addition, they are essentially indistinguishable from a normal discocyte. Echinocytes (spherical forms with pseudopods) for BSS have a main body diameter (i.e., excluding pseudopods) 1.6 times larger than normal and in addition a reduced number of pseudopods. The results demonstrate that the giant size of BSS platelets results from abnormal behavior of these platelets during the preparation of the blood smear. It is suggested that this disorder is associated with a defect in the mechanism of platelet shape change.

Abbreviations: Bernard-Soulier syndrome (BSS), platelet-rich plasma (PRP), hereditary giant platelet syndrome (HGPS), surface-connected canalicular system (SCCS)

The appearance of abnormally large “giant” platelets on peripheral blood smears is associated with a large number of bleeding disorders, including the hereditary BSS,1-12 hereditary dominant thrombocytopenic macrothrombocytopenia,13-15 including those associated with nephritis and deafness,16-17 May-Hegglin’s disease,18-20 the Gray platelet syndrome,20 myeloproliferative disorders,21-22 and some cases of hypersplenism.24 It has been suggested that careful measurements of size distributions of platelets on blood smear may be a useful diagnostic tool.24-26 Surprisingly, no quantitative work has appeared concerning the size of circulating platelets for these disorders, as measured with intact platelets in blood or plasma.

Here we examine the sizes of platelets for two cases of BSS. The platelets are abnormally large on blood smear. In contrast, it is shown that the platelet disc-form (discocyte), which comprises at least 80% of the circulating platelet population, is normal in size. Clearly the appearance of giant platelets must be associated with the preparation of the

From the Department of Physiology, McGill University, Montreal, Canada, and the Department of Hemostasis and Experimental Thrombosis, Institut de Recherches sur les Maladies du Sang, Hôpital Saint-Louis, Paris, France.
This work was partially supported by the Medical Research Council of Canada, grants MA-3612 and 4012, and by the Quebec Department of Education—DGES-FCAC.
Submitted for publication May 12, 1977; accepted Sept. 8, 1977.
Reprint requests: Dr. M. M. Frojmovic, Department of Physiology, McGill University, McIntyre Medical Sciences Building, 3655 Drummond St., Montreal, Que., Canada H3G 1Y6.
Table 1. Mean diameter (d) and percent of “giant” platelets for BSS and normal donors’ platelets on peripheral blood smears

<table>
<thead>
<tr>
<th>Donor</th>
<th>(\bar{d} (\mu m))</th>
<th>% of platelets with (d &gt; 2.5 \mu m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (3)</td>
<td>1.8 ± 0.2</td>
<td>7 ± 7</td>
</tr>
<tr>
<td>Normal*</td>
<td>1.8 ± 0.2</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>P (BSS)</td>
<td>3.1</td>
<td>80</td>
</tr>
<tr>
<td>BSS*</td>
<td>3.3</td>
<td>67</td>
</tr>
</tbody>
</table>

*Data from Weiss et al.*

blood smear. In addition, it is shown that the circulating echinocyte (spherical form with pseudopods) for BSS is larger than normal. On this basis it is suggested that the functional defect(s) for BSS may be related to the mechanism for shape change. The implications of these observations in light of recent findings concerning BSS are discussed.

Materials and methods

Case histories.* Normal donors were chosen from healthy men and women between the ages of 20 and 35 years. P was the first case described by Bernard and Soulier.1 Subsequent studies were reported by Bernard et al.2 and Tobelein et al.3 P is now deceased. Clinical descriptions of B and his mother, MB, were given by Bernard et al.2 P and B have been diagnosed as having BSS.1, 2, 3 MB does not have a bleeding disorder.2

Preparation of PRP. Blood was drawn by venipuncture into 3.8% citrate (1 vol to 9 vol of blood) and fixed with 4 vol of 1.3% glutaraldehyde at 37°C as described previously.22 PRP was collected by allowing the fixed blood to settle for 2 hr at room temperature in 5 ml plastic tubes. Platelet populations of normal and BSS-PRP typically contained 80% to 90% discocytes and 2% to 10% echinocytes, with the remaining fraction consisting of irregular forms. Sizes of platelets remaining trapped in the red blood cell residue did not differ from those in the PRP.

