Congenital Hemolytic Disease Associated with Red Cell Inclusion Bodies, Abnormal Pigment Metabolism and an Electrophoretic Hemoglobin Abnormality

By J. L. Scott, A. Haut, G. E. Cartwright and M. M. Wintrobe

Schmid et al. and Lange and Akeroyd have described cases of a hemolytic syndrome characterized by the presence of red cell inclusion bodies and the passage of urine containing an abnormal amount of a dark brown pigment considered to be a dipyrromethene, mesobilifuscin. Evidence of a severe hemolytic process appeared during the first three years of life in each of the three reported cases. In each instance the red cell and urinary abnormalities were first recognized after splenectomy had been performed. A familial type of the syndrome is suggested by its occurrence in a father and son reported by Schmidt et al. In these two cases there was probable elevation of the normal A2 hemoglobin component on starch electrophoresis in both and a possible increase in the fetal (alkali-resistant) component of the son’s hemoglobin. No hemoglobin abnormalities were found in the case reported by Lange and Akeroyd; subsequent studies have demonstrated abnormalities of red cell glutathione metabolism in this case.

The present report describes a fourth case of the syndrome of “congenital hemolytic anemia with abnormal pigment metabolism and red cell inclusion bodies” studied after splenectomy. On paper electrophoresis an abnormal hemoglobin component of reduced anodal mobility at pH 8.6 was found. This was shown on starch electrophoresis to migrate between the normal A1 and A2 fractions, appearing as a trail after the A1 fraction. The intracorpuscular defect was associated with an increased rate of erythrocyte autohemolysis in vitro. Family studies revealed no evidence of genetic transmission of the defect.

Methods

Routine hematologic studies and differential stains of the erythrocytes were done by standard methods. The Cr51-tagged red cells used in the survival studies were prepared by the addition of 50 ml of the patient’s blood to sterile ACD solution containing 10 μc.
of Cr.\textsuperscript{51}; the unwashed cells were injected following incubation at room temperature for 45 minutes and reduction of the excess chromate by the addition of ascorbic acid.

Rell cell hemolysates for electrophoretic and spectroscopic studies were prepared from thrice-washed cells by the addition of 1.5 volumes of distilled water and 0.5 volume of toluene, followed by manual shaking, storage overnight at 4 C. and aspiration of the clear layer following centrifugation.\textsuperscript{7} The resultant hemoglobin solutions were further cleared by centrifugation for one-half hour at 12,000 \times g. Alkali-resistant hemoglobin was determined by the method of Singer et al.\textsuperscript{7} The cleared hemolysates were subjected to electrophoresis, after oxygenation in room air or conversion to carbon-monoxo-, met- or cyanmethemoglobin derivatives, on paper by the use of conventional apparatus\textsuperscript{6} and on starch blocks by the technic of Kunkel et al.\textsuperscript{8} Spectroscopic examinations of hemolysates were done with a hand spectroscope and spectral curves determined with a Beckman DU spectrophotometer.

The erythrocyte osmotic fragility of fresh and incubated sterile blood was determined by the method of Emerson et al.\textsuperscript{9} Spontaneous autohemolysis of incubated sterile defibrinated blood was studied by the technic of Selwyn and Dacie\textsuperscript{10} and Young et al.\textsuperscript{11} Glucose-6-phosphate dehydrogenase activity of the red cells was assayed by the method of Marks\textsuperscript{12} and catalase activity by the method of Richardson et al.\textsuperscript{13}

**CASE HISTORY**

T. S., an eight year old Caucasian boy, was first seen in the Hematology Clinic of the Salt Lake County General Hospital on November 15, 1955, for evaluation of persistent leukocytosis of several months' duration. The patient was born at term following an uncomplicated pregnancy; he was first noted to be pale at the age of four months and was considered to be generally sickly as an infant. At the age of two years splenomegaly and anemia were found when the family physician was first consulted. Periodic transfusions were given until the age of 32 months, when a splenectomy was done. A spleen weighing 174 Gm. and an accessory spleen measuring 1.5 cm. in diameter were removed. Sections of the spleen revealed diffuse fibrosis with widely patent splenic sinusoids and an increase in splenic reticulum. The lymphoid follicles were decreased in number, and extensive hemosiderin deposits were present. Presplenectomy blood smears and data concerning preoperative blood values and transfusion requirements are not available.

