Albinism Associated with Hemorrhagic Diathesis and Unusual Pigmented Reticular Cells in the Bone Marrow: Report of Two Cases with Histochemical Studies

By F. Hermansky and P. Pudlak

HEMORRHAGIC DISORDERS with a prolonged bleeding time as a main laboratory finding and a normal platelet count may have a varying pathogenesis. They are usually caused by qualitative changes of the platelets or a disturbance of the vascular factor in the process of hemostasis. They are for the most part congenital and hereditary anomalies, which, despite the progress made in this field, are still difficult to classify into well-defined groups. We had the opportunity of observing a hemorrhagic disorder of medium severity with a prolonged bleeding time in two unrelated albinos. These observations were made independently in two separate institutions. Both cases had concomitant congenital nystagmus, and in both, large reticular cells with an unusual pigment were found in the bone marrow, which could not be identified with reference to similar cells described in the literature. The combination of these abnormalities independently observed in two unrelated patients suggested that we were confronted with a new, not previously described congenital anomaly.

CASE STUDIES

Case 1. E.B., a 33-year-old children’s nurse, was first admitted to the First Medical Clinic in Prague on December 22, 1953 because of severe, recurrent epistaxis not related to menstruation. From childhood the patient had noticed that she bruised easily and tended to bleed for a long time after injury. Severe menorrhagia had occurred from time to time since puberty, simultaneously with increased bruising and more frequent epistaxis. In 1951, she had severe bleeding after a dental extraction. During her several admissions to the hospital she was given repeated blood transfusions with doubtful hemostatic effect. Otherwise, the past history was not contributory and there was no family history of hemorrhagic disease or albinism.

The clinical examination showed a generalized lack of pigment in the skin and hair. The iris was a yellowish-green, and was reddish on lateral illumination. Furthermore, there was strabismus and continuous coarse nystagmus. There were scattered ecchymoses on the skin of the extremities. Otherwise, the clinical findings were within normal limits.

The blood pressure was normal, 130/90. There were subfebrile to febrile temperatures. The chest x-ray showed no focal changes. Ophthalmologic examination revealed myopia and confirmed the presence of albinism with oculogenetic nystagmus.

Laboratory findings: Urinalysis, negative; increased sedimentation rate, 51 mm. hr.; Wassermann reaction, WB; negative. Flocculation reactions: Weltmann 7; Takata negative; thymol turbidity test, 7.8 units; cholesterol, 214 mg. per cent. Serum bilirubin: total 0.8 mg. per cent, direct negative. Blood cultures were repeatedly negative. TB-
ALBINISM ASSOCIATED WITH HEMORRHAGIC DIATHESIS

Table 1.—Hematologic Findings

<table>
<thead>
<tr>
<th>Date</th>
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<th>Case 2</th>
<th>Case 2</th>
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<tbody>
<tr>
<td>2/22/53</td>
<td>12.8</td>
<td>13.4</td>
<td>10.2</td>
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<tr>
<td>2/24/54</td>
<td>13.3</td>
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<td>32.5</td>
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<tr>
<td>5/23/56</td>
<td>44</td>
<td>10.2</td>
<td>14</td>
</tr>
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</table>

Hemoglobin, Gm.
Hematocrit, Wintrobe
RBC, mil./cu.mm.
Retics, per 1000 RBC
WBC, thous. cu.mm.
Neutrophils, %
Eosinophils, %
Basophils, %
Lymphocytes, %
Monocytes, %

Table 2.—Bone Marrow Aspiration

<table>
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<td>18</td>
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<td>17.6</td>
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<td>52.5</td>
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<td>7600</td>
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<td>54</td>
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<td>29.6</td>
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<td>19.2</td>
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<td>57</td>
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<td>9.4</td>
<td>6</td>
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<td>13.2</td>
<td>15</td>
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<td>10/2/57</td>
<td>1.8</td>
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<tr>
<td>10/2/57</td>
<td>0.4</td>
<td>0.2</td>
<td>1.4</td>
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<tr>
<td>10/2/57</td>
<td>13.4</td>
<td>19</td>
<td>25.2</td>
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</tbody>
</table>

