“Impotent” Platelets in Albinos With Prolonged Bleeding Times

By Harold M. Maurer, James A. Wolff, Sue Buckingham, and Arthur R. Spielvogel

Functional, biochemical, and morphologic platelet abnormalities are reported in four children with the syndrome of albinism, mild bleeding tendency, prolonged bleeding time, and normal platelet count. In these children, primary platelet aggregation with adenosine diphosphate occurred normally, but secondary aggregation was impaired. Collagen and norepinephrine produced almost no platelet aggregation. Platelet content of serotonin (5-HT) was markedly reduced, and uptake and retention of 5-HT by the platelets in vivo and in vitro was poor. In one child who was given a tryptophan load, urinary tryptophan metabolites were normal, suggesting that there was no evidence of a block in the 5-HT synthetic pathway in the gastrointestinal tract. Electron microscopy revealed an absence of densely osmophilic granules in 5-HT poor platelets. Platelets from other albinos with no history of bleeding contained normal amounts of 5-HT and densely osmophilic granules.

The association of albinism with a hemorrhagic tendency characterized by a prolonged bleeding time, and normal platelet count is now well recognized.1-7,40,41 Our purpose is to report hematologic, coagulation, platelet function, biochemical, and histological studies in four albino children with this syndrome.

MATERIALS AND METHODS

Patients

Four children, three of whom were siblings, had oculocutaneous albinism and a bleeding tendency. L. R. and B. R. were brothers, ages 9 and 13 yr, respectively, and E. R. was their 16-yr-old sister. Their parents, Mr. and Mrs. R., were of Puerto Rican antecedents, and were neither “bleeders” nor albinos. The fourth albino, S. M., also Puerto Rican, was a 2-yr-old female whose parents were normally pigmented and in good health.

All four albinos gave a history of intermittent purpura since early childhood, excessive gingival bleeding, epistaxis, and prolonged oozing from cuts and bruises. In addition,
L. R. had hemorrhaged after tonsillectomy, and E. R. had had menorrhagia. Five other albinos, including three adults, who had no history of bleeding, also were investigated.

Methods

IVY bleeding time and tourniquet test were performed as described by Biggs and MacFarlane.8 Platelets were enumerated in a counting chamber using sodium citrate diluent.

Collection of Blood: Venous blood was collected with a disposable needle and plastic syringe and was diluted 9:1 with one of the following anticoagulants: 4% sodium citrate solution, 1.34% sodium oxalate solution; or 1% disodium ethylenediamine tetraacetate (EDTA) in 0.85% saline. Plasma fractions were separated by centrifugation at 4°C or at room temperature as follows: platelet-rich plasma (PRP), 600 rpm for 15 min; platelet-poor plasma (PPP), 9000 rpm for 20 min; routine plasma, 2000 rpm for 10 min. Supernatant plasma was removed and kept at room temperature or 4°C until tested, usually within 2 hr.

Coagulation Studies: The coagulation tests used were the following: Whole blood coagulation time,9 clot retraction,10 on stage prothrombin time,11 serum prothrombin time,12 celite partial thromboplastin time,13 and assays of Factors VIII,14 XIII,15 and fibrinogen.16

Platelet Function Studies: Platelet aggregation by adenosine diphosphate (ADP), (final concentration, 0.6 μg/ml), norepinephrine, (final concentration, 44 μg/ml), and collagen, (final concentration, 13.4 μg/ml) was determined by Dr. Hymie L. Nossel as previously described.17

Kaolin-induced platelet factor 3 activity was determined by the method of Spaet and Cintron.18 Platelet adhesion to glass was determined by Dr. Herbert I. Horowitz.19

Biochemical Studies: Platelet serotonin (5-HT) content was measured spectrophotofluorometrically.20 5-HT uptake was studied in duplicate aliquots of albino and control washed platelet suspensions incubated (37°C, 15 min) with exogenous 5-HT (1 μg base/ml washed platelet suspension).21 Leakage of newly acquired 5-HT was assessed by reincubating aliquots of amine-treated platelets, resuspended in buffer, for 15 min.