Following the operation no further transfusions were required, and the child was generally healthy. Development was normal. His physician noted the intermittent occurrence of dark urine; the icterus index was normal or only slightly elevated.

In August, 1955, leukocytosis was first found when an acute episode of diarrhea, abdominal cramps and vomiting necessitated hospitalization near his home. On admission the leukocyte count was 28,000/cu. mm., with 47 per cent polymorphonuclear neutrophils and 53 per cent lymphocytes; the blood hemoglobin was 11.2 Gm./100 ml. Prompt recovery from the symptoms of gastroenteritis followed the administration of parenteral fluids, but another leukocyte count two months later showed a persistent leukocytosis of 22,000/cu.mm. Two weeks before his referral for evaluation he became ill with anorexia, sore throat, dizziness and fever (103 F.). The symptoms subsided following a single intramuscular injection of penicillin and the administration of a sulfonamide for one week.

The past history and system review were otherwise unremarkable. No medications, other than occasional aspirin and the single course of sulfonamide, had been used in recent years. Vitamin preparations (ABDEC and Vidaylin) were given to the patient during infancy and in early childhood but had been discontinued two years before his first visit to this clinic.

**Family History**

The parents are descendants of large families of English ancestry residing in a rural area of southern Utah; both are living and well and are unaware of the presence of any familial disease in their relatives. There is remote consanguinity in the family of the patient's mother: her parents descended from great-grandparents who were siblings. A maternal
uncle of the patient was said to have died in childhood of Hodgkin’s disease. Two male siblings are living and well; a third male sibling died of pneumonia at ten days of age.

**Physical Examination**

Physical examination revealed a slender but well developed boy who did not appear to be anemic. His weight was 22.3 kilograms and his height 126 cm. There was diffuse cutaneous skin pigmentation of a tawny color. Scleral icterus was absent. Several soft, discrete 0.5 cm. lymph nodes were found in the posterior cervical, axillary and inguinal regions bilaterally. A soft, thin liver edge was palpable 3 cm. below the right costal margin; a left subcostal surgical scar was present. The remainder of the examination was within normal limits.

**Laboratory Examinations**

Routine hematologic studies were as follows: volume of packed red cells, 47 ml./100 ml. of blood; hemoglobin, 13.7 Gm./100 ml.; RBC 4,350,000/cu.mm.; MCV, 108 cu.μ.; MCH, 32 μg.; MCHC, 29 per cent; reticulocytes, 11 to 15 per cent (see below); white blood count, 16,000/cu.mm., with 40 per cent polymorphonuclear neutrophils, 7 per cent eosinophils, 1 per cent basophils, 44 per cent lymphocytes and 8 per cent monocytes; platelets, 341,000/cu.mm.

Morphologic examination of stained blood smears revealed the presence of stippling, polychromatophilic macrocytosis, anisocytosis and intraerythrocytic inclusions (see below). Routine urine analysis was normal. The urine was noted later to have an amber to dark brown color from time to time. The stool was dark brown and guaiac negative. The two hour urinary excretion of urobilinogen (2.00–4:00 p.m.) was 0.4 to 0.8 Ehrlich units. Total serum bilirubin was 1.2 to 1.5 mg.100 ml. with 1 minute values of 0.2 to 0.3 mg. 100 ml. The plasma iron was 207 μg.100 ml. Direct and indirect serum antiglobulin tests were negative. Presumptive screening tests for warm and cold agglutinins and hemolysins at physiologic and acid pH were negative.