Blasts %
Neutro myelocytes
nonsegmented
segmented
Eosinophils
Basophils
Lymphocytes
Retic. cells + monocytes
Plasma cells
Erythroid elements
Cellularity
Megakaryocytes

hemagglutination test (Middlebrook and Dubos), positive 1:16. The blood picture showed a moderate normochromic normocytic anemia (see table 1) that was corrected by transfusion, and occasional eosinophilia. In the bone marrow (table 2), large reticular elements were found packed with blackish-blue pigmented granules or particles on the usual panoptic stain. Megakaryocytes were present in normal amounts without major changes.

In view of the positive TB-hemagglutination reaction and a suspected gynecologic finding, the patient was treated with a combination of streptomycin (SM) and isoniazid (INH) without any particular influence on the raised temperature.

During the three years after discharge from the hospital the patient suffered from occasional epistaxis and more marked formation of subcutaneous hematomas, sometimes a light hemoptysis. In March 1957, she was admitted for reevaluation. In addition, she now showed hypertension 160/110 and microscopic hematuria, both of which later subsided. Renal investigation showed a decrease of GFR to 65 ml., with a normal re-absorption of 99.45 per cent. On pyelography, horseshoe kidney was found. The chest x-ray showed a fresh, right-sided subclavicular infiltration. The sputum was repeatedly negative for tubercle bacilli. On this admission the sedimentation rate was 10 mm./hr.; total serum proteins, 7.8 Gm. per cent; albumin, 4.8 Gm. per cent; globulin a, 0.98 Gm. per cent; globulin β, 0.9 Gm. per cent; globulin γ, 1.2 Gm. per cent. The finding of pigmented macrophages on repeated sternal puncture remained unchanged. There was prolonged bleeding after one sternal puncture, which necessitated treatment by compression.

Antituberculous treatment (SM, INH, PAS) led to slow regression of the subclavicular infiltration. At the same time, the hemorrhagic manifestations, especially epistaxis and
hemoptysis, continued, and on one occasion there was prolonged bleeding after dental extraction. At this stage, marked anemia developed which was not completely brought under control even by blood transfusion and iron therapy because of recurrent blood losses. In November 1957, the patient was transferred to a sanatorium for tuberculous patients. An almost complete resorption of the subclavicular infiltration was ascertained and the treatment with antituberculous bacteriostatics was continued.

Case 2. J.R., a 33-year-old farmer, was transferred from the University Chest Department to the Clinical Department of the Institute for Hematology and Blood Transfusion on May 22, 1956 for the examination of hemostasis prior to lung puncture. From childhood, the patient had suffered from nose bleeding and prolonged bleeding after dental extraction or injuries. There was no history of subcutaneous hematomas. In 1948, he was admitted to the hospital because of severe epistaxis which was treated by blood transfusion with doubtful hemostatic effect. From the age of 24, the bleeding tendency subsided except for occasional, moderate nose bleeding. During the past three years he had suffered from breathlessness on exertion. From 1955, he was treated at the University Chest Department for suspected pulmonary fibrosis. There was no family history of bleeding, albinism and no consanguinity.

As in the first case the clinical examination showed generalized lack of pigment. The ophthalmologic examination revealed astigmatism, hypermetropia and confirmed albinism. In the lungs there were occasional rales and rhonchi. The liver projected one finger below the costal margin. The chest x-ray revealed a large number of clearly defined shadows about the size of a small pea in both lung fields.

Laboratory findings: Urinalysis, negative. Blood sedimentation rate, 29 mm./hr.; WR negative. Flocculation reactions: Weltmann 8, thymol turbidity, 3 units. Serum bilirubin: total 0.69 mg. per cent, direct negative. Cholesterol, 156 mg. per cent. Serum calcium, 9.8 mg. per cent. Total serum proteins: 8.6 Gm. per cent; albumin, 3.95 Gm. per cent; globulin 1, 0.3 Gm. per cent; globulin 2, 0.7 Gm. per cent; globulin β, 1.0 Gm. per cent; globulin γ, 2.65 Gm. per cent. TB-hemagglutination test (Middlebrook and Dubos), 1:16. Sputum, repeatedly negative for tubercle bacilli.