Measurement of geometric parameters. Freely rotating platelets were filmed under phase-contrast microscopy (40× objective; Zeiss Universal Microscope) with a 16 mm Beaulieu movie camera utilizing a fine-grain film. Discocytes were characterized as oblate ellipsoids with a diameter (\(d\)), a thickness (\(t\)), and the calculated geometric axis ratio:

\[ r_o = \frac{t}{d} \] (1)

The diameter for the normal discocyte is chosen as the major axis of an ellipse whose outline is midway between the primary ellipse and secondary one (diffraction ring) seen for an edge-on orientation of a platelet in a photomicrograph. The thickness represents the minor axis of the primary ellipse.29 Microscopy, film analysis, and criteria for selecting an edge-on orientation and measuring \(t\) and \(d\) are the same, as previously reported.29 The surface area (SA) of an oblate ellipsoid is computed as follows:29

\[ SA = \frac{(\pi/2)}{d^2 + (\pi/4) \cdot t^2 \cdot (1 + r_o)/(1 - r_o)} \log_e r_o^{-1} \] (2)

Where \(d\) is diameter, \(t\) is thickness, and \(r_o\) is axial ratio. Good correlation had been shown between calculated mean \(r_o\) and \(V\) (volume) for normal, unactivated human platelets and values obtained by a number of other methods (including electronic particle counters) which could not, however, yield absolute frequency distributions for the geometric parameters under study.29

For the case of shape-changed platelets, the main body of these echinocytes, excluding pseudopods, was approximated as a sphere, with the diameter chosen as for the discocyte (midpoint between primary circle and outside of first diffraction ring). Similar measurements of diameters of commercial latex particles (\(d = 1.95 \mu m\); refractive index \((m) = 1.55\), Dow Chemicals) suspended in

*These experiments were performed according to the principles of the Declaration of Helsinki and informed consent was obtained.
Fig. 1. Histograms of diameter, thickness, and geometric axis ratio for discocytes from a normal mother (MB), her BSS son (B), and one further BSS donor (P). The histograms for MB's discocytes also represent those of a typical normal donor (see Table II).

Glycerol (m = 1.48) or water (m = 1.33) gave d values within 5% of the reported commercial value (d = 2.04 µm).

Platelet diameters on peripheral blood smears were measured by a morphometric technique.  

Volume distribution. Volumes of BSS platelets were determined with a Coulter Counter F (70 µ probe) or a Coulter Counter Z Bic, coupled with a multichannel analyzer, “channellyser” model C100, and Coultronix XY recorder (Coulter Electronics, Inc., Hialeah, Fla.). The diluting solution was “Isoton.”

Computations of volumes for discocytes based on microscopic geometric measurements were made using

\[ V = \left( \frac{\pi}{6} \right) \cdot d^3 t \]

and for echinocytes using equation 3 by setting t = d.

Previous studies have shown that volumes of platelets determined by these two methods agree to within 10%.  

Results

Platelets on blood smear. The mean diameter of BSS platelets is 1.7 to 1.8 times greater than that of normal platelets (Table I). In addition, a greater fraction (6 to 10 times more) have a diameter greater than 2.5 µm. These trends are typical for platelets from donors with giant platelet syndromes.
Table II. Mean diameters (\(\bar{d}\)), thicknesses (\(\bar{t}\)), axial ratios (\(\bar{r}_p\)), volumes (\(\bar{V}\)), and surface areas (\(\bar{SA}\)) for discocytes from normal, BSS, and HGPS donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>(\bar{d}) ((\mu m))</th>
<th>(\bar{t}) ((\mu m))</th>
<th>(\bar{r}_p)</th>
<th>(\bar{V}) ((\mu m^3))</th>
<th>(\bar{SA}) ((\mu m^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (9)</td>
<td>3.2 ± 0.5</td>
<td>1.1 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>6.5 ± 3.0</td>
<td>17.2 ± 5.2</td>
</tr>
<tr>
<td>MB (normal)</td>
<td>3.2</td>
<td>1.1</td>
<td>0.4</td>
<td>6.2</td>
<td>18.5</td>
</tr>
<tr>
<td>B (BSS)</td>
<td>3.2</td>
<td>1.1</td>
<td>0.4</td>
<td>6.4</td>
<td>17.8</td>
</tr>
<tr>
<td>P (BSS)</td>
<td>3.5</td>
<td>1.5</td>
<td>-</td>
<td>0.4</td>
<td>9.9</td>
</tr>
<tr>
<td>I*(HGPS)†</td>
<td>2.9</td>
<td>1.3</td>
<td>0.5</td>
<td>6.2</td>
<td>16.6</td>
</tr>
<tr>
<td>I (HGPS)†</td>
<td>3.0</td>
<td>1.6</td>
<td>0.5</td>
<td>7.8</td>
<td>18.4</td>
</tr>
</tbody>
</table>

*Unfixed platelets.
†Patient I has an HGPS (not BSS),* with platelets on blood smear having a mean diameter equal to 3.3 \(\mu m\), and 80% having \(d > 2.5 \mu m\) (compare with Table I).