X-ray examinations of the chest and skull were normal. A normally functioning gallbladder was visualized after the oral administration of a radiopaque dye; neither radiolucent or radiopaque stones could be demonstrated.

**Subsequent Course**

Leukocytosis was observed to persist during the ensuing six months in the absence of symptomatic or physical evidence of illness. This finding was considered to be the result of active hemolysis and hematopoiesis in combination with the usual postsplenectomy leukocytosis. The volume of packed red cells has been maintained between 43 and 47 ml. per cent, and the patient’s health has been generally good, except for an episode of increased icterus and anemia which occurred in December 1957, and subsided spontaneously.

**Special Studies**

**Morphology**

Wright-stained smears of the patient’s blood showed faintly-stained, irregular inclusions in a few of the red cells, while many were stippled and contained basophilic material of variable size and shape (fig. 1A). In addition, there was the macrocytosis and polychromatophilia characteristic of increased erythropoiesis. Spherocytosis was not observed. Supravital staining with brilliant cresyl blue and methyl violet revealed the presence of basophilic inclusions which were more numerous than the faintly stained large inclusions present in the Wright-stained smears; these were found in about 90 per cent of the cells (fig. 1B). These inclusions usually occurred singly,
Fig. 1A (at left).— Blood smear, Wright's stain. Four cells are seen to contain fine densely basophilic material. In other cells there are larger, less dense, irregular basophilic bodies similar to those inclusions which are refractile under the phase microscope and stain supravitaly with aqueous stains (fig. 1B).

Fig. 1B.— Wet blood film stained supravitaly with aqueous methyl violet and allowed to dry. The larger inclusions are densely basophilic, variable in size and shape and usually occur singly.

were somewhat irregular in shape and were refractile under phase microscopy. A smaller percentage of the cells contained such inclusions after counterstaining the dried methyl violet preparations with methanolic Romanowsky stain. Differential counting of such preparations was not accurate due to the wide morphologic variation of the intraerythrocytic basophilic material. After supravital staining with brilliant cresyl blue and counterstaining with Wright's stain, 11 to 15 per cent of the cells contained basophilic material characteristic of reticulum. Siderotic granules were present in 18 to 25 per cent of the cells in smears stained for iron with the Prussian blue reaction.

Bone marrow smears made from material aspirated from the iliac crest were very cellular. There was erythroid hyperplasia, with a myeloid : erythroid ratio of 0.7:1. Erythroid maturation was morphologically normal. Inclusion bodies could not be identified in the nucleated erythrocytes in either methyl violet or Wright-stained preparations.

**Inclusion Bodies**

During the preparation of hemolysates of the patient's cells it was noted that dilute hemolysates appeared opalescent and that the volume of sediment was greater than that obtained from hemolysates of an equal volume of normal red cells. After mixture of the stromal sediment with aqueous methyl violet, large numbers of basophilic bodies similar to those in the supravitally stained intact erythrocytes were seen. This suggested that the inclusion.
CONGENITAL HEMOLYTIC DISEASE

were insoluble in water, as were those in the case of Lange and Akeroyd. These authors found an increase in the nucleic acid content of this stromal residue.3

To investigate this, the stromal residue was washed repeatedly by suspension in distilled water and centrifuged until hemoglobin was no longer detectable in the wash water. After drying with alcohol and ether, the residue was extracted with hot hypertonic salt solution to solubilize any nucleic acid present. The addition of ethanol to the chilled extract resulted in the precipitation of a small amount of material with the gross appearance of nucleic acid; after perchloric acid hydrolysis, this was shown by paper chromatography to contain adenine, guanine, cytosine and uracil.14 Thymine was not detected, and the isolated nucleic acid was thus ribose nucleic acid (RNA). It is possible that this finding is indicative only of the presence of reticulocytosis, since reticulocytes are believed to contain RNA3 and were present in significant numbers. Therefore, further investigation of the composition of the stromal residue would be necessary before it could be concluded that the inclusions themselves contain RNA.