Ethanol extracts of PRP were prepared according to Mills et al.22,23 modification of the procedure of Holmsen et al.24 and ATP determinations were carried out by the firefly method.22,24

A tryptophan load test was carried out simultaneously on B. R. (one of the albinos with a prolonged bleeding time) and a normally pigmented volunteer of similar age using 1.5 and 2.0 g, respectively, of 1-tryptophan orally. Blood was sampled for platelets 5-HT content before tryptophan supplementation and at 1, 2, and 4 hr following the ingestion of the amino acid. Twenty-four hour urine specimens were collected before and after tryptophan supplementation for determination of tryptophan metabolites. Toluene was used as a preservative and the urine was kept in the dark. Upon completion of each collection the urine was frozen, packed in dry ice, and shipped air freight to Dr. Raymond R. Brown, University of Wisconsin, who performed the assays.25

Electron Microscopy: Platelet samples for electron microscopy were prepared at 4°C, fixed in glutaraldehyde phosphate buffer, pH 7.4, and postfixed in Dalton’s chromo-osmium.26,29

RESULTS

All four albinos with symptoms of bleeding had variably prolonged bleeding times ranging from 6 to more than 20 min, normal platelet counts, and negative tourniquet tests. The three affected siblings also had course irregular bluish-black cytoplasmic inclusions in bone marrow histiocytes stained with Wright stain. These inclusions stained negatively for iron with Prussian Blue. The parents of the siblings and control albino subjects had no symptoms of bleeding and had normal platelet counts and bleeding times. The tourniquet test was negative in one control albino. Bone marrow aspirations were not performed on those with normal bleeding times.
Coagulation Studies: All coagulation test values fell within the normal range except for fibrinogen which was elevated above 500 mg/100 ml in three of the four affected albinos and in one control albino.

Platelet Adhesion to Glass: Platelet stickiness to glass varied widely (17.5–71.1%; normal values 20–65%) and appeared to be unrelated either to the history of bleeding or the length of the bleeding time.

Platelet Aggregation: Platelet aggregation by ADP, collagen, and noradrenaline was studied in the two brothers with prolonged bleeding times (B.R. and L.R.) and in two normal subjects (Fig. 1). The results obtained in B.R., illustrated in Figure 1B, and his brother (not shown) were similar. B.R.'s platelets aggregated as rapidly as those of the control after the addition of ADP. Subsequently, however, his platelets rapidly disaggregated, while the control's platelets remained clumped. When collagen and norepinephrine were added to the albino's PRP, aggregation was either diminished or absent, findings not characteristic of normal platelets. The control results are similar to those obtained in ten normal individuals previously studied.

Platelet Factor 3 Activity: Platelet factor 3 activity was normal in two albinos with prolonged bleeding times (B.R. and L.R.) and two control albinos with normal bleeding times, as determined by shortening of the Russell Viper Venom clotting times obtained at 30 min of incubation.

Biochemical Studies: In all of the albinos with prolonged bleeding times, platelet 5-HT stores were abnormally low with values of 10% of normal (Table 1). When these platelets were incubated in vitro with exogenous 5-HT, their uptake of amine was also subnormal in total quantity. Further, taken as a group, these platelets after 15 min of incubation, leaked the recently acquired amine, a finding not characteristic of the group of normal platelets (p <0.05). Platelets from albinos with normal bleeding times and from the parents of the affected siblings contained amounts of 5-HT within normal range (Table 2).
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Table 1. Serotonin (5-HT) Levels in Platelets of Albinos With Prolonged Bleeding Times:
In Vitro Uptake and Retention of Exogenous 5-HT