The blood picture was normal (table 1). In the bone marrow (table 2) the same pigmented macrophages were found as in the first case. The megakaryocytes showed no abnormalities. After discharge the patient remained under observation at the University Chest Department as a case of chronic interstitial pulmonary fibrosis or of a pulmonary form of M.Boeck-Besnier-Schaumann. He died at home a year later; no autopsy was performed.

Tests of hemostasis: The results of the tests of hemostasis are summarized in table 3. The one consistent abnormality was a moderate to marked prolongation of the bleeding time (Duke). At the same time the platelet count (Feissly-Ludin)* was within normal limits and the platelets did not display any definite morphologic changes in the smears. When observing the native blood under the phase contrast microscope, in Case 1 the

<table>
<thead>
<tr>
<th>Test</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Control</th>
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<tr>
<td>Platelets</td>
<td>108,000-216,000</td>
<td>270,000-399,000</td>
<td>150,000-300,000</td>
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<tr>
<td>Bleeding time, min.</td>
<td>8-13</td>
<td>12-&gt;20</td>
<td>3-5</td>
</tr>
<tr>
<td>Clot retraction</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Tourniquet test</td>
<td>Negative</td>
<td>Slightly positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Clotting time, min. Lee-White</td>
<td>6-8</td>
<td>9-11</td>
<td>4-10</td>
</tr>
<tr>
<td>Prothrombin time, sec. Quick³⁵</td>
<td>14.1-14.7</td>
<td>12.2-16.6</td>
<td>12-15</td>
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<tr>
<td>Prothrombin consumption, sec.</td>
<td>Normal</td>
<td>Normal</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Fibrinolysis³⁴</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Antithrombin titer¹⁰</td>
<td>Normal</td>
<td>—</td>
<td>Normal</td>
</tr>
<tr>
<td>Fibrinogen, mg. per cent</td>
<td>287</td>
<td>—</td>
<td>200-400</td>
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TABLE 3.—Tests of Hemostasis
Platelets showed the formation of pseudopods and aggregation in the same way as in the controls. Similarly, the aggregation and disintegration of the platelets studied in recalcified citrated platelet-rich plasma proceeded normally. Clot retraction was repeatedly normal. In patient 2, the tourniquet test was slightly positive. In both patients prothrombin consumption was repeatedly normal; only once was it slightly decreased in patient 1 (36 sec.). The thromboplastin generation test (Duckert et al.)* in our modification also gave repeatedly normal values when using BaSO₄ plasma, serum or the platelets of the patients consecutively or simultaneously in the generation mixture. The antithrombin titer which was examined in the woman patient was normal, as was the inactivation of tissue thromboplastin by her serum. Thrombelastography in the woman patient gave normal results when using whole blood (reaction time r 9 sec., K value 5'15 sec., max. amplitude 56 mm.) and recalcified citrated plasma (r 7'15 sec., K 2'30 sec., ma. 60 mm.). No morphologic abnormalities on capillary loops of the nail bed could be found in this patient.

Histochemical studies: The large pigmented reticuloendothelial cells were present in the bone marrow smears in about the same amount as megakaryocytes. Their cytoplasm was packed with coarse granules or particles of varying size and form which were in places badly defined against the cytoplasm. In unstained smears these particles had a yellow color; on panoptical Pappenheim's stain they became a blackish-blue or greenish-blue (fig. 1).

For closer analysis of the chemical nature of these granules some special staining methods were used with the following results:

1. The periodic acid Schiff reaction (PAS) gave a clear positive result. The red staining of the examined substance remained unchanged after previous digestion with saliva for one hour which abolished the PAS reaction in granulocytes.