Incubation of the patient's serum with normal compatible red cells for 24 hours at 37 C. failed to produce inclusions in the normal cells. After incubation of the patient's cells with phenylhydrazine, each cell was seen to contain many methyl violet-staining inclusions. These bodies were smaller and more numerous than the naturally occurring inclusions and were indistinguishable from those found in the similarly treated cells of normal donors and the patient's parents and siblings.

Red Cell Survival

The survival of the patient's Cr1-tagged cells after autologous transfusion and after transfusion into a normal compatible recipient with an intact spleen is depicted in figure 2. The survival of the autotransfused cells (curve B) was moderately shortened, with a half-life of about eight days (normal approximately 25 days). More rapid disappearance of the patient's tagged cells was observed in the normal recipient with an intact spleen (curve C), their half-life being only about two days. Studies of the recipient's blood for the presence of inclusion-containing erythrocytes were not made because of the small amount of blood transfused.

Hemoglobin Studies

Paper electrophoresis of the patient's hemoglobin (barbital buffer, pH 8.6, 0.05 M) repeatedly showed the presence of a trail of pigment following a major component of normal anodal mobility (fig. 3A). Prolonged electrophoresis (12 hours at 380 volts, 15 m.amp.) failed to resolve the trail into a separate component. When run simultaneously with known hemoglobins of reduced anodal mobility at alkaline pH, the trail was seen to extend backward toward the origin from the normal component through the migratory zone of fetal hemoglobin to that of sickle hemoglobin (fig. 3B). Normal
cathodal migration as a single homogeneous focus was observed on paper at acid pH (M/15 phosphate buffer, pH 6.5).

On starch block electrophoresis at pH 8.6 the trailing component migrated as a band between the A₁ and A₂ components (fig. 3C). A similar electrophoretic pattern was observed on starch gel. The diffuse trail was incompletely separable from the normal components. Quantitative elution of the different zones from the starch block showed that this band comprised about 10 per cent of the total pigment. The A₂ component was approximately normal in amount, i.e., about 2.5 per cent.

When eluates of the abnormal component and the patient's A₁ component were concentrated by lyophilization and resubmitted to starch electrophoresis, no change in the relative mobility of the two fractions was observed; there was no further trailing of the patient's isolated A₁ fraction, and the trailing fraction showed the same shape and appearance found in the whole hemolysate. Alkali denaturation of the concentrated trailing and A₂ hemoglobins showed that 34 per cent of the fraction was resistant to alkali. The hemolysate contained 2.3 per cent alkali-resistant hemoglobin. Thus, practically all of the alkali-resistant hemoglobin in the original hemolysate was found in the abnormal fraction. However, ultraviolet spectroscopy failed to show the characteristic "tryptophane notch" of fetal hemoglobin;¹⁵ in contrast, such a notch could be demonstrated in normal cord blood containing little more alkali-resistant hemoglobin (40 per cent) than did the concentrated trail. Spectral curves of the patient's whole hemolysate in the form of oxyhemoglobin, carbonmonoxyhemoglobin and cyanmethemoglobin were identical with normal control samples in the range between 500 and 700 mμ. Eluates of the abnormal
and A1 components of the patient's hemoglobin after separation by starch electrophoresis in the form of oxyhemoglobin, methemoglobin and cyanmethemoglobin also showed spectral absorption curves which were identical with similarly treated normal control samples.

Erythrocyte Metabolism

Repeated determinations of erythrocyte osmotic fragility in fresh and incubated defibrinated blood showed no consistent abnormality when compared with simultaneously studied normal control cells. However, a manyfold increase in the rate of spontaneous autohemolysis was regularly observed during sterile incubation of the patient's defibrinated blood at 37 C. for 24 and 48 hours. Partial correction of this phenomenon was observed after the addition of glucose, adenosine or inosine; under these conditions, the degree of hemolysis nevertheless remained greater than that of similarly treated normal control cells (table 1).