<table>
<thead>
<tr>
<th>Subject</th>
<th>Initial 5-HT Content (µg/mg Protein)</th>
<th>5-HT Content After Incubation With Exogenous 5-HT* (µg/mg Protein)</th>
<th>5-HT Content After Reincubation With Buffer† (µg/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. R.</td>
<td>(6) 0.034 (0.022-0.047)</td>
<td>(2) 0.281 (0.270, 0.292)</td>
<td>(2) 0.244 (0.239, 0.248)</td>
</tr>
<tr>
<td>B. R.</td>
<td>(4) 0.047 (0.032-0.073)</td>
<td>(2) 0.295 (0.290, 0.300)</td>
<td>(2) 0.251 (0.243, 0.258)</td>
</tr>
<tr>
<td>E. R.</td>
<td>(2) 0.024 (0.023, 0.025)</td>
<td>(2) 0.130 (0.129, 0.131)</td>
<td>(2) 0.123 (0.119, 0.127)</td>
</tr>
<tr>
<td>S. M.</td>
<td>(2) 0.022 (0.020, 0.024)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>(4) 0.462 (0.335-0.512)</td>
<td>(4) 1.20 (1.19-1.121)</td>
<td>(2) 1.20 (1.19, 1.21)</td>
</tr>
<tr>
<td>Control</td>
<td>(3) 0.421 (0.397-0.500)</td>
<td>(4) 0.817 (0.653-1.10)</td>
<td>(4) 0.810 (0.622-0.992)</td>
</tr>
</tbody>
</table>

Values represent means. Number of observations are shown in parenthesis preceding mean value.

*15 min, 37°C, 1µg/ml 5-HT added to incubate.
†After incubation with exogenous 5-HT, platelets were washed, resuspended in buffer, and incubated for 15 min, 37°C.
‡Significance of 5-HT loss from all albino platelets as compared to all control platelets.

L-Tryptophan, a 5-HT precursor, was given simultaneously to albino B.R. and to a healthy volunteer of similar age. The results of platelet studies are illustrated in Fig. 2. A small rise in platelet 5-HT content was observed in the albino followed by an apparent leakage of these amine stores. In contrast, the 5-HT content of control platelets increased markedly and remained at approxi-

Table 2. Serotonin (5-HT) Levels in Platelets of Albinos With Normal Bleeding Times, and in Albino B. R.’s Parents

<table>
<thead>
<tr>
<th>Subject</th>
<th>5-HT Content (µg/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mrs. R.</td>
<td>0.168 (0.154, 0.182)</td>
</tr>
<tr>
<td>Mr. R.</td>
<td>0.168 (0.158, 0.178)</td>
</tr>
<tr>
<td>Albino A</td>
<td>0.303 (0.292, 0.314)</td>
</tr>
<tr>
<td>Albino B</td>
<td>0.766 (0.752, 0.780)</td>
</tr>
<tr>
<td>Albino C</td>
<td>0.234 (0.211, 0.258)</td>
</tr>
<tr>
<td>Controls*</td>
<td>0.327 ± 0.01 (0.156-0.512)</td>
</tr>
</tbody>
</table>

Except for controls, values represent means of two observations.

*Mean value (± SE) of 18 determinations.
Fig. 2. Effect of oral loading doses of l-tryptophan on the platelet content of serotonin in albino B.R. and control.

Fig. 2. Effect of oral loading doses of l-tryptophan on the platelet content of serotonin in albino B.R. and control.

mately the initial level for 4 hr. At the same time the albino's urinary excretion of tryptophan metabolites, including 5-hydroxyindoleacetic acid, the chief product of the 5-HT pathway, was comparable to that in the control patient (Table 3). Thus, no evidence was found to suggest a specific block in the 5-HT synthetic pathway in the gastrointestinal tract.

The concentration of ATP in the 5-HT deficient platelets (30–46 μg/10⁹ platelets) was not substantially different from that of the control (38.3–51.7 μg/10⁹ platelets). Our control values approximate the normal values reported by Mills and Thomas.

Electron Microscopy: Serotonin-poor platelets were structurally normal except for complete absence of the densely osmophilic granules that have been identified with 5-HT storage (Fig. 3). The platelets from albinos with normal bleeding times and normal platelet 5-HT levels could not be distinguished morphologically from control platelets obtained from normally pigmented volunteers in good health.