2. Previous acetylation with acetic acid anhydrid in pyridine (MacMannus and Cason), used for the bone marrow cells by Storti et al., completely abolished the PAS reaction of the particles in the smears fixed in one instance in Rossmann's mixture and the second time with methanol. Surprisingly, however, the positive reaction of granulocytes was still present. After deacetylation with 0.1 N KOH, the substance of the macrophages again stained red.

3. The reaction for acid mucopolysaccharides (Halle) did not give a distinct blue color. We therefore considered it as most probably negative.

4. The use of Sudan orange for staining lipids (Bomeis) gave the granules an orange color.

5. Sudan Black B in 70 per cent alcohol stained the examined substance a dark blue, sometimes with a greenish tinge.
6. On staining with Nile blue sulphate (Lorraine and Smith) the cytoplasmic inclusions stained blue, while the neutral fat droplets stained pink.
7. The plasmal reaction for acetal-lipids gave negative results.
8. Extraction with methanol-chloroform for 24 hours at 60 C. (Keilig) only slightly reduced the intensity of the following staining with Sudan Black B. Also the PAS reaction carried out after this extraction remained positive in most cells; only in a few cases the small areas of cytoplasm were stained a yellow-orange.
9. By extraction with pyridine (Baker) the intensity of staining with Sudan Black B was slightly more reduced. The yellow-orange color of the particles occurred more frequently in the cytoplasm of macrophages with the PAS reaction.
10. The test of phospholipids with Nile blue sulphate (Menschik) gave negative results.
11. Millon’s reaction for tyrosine (Serra and Queiroz Lopes) gave doubtful results.
12. The Prussian Blue reaction did not demonstrate stainable iron in these cells.
13. By using the staining procedure according to Lignac the presence of melanin could be excluded.

**Discussion**

It is assumed that generalized albinism is due to the lack of the main chromogenic factor C which results in the complete inhibition of the process of pigmentation. The yellowish-green color of the iris in our patients is evidence of an incomplete form of generalized albinism. The heredity of this anomaly, which is probably transferred as a recessive trait, was not possible to ascertain in the family history, nor was there a history of consanguinity. Albinism is often associated with other anomalies such as deafness, oligophrenia and polydactyliu. However, in the accessible literature we could not find an association with a hemorrhagic disease, which, in view of the early manifestation, can also be considered a congenital abnormality in our patients.

This hemorrhagic disorder falls in the group of so-called pseudohemophilias, the clinical manifestations and laboratory data of which are summarized in a recent review by Buchanan and Leawell. The only consistent laboratory abnormality in these cases is a prolonged bleeding time which was repeatedly found in both our patients. The normal prothrombin consumption and thromboplastin generation test exclude a simultaneous defect in plasma thromboplastic factors. These tests also confirmed the normal thromboplastic activity of the platelets which did not display any morphologic changes or functional disturbances in the tests used. These tests, however, did not cover all partial platelet functions as, for example, the amount of serotonin in the platelets, which we are not able to investigate. It was, therefore, not possible to exclude the possibility of both vascular and platelet defects playing a part in the pathogenesis of the bleeding.

In the female patient, the horseshoe kidney is to be considered a developmental anomaly. At the time this finding was made, it was unfortunately impossible to carry out a pyelogram on the other (male) patient.

The pulmonary disease in both our patients was obviously an associated process. In patient 1, the typical x-ray finding and the clinical course with slow regression of the pulmonary infiltration after prolonged treatment with bacteriostatic antituberculous drugs gave evidence of tuberculosis in spite of repeated negative findings in the sputum. In the male patient the nature of the pulmonary process could not be precisely determined.
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A common abnormality was the finding of unusual pigmented macrophages in the bone marrow. The positive PAS reaction and the staining with Sudan Black B would suggest according to Pearse\textsuperscript{18} that we were most probably concerned with an accumulation of glycolipids, phospholipids or lipoproteins in these cells. Glycogen was excluded by the resistance to digestion by saliva, neutral fats by blue staining with Nile blue, and acetal-lipids by the negative plasmal reaction. The easy reversible blocking of the PAS reaction in the examined cells by acetylation should suggest the presence of numerous 1:2-glycol groups and therefore the probable presence of a carbohydrate component.\textsuperscript{18} According to Wolman,\textsuperscript{3} even sphingolipids may behave in a similar way regardless of whether they contain a carbohydrate component or not. The evaluation of the acetylation test is made difficult because granulocytes containing glycogen gave a positive PAS reaction even after acetylation.