Since the subjects could not come to the laboratory for the study of glucose-6-phosphate dehydrogenase activity, samples from the family members and from two normal control subjects were collected in ACD solution, shipped by mail and the enzyme activity assayed 24 hours later. The results are summarized in table 1; in spite of the delay, the normal control values approximate those found by Marks2 using the same method. The elevation of activity found in the patient's red cells is similar to that found by this investigator to be associated with reticulocytosis, which was consistently present in our patient, and thus may have no other significance. Normal values were observed in the erythrocytes of his siblings and parents. Red cell glutathione and glutathione stability in the presence of acetylphenylhydrazine were not determined, due to our inability to obtain fresh cell samples. An earlier determination of the catalase activity of the patient's fresh red cells was within normal limits.

Pyrrol Excretion

In view of the abnormal excretion of dipyrrylmethene compounds reported in two similar cases of hemolytic anemia, quantitative determinations of the excretion of these compounds were made in the present case. These values were found to be about ten times the normal adult values in both the stool and urine (table 2). Fecal urobilinogen excretion was also increased, and a normal ratio of fecal urobilinogen to mesobilifuscin (8:14) was maintained.18 Reactions for other pyrrol compounds capable of causing a dark amber color of the urine, namely porphobilinogen, bilirubin, hemochromogen and pentapyrrol, were negative. Coproporphyrin excretion was within normal limits. The marked elevation in fecal urobilinogen and bilirubin excretion confirmed the greatly increased rate of hemoglobin catabolism. From the pyrrol excretion data it can be calculated that approximately 16.5 Gm. of hemoglobin were destroyed daily, resulting in the total daily excretion of 575 mg. of tetrapyrrrol compounds. This would indicate that the patient's red cells were destroyed at a rate approximating the maximum rate of marrow erythropoiesis in severe hemolytic processes.20
Fig. 3A, B, C.—See legend, facing page.
Table 1.—Studies of Erythrocyte Metabolism

<table>
<thead>
<tr>
<th></th>
<th>No substrate added</th>
<th>Glucose added</th>
<th>Adenosine added</th>
<th>Inosine added</th>
<th>Glucose-6-phosphate dehydrogenase activity Δ O.D., min./ml. RBC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.3</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>4.9 (two subjects)</td>
</tr>
<tr>
<td>Published mean normal</td>
<td>1.511</td>
<td>0.011</td>
<td>0.511</td>
<td>—</td>
<td>4.95 ± 0.6512</td>
</tr>
<tr>
<td>values, with range, or ± 1 S.D.</td>
<td>(0.0-5.5)</td>
<td>(0.0-0.5)</td>
<td>(0.0-1.0)</td>
<td>—</td>
<td>8.86 ± 1.2412</td>
</tr>
<tr>
<td>Patient</td>
<td>8.0†</td>
<td>1.5†</td>
<td>1.4</td>
<td>1.2</td>
<td>7.7†</td>
</tr>
<tr>
<td>Mother</td>
<td>1.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.7</td>
</tr>
<tr>
<td>Father</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.3</td>
</tr>
<tr>
<td>Sibling (K. S.)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.9</td>
</tr>
<tr>
<td>Sibling (D. S.)</td>
<td>1.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*Change in optical density at 340 mp min. ml. of packed RBC.
†Patients with reticulocytosis.
‡Two separate determinations.

Family Studies

Physical and hematologic studies of the patient's parents and siblings were within normal limits: splenomegaly, anemia, morphologic red cell abnormalities and elevations of the serum iron and serum bilirubin were absent. Hemoglobin electrophoresis at pH 8.6 on both paper and starch showed no abnormalities. Red cell glucose-6-phosphate dehydrogenase activity of the red corpuscles of the parents and both siblings and red cell autohemolysis studies of the patient's mother and one sibling were similarly normal. Pyrrol excretion studies of all of the family members could not be obtained but were within normal limits in the patient's mother.