DISCUSSION

Our data demonstrate associated functional, biochemical, and morphologic platelet abnormalities in individuals with albinism and prolonged bleeding times. In these individuals, the initial phase of platelet aggregation (primary aggregation) by ADP occurred normally, but the second phase (secondary aggregation), which amplifies aggregation and follows the release of additional ADP from the platelets themselves, failed to occur and the platelets rapidly dispersed. Collagen and norepinephrine, agents dependent to a large extent on the release of intrinsic platelet ADP for their aggregating activity, produced almost no response. Similar platelet responses were first reported by Hardisty and Hutton in two such individuals, and suggest defective release of intrinsic ADP from the platelets. Mills and Hardistry also found lower (0.6–1.3 μmoles/10¹¹ platelets) than normal (4.01±0.27 μmoles/10¹¹ platelets) platelet ADP levels while ATP (patients, 4.0–6.7 μmoles/10¹¹ platelets; control, 7.04±0.37 μmoles/10¹¹ platelets) was only slightly reduced. In one of our patients, platelet ATP content was determined and was not substantially different from
Table 3. Tryptophan Metabolic Studies

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Micromoles of Metabolite Excreted in the Urine per 24 hr</th>
<th>Pretryptophan Load</th>
<th>Posttryptophan Load</th>
<th>Albino B. R.</th>
<th>Control</th>
<th>Normal Values*</th>
<th>Albino B. R.</th>
<th>Control</th>
<th>Normal Values*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Micromoles of Metabolite Excreted in the Urine per 24 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridone</td>
<td>89</td>
<td>160</td>
<td>103</td>
<td>232</td>
<td>132</td>
<td>135.6</td>
<td>± 28.0</td>
<td>± 37.3</td>
<td></td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>12</td>
<td>15</td>
<td>13.2</td>
<td>49</td>
<td>59</td>
<td>56.6</td>
<td>± 5.6</td>
<td>± 21.5</td>
<td></td>
</tr>
<tr>
<td>Xanthurenic acid</td>
<td>6</td>
<td>6</td>
<td>10.1</td>
<td>27</td>
<td>20</td>
<td>29.4</td>
<td>± 2.4</td>
<td>± 25.4</td>
<td></td>
</tr>
<tr>
<td>Indican</td>
<td>132</td>
<td>178</td>
<td>383</td>
<td>314</td>
<td>183</td>
<td>544</td>
<td>± 121</td>
<td>± 312</td>
<td></td>
</tr>
<tr>
<td>Anthranilic acid</td>
<td>4</td>
<td>3</td>
<td>5.4</td>
<td>6</td>
<td>4</td>
<td>9.1</td>
<td>± 1.6</td>
<td>± 2.4</td>
<td></td>
</tr>
<tr>
<td>glucuronide</td>
<td>25</td>
<td>23</td>
<td>26.5</td>
<td>45</td>
<td>41</td>
<td>49.6</td>
<td>± 5.4</td>
<td>± 14.2</td>
<td></td>
</tr>
<tr>
<td>0-Aminohippuric acid</td>
<td>9</td>
<td>6</td>
<td>12.7</td>
<td>13</td>
<td>14</td>
<td>17.1</td>
<td>± 3.2</td>
<td>± 4.4</td>
<td></td>
</tr>
<tr>
<td>Acetylkynurenine</td>
<td>12</td>
<td>4</td>
<td>13.2</td>
<td>30</td>
<td>8</td>
<td>35.5</td>
<td>± 4.2</td>
<td>± 21.0</td>
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</tr>
<tr>
<td>Kynurenine</td>
<td>0</td>
<td>16</td>
<td>19.3</td>
<td>10</td>
<td>18</td>
<td>37.6</td>
<td>± 9.1</td>
<td>± 20.4</td>
<td></td>
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<tr>
<td>Hydroxykynurenine</td>
<td>44</td>
<td>64</td>
<td>-</td>
<td>63</td>
<td>60</td>
<td>-</td>
<td>± 63.6</td>
<td>± 20.4</td>
<td></td>
</tr>
<tr>
<td>N-Methylnicotinamide</td>
<td>3.87</td>
<td>2.16</td>
<td>4.10</td>
<td>3.53</td>
<td>4.44</td>
<td>3.89</td>
<td>± 0.56</td>
<td>± 1.33</td>
<td></td>
</tr>
<tr>
<td>5-Hydroxyindole-acetic acid†</td>
<td>6.1</td>
<td>4.8</td>
<td>-</td>
<td>4.9</td>
<td>4.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tryptophan given</td>
<td>2.0g</td>
<td>1.5g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Normal adult values in Dr. Raymond R. Brown’s laboratory.
†Milligrams per 24 hr.

