Platelet Amino Acid Levels in Essential Thrombocytosis

By Ralph L. Nachman, Herbert I. Horowitz and Richard T. Silver

Essential thrombocytosis is a disorder of unknown etiology characterized by a marked increase in the number of circulating platelets, and may be related to the myeloproliferative diseases.1 In the course of this condition, many patients develop hemorrhagic and/or thrombotic complications, which may result from an abnormal number of platelets, from a qualitative defect in platelets or from a combination of both factors.

If the platelets in this disorder are truly defective, the abnormality might be a consequence of deranged levels of certain intracellular constituents. In order to evaluate this possibility, the intraplatelet free amino acid composition was studied. We have also studied the pseudohyperkalemic phenomenon which represents a spurious elevation of serum potassium levels owing to potassium release from platelets when whole blood containing increased numbers of platelets clots in vitro. In addition, platelet factor 3 availability was evaluated in the thromboplastin generation test.

Materials and Methods

Preparation of Platelet Concentrates

On the day of each experiment blood was collected from fasting patients by venipuncture directly into plastic tubes containing EDTA in a final concentration of 0.2 per cent, as anticoagulant. Platelet rich plasma (PRP) was prepared by centrifugation of the anticoagulated whole blood at 330 g for 15 minutes at room temperature. The platelets were then separated from the PRP using the oil bottle technic of Green.2 The platelet rich plasma was introduced into a 100 ml. siliconized oil bottle 4 cm. long, with a diameter of 3.8 mm.* The oil bottle was spun at 3000 r.p.m. for 30 minutes at room temperature in a No. 1 International centrifuge using a special cup holder (International No. 395). The pure platelet button with negligible numbers of white and red cells was harvested using siliconized Pasteur pipettes and washed twice at 4 C. with Asever’s solution and once with isotonic Veronal buffer, pH 7.4.3 The platelets were then counted by phase microscopy and the packed platelet volume measured. The platelets were resuspended in Veronal buffer in a concentration of 5 × 10⁹ cells per ml. buffer. Determination of amino acids was performed on 0.2 ml. aliquot containing 1 × 10⁹ platelets.

*Corning Glass Co., Corning, New York.
Table 1.—Clinical Features of Three Patients with Essential Thrombocytosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Platelets/mm.²</th>
<th>Clinical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>1 × 10⁶</td>
<td>Hemorrhagic tendency</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>2 × 10⁶</td>
<td>Hemorrhage and thrombosis</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>7 × 10⁵</td>
<td>Long-term P. vera with hemorrhage</td>
</tr>
</tbody>
</table>

Chemical Determinations

Platelets, 1 × 10⁹, were brought to 1 ml. volume with distilled water. They were lysed by rapidly freeze-thawing 4 times in carbon dioxide and water. The platelet lysate was deproteinized with 4 volumes of 10 per cent trichloracetic acid at 4 C. Alpha amino nitrogen (AAN) determinations were performed by the method of Rosen on the deproteinized lysate.

Electrophoresis of Platelet Amino Acids

Deproteinization of 1 × 10⁹ lysed platelets was performed with 100 mg. of picric acid. The deproteinized material was placed on Whatman #3 MM paper and high voltage electrophoresis was performed in an EMC cooled Savant chamber at 90 V/cm. for 35 minutes using a low ionic strength buffer (pH 2.0) made of formic acid, glacial acetic acid, and distilled water in the ratio of 6:24:170. The amino acids were developed using .25 per cent ninhydrin in acetone.

Clinical Material

Pertinent clinical features of the three patients studied are shown in Table 1. The first two patients had essential thrombocytosis. The third patient developed thrombocytosis in the setting of previously diagnosed polycythemia vera. The first patient, a 70-year-old female, had numerous hemorrhagic episodes, associated with marked splenomegaly, leukocytosis, megakaryocytic hyperplasia of the bone marrow and a platelet count greater than 1 × 10⁶/mm.³ The second patient, a 36-year-old male, was splenectomized 5 years earlier due to a traumatic rupture of the spleen. Subsequently, numerous hemorrhagic and thrombotic episodes developed. He was admitted to the hospital due to thrombosis of a mesenteric vein, with a platelet count greater than 2 × 10⁶/mm.³ The third patient, a 66-year-old male, had antecedent polycythemia vera for 15 years with frequent minor hemorrhagic episodes. At the time of evaluation, the hematocrit and red blood cell values were normal, but the platelet count was 750,000/mm.³

RESULTS

The AAN expressed as micrograms per 10⁹ platelets in two groups of controls and in the three patients with thrombocytosis is shown in Figure 1. The mean value for the 12 normals was 5.6 µg./10⁹ platelets. The second control group consisted of 6 patients with postsplenectomy thrombocytosis. The platelet counts in this group were in the range of the platelet counts of the three patients with essential thrombocytosis. The mean AAN value for this group was 5.9 µg./10⁹ platelets. These values are in accord with values recently reported in a similar group of patients. In contrast, the mean value for the three patients with essential thrombocytosis was 13.5 µg./10⁹ platelets. Points A and B represent values from studies on the same patient performed at different times in the course of his disease.

*Savant Instrument Co., Hicksville, N. Y.
Fig. 1.—Platelet amino acid levels in controls and in essential thrombocytosis. Point X in the normal population represents a patient with polycythemia vera with a normal platelet count. Points A and B represent values from studies on the same patient performed at different stages in the disease: A, before therapy; and B, following 3 months of busulfran.

The high voltage electrophoretic separation of free amino acids from 10⁹ platelets of patient No. 1 compared to a normal control is shown in Figure 2. There is a diffuse increase of all the amino acids in the platelets from the patient. Similar results were obtained in the other two patients.