Discussion

The present case is similar to those reported previously by Schmid et al. and Lange and Akeroyd with respect to the early age of onset, the occurrence of erythrocytic inclusions and an intracellular defect of red cell survival in the absence of exposure to agents associated with Heinz body anemia, and the increased urinary excretion of dipyrormethene pigments. The characteristics of these four cases are compared in Table 3. It is apparent that the present case is distinguished primarily by the finding of an abnormal minor hemoglobin component. This component appears to be heterogeneous in composition.
Table 2.—Daily Excretion of Pyrrol Compounds

<table>
<thead>
<tr>
<th></th>
<th>Mesobilifuscin (mg.)</th>
<th>Urobilinogen (mg.)</th>
<th>Bilirubin (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Patient 46-63</td>
<td>0.4-3.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Normal adult 0-4.016</td>
<td>0-3.55</td>
<td>0</td>
</tr>
<tr>
<td>Stool</td>
<td>Patient (4 day mean)</td>
<td>98</td>
<td>518</td>
</tr>
<tr>
<td></td>
<td>Normal adult 7-1818</td>
<td>140 (mean)19</td>
<td>5-2019</td>
</tr>
</tbody>
</table>

The abnormal fraction is dissimilar to the well characterized major abnormal hemoglobins migrating in the same area at pH 8.6. Its trailing characteristic at this pH bears some resemblance to "Alexandra" hemoglobin, which is probably a variant of fetal hemoglobin, and to hemoglobin 0, or "Buginese X". However, the trailing component in the present case differs from these other minor fractions in its association with severe hemolytic disease and from the "Alexandra" hemoglobin in its heterogeneity with respect to alkali denaturation. With further characterization, other distinctions may become evident.

The intraerythrocytic inclusion in the present case closely resemble those described in detail by Lange and Akeroyd, who have cited subtle differences between these inclusions and the Heinz-Ehrlich bodies which occur with exposure to various chemical compounds. The RNA content of the insoluble inclusions, if not due to the presence of contaminating reticular material, may be another distinguishing characteristic. However, this point requires further investigation. Previous analyses of Heinz body composition have been somewhat inconclusive. The inclusions are generally thought to contain denatured globin, in addition to other compounds. Specific studies of nucleic acid content have not yet been made, to our knowledge.

The hemolysis and Heinz body formation which occurs when certain susceptible individuals are exposed to drugs or chemicals is under active investigation in several laboratories. Since Heinz body formation is sometimes associated with the presence of a degradation product of hemoglobin, variously called verdoglobin, verdochromogen or choleglobin, the characteristic absorption peak of this material was sought in both the whole hemolysate and the isolated trailing fraction of our patient's hemoglobin but could not be demonstrated. Of similar interest in the Heinz body anemias is the erythrocyte level of glucose-6-phosphate dehydrogenase activity, which is decreased in the majority of cases of drug-sensitive hemolytic anemias. A decreased level of enzyme was not demonstrable in the cells of our patient, and it is therefore unlikely that the hemolysis was due to drug sensitivity. However, glutathione
Table 3.—Summarized Clinical and Laboratory Findings of Reported Cases of Congenital Hemolytic Disease with Red Cell Inclusions and Mesobilifuscinuria