that of the control subject. These investigators also showed that nucleotides were not released from these platelets with thrombin, which caused the release of about 60% of the nucleotides from normal platelets. It appears that these abnormal platelets may lack the storage or nonmetabolic pool of ADP which is selectively released from platelet granules during the platelet release reaction.30 43

In addition to impaired platelet aggregation, the four albinos with prolonged bleeding times had abnormally low platelet 5-HT stores. Levels in those with normal bleeding times, including the parents of the three affected siblings, were normal. The reduced content of 5-HT in the platelets could reflect either a block in amine synthesis in the gastrointestinal tract or defective 5-HT accumulation in the platelets. Poor uptake and leakage of 5-HT by these platelets in vitro and in vivo, and the normal urinary excretion of tryptophan metabolites after tryptophan supplementation both suggest the presence of an intrinsic platelet defect. Mills and Hardisty similarly found low platelet 5-HT content and poor uptake of 5-HT by the abnormal platelets in their patients.7

It has been suggested that platelet ATP is involved in accumulation and bind-
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The amount of 5-HT is proportional to the amount of ATP present. Inasmuch as normal platelet ATP levels were found, 5-HT binding sites other than ATP may thus be implicated.

The association of low ADP and 5-HT stores in the abnormal platelets is in keeping with experimental observations that show a close correlation between platelet liberation of adenine nucleotides and that of 5-HT. Apparently, in the albinos with prolonged bleeding times, as a consequence of low stores of these compounds, the platelet release reaction is ineffective in promoting aggregation; in this respect, the platelets are impotent. This inherent lack of granule-stored compounds remains unexplained. With respect to 5-HT the evidence is suggestive of impaired platelet binding of the amine or changes in membrane permeability permitting its leakage.

Recently, studies in rabbits and in man indicate that 5-HT is primarily concentrated in densely osmophilic granules and is found to a lesser extent in other platelet sites after its uptake and transfer into the platelet cytoplasm. These 5-HT storage granules disappear in the presence of reserpine or amphetamine-like compounds. Our platelet electron microscopy studies disclosed that those with a deficiency of 5-HT lacked densely osmophilic granules. Mills and Hardisty are not certain that the densely osmophilic bodies can be recognized in all normal individuals; they found no characteristic differences between 5-HT-poor platelets and controls. On the other hand, White et al. have lately described a child with albinism and a mild hemorrhagic disorder in whom the defects included a decrease in the number of platelet dense bodies, a reduction in platelet 5-HT content, defective platelet factor 3 activation by collagen and increased urinary excretion of glycolipid.

In our patients and the one described by Logan et al., platelet factor 3 activity was found to be normal. Platelet factor 3 was defective, however, in Hardisty and Hutton’s two albinos with prolonged bleeding times, though the total coagulant activity of the platelets after freezing and thawing was normal. Similarly, the patient reported by White et al. had abnormal platelet factor 3 activation by collagen. The apparent lack of agreement in these findings is perplexing.

The nature of the link between the platelet disturbance and albinism, a genetic disorder in which there is a deficiency of tyrosinase activity in melanocytes, is unknown. Preliminary results of chromosome studies performed by McGilvray et al. have indicated that the four albinos with prolonged bleeding times all had a highly significant increase in the frequency of cells with breaks (affected albinos, 16–20%; eight normally pigmented control subjects, 2%), and some reunion figures consisting of dicentric chromosomes and quadiradial configurations. Moreover, 4 heterozygous parents also showed significantly increased frequencies of such cells (6–15%). In contrast, almost no chromosomal abnormalities were seen either in two normally pigmented brothers of the sibling albinos or in the albinos with normal bleeding times. The cytogenetic defect may reflect a genetic disturbance related to the platelet disorder rather than to albinism.
ACKNOWLEDGMENT

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REFERENCES


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