**BSS discocytes.** \(\bar{d}\), \(\bar{t}\), \(\bar{r}_p\), \(\bar{V}\), and \(\bar{SA}\) for BSS discocytes fall within the range observed for normal discocytes (Table II). There is a slight tendency for \(\bar{t}\) and \(\bar{r}_p\) in BSS discocytes to be larger. A comparison of geometric parameters for both glutaraldehyde-hardened discocytes (as prepared for BSS studies) and of unfixed discocytes observed directly in plasma, made with a donor having an HGPS (see Table II footnote), indicates that the normal size of fixed discocytes in our BSS studies does not result from shrinkage during fixation. Frequency histograms for \(d\), \(t\), and \(r_p\) are given in Fig. 1. The over-all shape of the distributions for the normal mother (MB) and her son with BSS (B) are similar. Differences, particularly with respect to \(t\), can be seen between the distributions for MB and P. These observations would seem to imply that there are subtle differences in the shape of normal and BSS discocytes. However, the platelets obtained from donor P may not be truly representative for this disorder, since P, at the time the sample was obtained, had abnormally low platelet counts (<10,000 \(\mu l^{-1}\)) and had an antibody which induces a BSS-like syndrome with normal platelets; he died 1 week later from a meningeal hemorrhage.* It is possible that these additional physiological stresses had an influence on the shapes of the discocytes observed for this patient.

**Volume distribution for BSS platelets.** For normal donors, the volume distributions for platelets determined by Coulter Counter agree very well with those determined from geometric measurements of discocytes alone. A striking discrepancy between these methods is observed for BSS platelets (Table III). Whereas Coulter Counter measurements indicate that ~40% of the BSS platelets have volumes greater than 30 \(\mu m^3\), geometric measurements of BSS discocytes suggest that the volume distribution is within normal limits.

**BSS echinocytes.** Blood, immediately fixed from normal or diseased donors, always contained from 2% to 10% of echinocytes ("spheres" with pseudopods). BSS echinocytes differ from normal echinocytes in two ways. Observations of echinocytes under a dark-field microscope indicated that the number of pseudopods per echinocyte was greatly reduced. Second, the mean diameter for the main body of BSS echinocytes was 1.6 times greater than normal (Table IV). This increased size of BSS echinocytes is of the same order as the increased size observed on blood smear. Similar observations were obtained for unfixed echinocytes studied with PRP from donor I (HGPS). In addition, the volume distribution for P's echinocytes rather than the discocytes (Table III) is in better agreement with that determined by Coulter Counter, suggesting that shape change had occurred before Coulter Counter measurements were made.

*Donor P had not received any platelet transfusion during the 14 months prior to these studies.
Table III. Comparison of volume distributions for platelets from normal and BSS donors, determined from Coulter Counter and geometric measurements

<table>
<thead>
<tr>
<th>Donor</th>
<th>% of platelets with volumes (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.65-2.8</td>
</tr>
<tr>
<td>Coulter Counter:</td>
<td></td>
</tr>
<tr>
<td>Normal (5)</td>
<td>8</td>
</tr>
<tr>
<td>P (BSS)</td>
<td>8</td>
</tr>
<tr>
<td>B (BSS)</td>
<td>9</td>
</tr>
<tr>
<td>Geometric measurements:</td>
<td></td>
</tr>
<tr>
<td>Normal (8)</td>
<td>10 ± 7</td>
</tr>
<tr>
<td>P (BSS)</td>
<td>3</td>
</tr>
<tr>
<td>B (BSS)</td>
<td>7</td>
</tr>
<tr>
<td>MB (normal)</td>
<td>3</td>
</tr>
</tbody>
</table>

Table IV. Mean diameters (\(\overline{d}\)) and volumes (\(\overline{V}\)) for normal and BSS circulating echinocytes

<table>
<thead>
<tr>
<th>Donor</th>
<th>(\overline{d}) (μm)</th>
<th>(\overline{V}) (μm³)</th>
<th>% platelets with volumes &gt;30 μm²</th>
<th>(\overline{r}_s^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.1</td>
<td>5.0</td>
<td>0</td>
<td>0.85</td>
</tr>
<tr>
<td>P (BSS)</td>
<td>3.4</td>
<td>21.0</td>
<td>18</td>
<td>2.12</td>
</tr>
<tr>
<td>I (HGPS)†</td>
<td>2.6</td>
<td>9.9</td>
<td>0</td>
<td>1.60</td>
</tr>
</tbody>
</table>

\(\overline{r}_s^* = \overline{V}_{echinocyte} / \overline{V}_{discocyte}\)

†Undixed platelets from a donor with a hereditary giant platelet syndrome (see Table II).