<table>
<thead>
<tr>
<th></th>
<th>Lange and Akeroyd</th>
<th>Schmid et al.</th>
<th>Father</th>
<th>Son</th>
<th>Present case</th>
</tr>
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<tr>
<td><strong>Age</strong></td>
<td>14</td>
<td>33</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td><strong>Age at onset of anemia</strong></td>
<td>30 mo.</td>
<td>birth</td>
<td>20 mo.</td>
<td>4 mo.</td>
<td></td>
</tr>
<tr>
<td><strong>Post-splenectomy improvement</strong></td>
<td>&quot;excellent&quot;</td>
<td>slight but transient</td>
<td>none</td>
<td>compensation of the hemolytic disease</td>
<td></td>
</tr>
<tr>
<td><strong>Family history</strong></td>
<td>negative*</td>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>English</td>
</tr>
<tr>
<td><strong>National origin</strong></td>
<td>Italian, German, Irish</td>
<td>Italian, German, Irish</td>
<td>Italian, German, Irish</td>
<td>English</td>
<td>English</td>
</tr>
<tr>
<td><strong>Hgb. Gm./100 ml.</strong></td>
<td>8.5-10.0</td>
<td>8.2-8.6</td>
<td>8.4-9.7</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td><strong>RBC x 10^6/cu.mm.</strong></td>
<td>3.0</td>
<td>3.0</td>
<td>2.6-3.1</td>
<td>4.35</td>
<td></td>
</tr>
<tr>
<td><strong>Volume of packed red cells (ml./100 ml.)</strong></td>
<td>31</td>
<td>31-36</td>
<td>31-32</td>
<td>43-47</td>
<td></td>
</tr>
<tr>
<td><strong>Reticulocytes (%)</strong></td>
<td>19.5</td>
<td>35-42</td>
<td>16-27</td>
<td>11-15</td>
<td></td>
</tr>
<tr>
<td><strong>Normoblasts/100 WBC</strong></td>
<td>3</td>
<td>15-130</td>
<td>8-35</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>RBC inclusion (%)</strong></td>
<td>14</td>
<td>37-38</td>
<td>29</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td><strong>Siderocytes (%)</strong></td>
<td>—</td>
<td>58</td>
<td>—</td>
<td>18-26</td>
<td></td>
</tr>
<tr>
<td><strong>Serum direct bilirubin (mg.%)</strong></td>
<td>1.3</td>
<td>10.7-11.2</td>
<td>2.3-3.2</td>
<td>1.2-1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Fecal urobilinogen (E.U./day)</strong></td>
<td>—</td>
<td>1450</td>
<td>609</td>
<td>518</td>
<td></td>
</tr>
<tr>
<td><strong>Cr^1 RBC survival (T/2, days)</strong></td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Urine mesobilifuscin</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>98 mg./24 hr.</td>
<td></td>
</tr>
<tr>
<td><strong>Fecal mesobilifuscin</strong></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>46-63 mg./24 hr.</td>
<td></td>
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<tr>
<td><strong>Fecal bilirubin</strong></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>57 mg./24 hr.</td>
<td></td>
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<tr>
<td><strong>Hgb. electrophoresis</strong></td>
<td>A</td>
<td>A; probably elevated A;</td>
<td>A; probably elevated A;</td>
<td>A plus trait;</td>
<td></td>
</tr>
<tr>
<td><strong>Alkali-resistant Hgb.</strong></td>
<td>2.8-3.4%</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td>2.3-3.2%</td>
</tr>
<tr>
<td><strong>Hgb. spectroscopy</strong></td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td><strong>RBC osmotic fragility (% saline beginning—complete)</strong></td>
<td>0.56-0.10</td>
<td>0.9-0.15</td>
<td>0.65-0.20</td>
<td>0.52-0.30</td>
<td></td>
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<tr>
<td><strong>RBC catalase</strong></td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td></td>
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<tr>
<td><strong>G-6-P dehydrogenase</strong></td>
<td>increased</td>
<td>normal</td>
<td>normal</td>
<td>increased</td>
<td></td>
</tr>
<tr>
<td><strong>RBC glutathione</strong></td>
<td>unstable</td>
<td>—</td>
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</tbody>
</table>

*Increased glucose-6-phosphate dehydrogenase and decreased amount and stability of reduced glutathione have been found in the erythrocytes of this patient's father and may indicate the presence of a genetically transmitted defect.*

instability, which is characteristic of the drug-sensitive erythrocytes; has recently been observed in the erythrocytes of both the patient reported by Lange and Akeroyd and her father. This finding suggests that a more thorough study of red cell glutathione metabolism in this syndrome is needed. The pathologic significance of the dipyrrol pigment excretion, which has been in the same general range in all of the studied cases of this syndrome, is not yet understood. Schmid concluded, with substantial basis, that the urinary pigments were products of erythrocyte catabolism. The studies of Gilbertson and co-workers, on the other hand, are more consistent with the theory that these pigments are related to heme synthesis. The increased pigment is considered by these workers to be another index of an increased

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rate of erythropoietic activity.\textsuperscript{18,19} This facet of the syndrome also requires further investigation.