We were not able to remove the substance of the macrophages noticeably either with methanol-chloroform or pyridine. Similarly, Menschik's reaction for phospholipids, with which there is little experience in hematologic practice, was also negative. It cannot be excluded that some of the tests were influenced by the age of the bone marrow smears which were examined from several weeks to one year after the puncture. In view of their staining properties, the pigmented macrophages of our patients did not appear to be identical with the blue-pigmented macrophages of Moeschlin\textsuperscript{15} nor with the reticuloendothelial elements of Sawitsky, Hyman and Hyman.\textsuperscript{24} The cells described by the latter authors stained only weakly with the PAS reaction, and staining for lipids gave negative results. In the opinion of these authors, the granules of these cells appear to contain mucopolysaccharides.

The relationship of the pigmented macrophages and albinism is quite obscure in view of the complete lack of information about the bone marrow findings in albinos. It seems, however, quite improbable that the occurrence of these cells forms an integral part of albinism. The relationship of these cells to the disturbance in hemostasis is equally unclear. The substance of the granules could theoretically contain some complex with a coagulation inhibitor, for example, inositolphosphatid.\textsuperscript{17} The coagulation tests, however, did not provide evidence of an increased level of an inhibitor in the peripheral blood. It is interesting that the patient of Sawitsky et al.\textsuperscript{24} suffered from hemorrhagic manifestations; the bleeding time, however, was normal. A hemorrhagic diathesis from the group of so-called pseudohemophilias, with a simultaneous decrease in the thromboplastic activity of the platelets, was described in association with another congenital anomaly, i.e., osteogenesis imperfecta.\textsuperscript{26} It is to be hoped that analysis of a larger number of similar cases might contribute to the discovery of hitherto unknown pathogenetic relations.

**Summary**

1. A description is given of two unrelated albinos with hemorrhagic diathesis and peculiar, pigmented reticular cells in the bone marrow.
2. On examining hemostasis, the only consistent abnormal laboratory finding was a prolonged bleeding time, so that the hemorrhagic disorder fell into the group of so-called pseudohemophilias.
3. Unusual reticuloendothelial cells in the bone marrow were packed with blackish or greenish blue granules or particles. According to the histochemical study, the substance in the cytoplasm was probably of a lipid nature. These cells could not be identified with similar cells of this kind which have so far been described.

4. The combination of the above described congenital abnormalities (albinism, pseudohemophilia and unusual pigmented macrophages in the bone marrow) in two unrelated patients suggests that a common syndrome is present.

**SUMARIO IN INTERLINGUA**

1. Es reportate le casos de duo albinos non-consanguinee con diathese hemorrhagie e con peculiar cellulas reticular pigmentate in le medulla ossee.

2. Con respecto al hemostase, le sol regular constatatation laboratorial eseva un prolongate tempore de sanguination, de maniera que le disorde hemorrhagie se classava inter le si-appellate pseudohemophilias.

3. Inusual cellulas reticuloendothelic in le medulla ossee esseva plenate die granulos o particulas de color nigrastre o verde-blau. Secundo le studio histo-chimic, le substantia in le cytoplasma esseva probablemente de natura lipidic. Iste cellulas non poteva esser identificate con simile cellulas de iste genere usque nune describite.

4. Le combination del supra-describite anormalitates congenite (i.e., albinismo, pseudohemophilia, e macrophagos pigmentate inusual in le medlulla ossee) in duo patientes non-consanguinee suggere le presentia de un syndrome commun.

**REFERENCES**


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