Discussion

We have demonstrated that circulating platelets for BSS and HGPS donors exist primarily as discocytes which do not differ significantly with respect to geometry from those of a normal donor. This observation implies that the giant size observed for these platelets on blood smear results from abnormal behavior during its preparation. The mean diameter of normal platelets on blood smears (1.8 μm) is comparable to that of echinocytes (2.1 μm) but differs from that of the face-on discocyte (3.2 μm). This observation indicates that platelets typically undergo shape changes in the preparation of the blood smear. One explanation for the observation of "giant" platelets is that such platelets are able to resist these induced shape changes. This would mean that giant platelets on blood smear are equivalent to face-on normal-sized discocytes.

A more attractive explanation for the appearance of giant platelets is that the preparation procedure for blood smear induces the formation of a larger than normal echinocyte. This interpretation is supported by the observation that circulating echinocytes for BSS and HGPS are larger than normal echinocytes. In addition, preliminary studies indicate that ADP can cause the formation of larger echinocytes for HGPS. If we assume that the platelets on blood smear are spherical and utilize the data given in Tables I and II, then we can see that this shape change must occur with a 1.5- to 1.8-fold increase in mean platelet main body surface area. This increase in surface area does not necessarily imply that BSS and HGPS platelets have an excess amount of membrane. Recently, Milton and Frojmovic56 have demonstrated that it is possible to externalize the SCCS of normal platelets to form a large, smooth spherical platelet for which the mean surface area has increased by about threefold. This suggests that platelets in some giant platelet syndromes may be
defective in the mechanism which normally prevents the SCCS from being externalized.

A number of observations indicate that a membrane abnormality is linked with BSS.
BSS platelets have a decreased electrophoretic mobility, and a reduced content of sialic acid. Receptor site defects on the platelet membrane are suggested by the observations that BSS platelets have a decreased adhesion to rabbit aorta sub endothelium and that they are not aggregated by bovine fibrinogen preparations or the antibiotic ribostamycin. As is not the case in von Willebrand's disease, the impaired ristocetin-induced aggregation is not corrected by normal plasma or purified factor VIII. Several recent studies point to the possibility that the lesion in BSS is related to an abnormality in platelet membrane glycoprotein; in particular, a reduction in a 155,000 molecular weight surface protein referred to as glycoprotein I. This glycoprotein has been implicated as the acceptor-receptor for the interaction between platelets and sub endothelium and macromolecular aggregating agents. On the basis of the observation reported here that BSS discocytes are normal sized but their echinocytes are larger, we suggest that the membrane defect must also be associated with morphological defects during the discocyte-echinocyte transformation.

Platelet pseudopods are important for platelet-platelet interactions and adhesion. Our observation that BSS echinocytes have a reduced number of pseudopods is consistent with the observed decreased adhesion of these platelets to sub endothelium.

In the case of some giant platelet diseases characterized by the appearance of giant platelet forms on blood smear, it would appear that giant discocytes may indeed be present, as for example in May-Hegglin's disease. However, in the particular case of BSS, it would appear that a structural defect probably exists associated with an abnormality(ies) in the shape change, possibly related to the SCCS. Indeed, a recent ultrastructural study of BSS platelets has revealed hypertrophic and widely dilated SCCS. Moreover, it has been reported that adenosine diphosphate does not cause a normal shape change for BSS platelets. Results for our donor I (Table II) indicate that a similar phenomenon exists for platelets from HGFS. These observations suggest a novel relationship between defective platelet anatomy and abnormal function for a broad class of bleeding disorders.

Whatever the true nature of the abnormal behavior of giant platelets on blood smear may be, the observations reported in this communication may provide a useful clue for unraveling the functional defects associated with platelets in giant platelet syndromes and may shed more light on the contractile mechanisms regulating platelet structure and function.

We acknowledge the technical assistance of C. Vaitiekuna for blood smear preparations and of Dr. P. Desnoyers and Mrs. D. Saint-Dizier for estimation of platelet volume with the Coulter Counter. We thank Dr. M. Lacombe (Montreal) for referring patient I to us.

REFERENCES