The pathogenesis of the hemolytic process in the present case could not be clarified. However, a likely hypothesis would be that the patient’s cells contain an unstable hemoglobin, analogous to hemoglobin H, which somehow results in shortening of erythrocyte survival.\textsuperscript{29} Genetic transmission of such a defect could not be demonstrated, but the possibility remains that the condition may arise somehow from interaction of parental genes which in themselves are unassociated with detectable hematologic abnormality.

SUMMARY

1. A fourth case of the syndrome of congenital hemolytic anemia with abnormal pigment metabolism and red cell inclusion bodies following splenectomy is described.
2. In this case an abnormality was found on paper and starch electrophoresis of the red cell hemolysate at pH 8.6. A similar abnormality has not been reported previously.
3. An increased rate of erythrocyte autohemolysis was found during in vitro incubation. Partial correction of this defect occurred in the presence of glucose or purine ribosides.
4. Genetic transmission of the defect could not be demonstrated.

SUMMARIO IN INTERLINGUA

1. Es describite un quarte caso del syndrome de congenite anemia hemolytic con anormal metabolismo de pigmento e corpores de inclusion in le erythrocytos post splenectomia.
2. In iste caso, un anormalitate del hemolysato erythrocytic esseva constatate a pH 8,6 in studios electrophoretic a papiro e a amylo. Un simile anormalitate ha non previemente essite reportate.
3. In incubation in vitro, un accelerate autohemolyse erythrocytic esseva constatate. Un correction partial de iste defecto esseva notate in le presentia de glucosa o de ribosidos de purina.
4. Le transmission genetic del defecto non poteva esser demonstrate.

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REFERENCES


PRESENCE OF K AND J HEMOGLOBIN IN THE POPULATION OF SOUTHERN ITALY.

Out of 2186 subjects from Southern Italy and the Italian Islands, a carrier of K hemoglobin and a carrier of J hemoglobin were found. The first case was a man with pernicious anemia, in which the hemoglobin patterns did not vary during remissions or relapses. The hereditary pattern of the alteration was demonstrated, having been detected in a normal son. Slightly increased values of A1 hemoglobin were found in the carrier of K hemoglobin as well as in some normal members of his family. Spectrophotometric and alkali denaturation in studies of K and J hemoglobin showed properties similar to those of A1 hemoglobin.—P. d. N.


A detailed case report of a patient with sickle cell–Hgb. D disease in a young white male. Since so few cases of this syndrome have been reported, the additional clinical information is indeed welcome. The authors point out that all seven cases so far documented in the literature have been in Caucasians but suggest that cases occurring among the Negro are more likely to be accepted as homozygous Hgb. S disease. The diagnosis in this instance is supported by an extensive family study and agar electrophoresis determinations.—A. I. C.


A very full review, with nearly 500 references consisting mainly of an appraisal of the various criteria for heterogeneity of hemoglobins. Nearly half of the first part is devoted to a critical consideration of the factors influencing the behavior of hemoglobin, with particular emphasis on hemoglobin binding and denaturation of the hemoglobin molecule. The limitations of the various methods of identification of hemoglobins are stressed, and a warning given against the too ready acceptance of “new” variants. Clinical, genetic and ethnologic aspects of the subject are not considered.

—R. M. H.
Congenital Hemolytic Disease Associated with Red Cell Inclusion Bodies, Abnormal Pigment Metabolism and an Electrophoretic Hemoglobin Abnormality

J. L. SCOTT, A. HAUT, G. E. CARTWRIGHT and M. M. WINTROBE

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