Pseudohyperkalemia and Platelet Factor 3 Evaluation

In each patient, potassium determinations were performed both on serum obtained from whole blood 3 hours after the clot had formed and from serum separated from platelet-poor native plasma. The results are given in Table 2.
After 3 months of busulfan therapy (Table 3), the platelet count of patient

Fig. 2.—High voltage electrophoresis separation of platelet amino acids in a normal and in patient No. 1.

In each case the potassium concentration was greater in the serum obtained from whole blood than in the serum obtained from native platelet-poor plasma. The values found were those in the range frequently associated with clinical hyperkalemia. None of these patients, however, were symptomatic.

Thromboplastin generation tests were performed using the patient's platelets over a wide range of concentration. As a control, platelets from a normal individual were tested at identical concentrations. In each, the thromboplastin generation was completely normal and no difference could be detected between the 3 patients tested and the normal controls.

Effect of Busulfan

After 3 months of busulfan therapy (Table 3), the platelet count of patient
Table 2.—Comparison of K⁺ in Sera from Platelet-Poor Plasma (PPP) and from Whole Blood

<table>
<thead>
<tr>
<th>Patient</th>
<th>Platelets/mm.³</th>
<th>PPP Serum K⁺ (mEq./L.)</th>
<th>Whole Blood Serum K⁺ (mEq./L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 × 10⁶</td>
<td>5.5</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>2 × 10⁶</td>
<td>4.8</td>
<td>7.8</td>
</tr>
<tr>
<td>3</td>
<td>7.5 × 10⁵</td>
<td>4.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Normal</td>
<td>2.3 × 10⁵</td>
<td>4.5</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Table 3.—Effect of Busulfan on Patient No. 2

<table>
<thead>
<tr>
<th>Platelets/mm.³</th>
<th>PPP Serum K⁺ (mEq./L.)</th>
<th>Serum K⁺ (mEq./L.)</th>
<th>µg. AAN/10⁵ Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before therapy</td>
<td>2 × 10⁶</td>
<td>4.8</td>
<td>7.8</td>
</tr>
<tr>
<td>After therapy</td>
<td>2 × 10⁵</td>
<td>4.5</td>
<td>4.8</td>
</tr>
</tbody>
</table>

No. 2 fell to normal and the pseudohyperkalemic phenomenon was no longer observed. Although the platelet amino acid level fell, the values observed remained in the abnormal range (Points A and B, Fig. 1).

DISCUSSION

It is unclear whether the fundamental defect of the platelet is essential thrombocytosis represents a quantitative or a qualitative derangement. Hartmann⁷⁻⁸ and others⁹ who have studied the pseudohyperkalemic phenomenon have concluded that the increased potassium content of the serum following clotting of whole blood was a function of the increased platelet mass of the clot. Others have suggested, however, that these platelets are, in addition, functionally defective in releasing increased amounts of potassium during clotting.¹⁰ Diminished platelet factor 3 availability has been described as another functional aberration of thrombocytemic platelets.¹⁰,¹¹ We have not been able to confirm such a defect. It must be pointed out, however, that the method of measuring platelet factor 3 activity used in this study subjects the platelets to three vigorous washings and is thus not likely to detect subtle changes in the availability of platelet factor 3. Serotonin deficiency has also been found in platelets from some patients with thrombocytosis.¹¹,¹² Recent studies¹³ suggest that there may be diminished adhesiveness of these platelets to glass.

Nour Eldin⁵ recently studied the AAN level of platelets in various conditions. The normal range of five patients varied from 5.8 to 9.2 µg. AAN per 10⁹ platelets and is similar to the range we observed in our control population. One patient with thrombocytemia, reported by Nour Eldin, had an elevated level of 10 µg. AAN/10⁹ platelets.

In one of our patients the pseudohyperkalemic phenomenon was no longer observed following the return of the platelet count to normal after busulfan induced remission. Concomitant with this change, the AAN level fell, but remained in an abnormal range. These data suggest that the amino acid abnormality described may represent a more sensitive parameter of the platelet defect.
The increased amino acid content of platelets in essential thrombocytosis may be a function of (1) increased mass of the individual platelet or (2) a deranged internal environment of the platelet or (3) a combination of both of these factors. Despite the fact that some of the platelets from these patients were large and bizarre in shape, there was no significant difference in the packed cell mass of thrombocythemic platelets as compared to an equal number of normal platelets. This would imply that the increase in total AAN was not a function of increased platelet mass. A more precise method of measuring individual platelet volume using the Coulter Counter, recently described by Bull and Zucker, may further facilitate comparisons of normal and thrombocythemic platelets.

**Summary**

Studies on the platelets from three patients with essential thrombocytosis were presented. The intraplatelet free amino acid levels were elevated and the pseudo-hyperkalemic phenomenon was present. In one subject the pseudo-hyperkalemic phenomenon was reversed after a therapeutic remission, but the elevation of amino acids persisted. The data suggest that a qualitative abnormality may exist in the platelets of patients with essential thrombocytosis.

**Summario in Interlingua**

Es presentate studios concernite con le plachettas ab tres patientes con thrombocytosis essential. Le concentrationes intraplachettal de libere amino-acido esseva elevate, e le phenomeno pseudo-hyperkaliemic esseva presente. In un del subjectos, le phenomeno pseudo-hyperkaliemic esseva revertite post un remission therapeutic, sed le elevation del amino-acidos persisteva. Le datos suggere que un anormalitate qualitative existe possibilmente in le plachettas de patientes con thrombocytosis essential.

**REFERENCES**

PLATELET AMINO ACID LEVELS

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RALPH L. NACHMAN, HERBERT I. HOROWITZ and RICHARD T. SILVER