Hereditary Nonspherocytic Hemolytic Disease

A Study of a Singular Familial Hemolytic Syndrome

By Arno G. Motulsky, William H. Crosby, and Henry Rapaport

Hereditary hemolytic disease distinct from other genetically determined hemolytic anemias has been described in several families in recent years. In contrast to the striking peripheral blood picture of sickle-cell anemia, Mediterranean anemia, hereditary spherocytosis, and hereditary elliptocytosis, no consistent morphologic abnormality of the red cells has been recognized in these atypical cases. Osmotic and mechanical fragility tests of the red cells are normal. Erythrocyte survival time studies show clearly that the disease is due to an intrinsic defect of the red cells which cause their premature disintegration. The disease usually appears to be transmitted as a Mendelian dominant. The specific nature of the red cell anomaly has remained unknown. Indeed, it seems likely that the category may include a number of different anomalies.

The recognition of hereditary nonspherocytic hemolytic disease is clinically important since splenectomy is of little or no benefit. Because of jaundice, the condition may be confused with chronic infectious hepatitis and cause prolonged hospitalization and invalidism. When the anemia is mild or absent the disease may be mistaken for the so-called benign retention jaundice or constitutional hyperbilirubinemia. In one of the cases presented below the full-blown syndrome of hemolytic anemia was present at birth and led to an erroneous diagnosis of erythroblastosis fetalis.

During the past year we have studied four patients with hereditary nonspherocytic hemolytic disease. In addition to the usual clinical and hematologic examinations, we were able to perform a number of special investigations. These dealt with iron metabolism, osmotic and mechanical fragility after incubation, abnormal hemoglobins, red cell survival, and histologic features of spleens and liver biopsy specimens.

Materials and Methods

Family Studies

The propositus in each family group was a patient at Walter Reed Army Hospital. Some relatives who visited the patients were examined, but it was not possible to examine di-
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rectly those who lived elsewhere. An attempt was made to screen the immediate relatives of each propositus by way of a "mail-order reticulocyte count" as the simplest test for the presence of the hemolytic disease under investigation. Capillary pipets were filled with reticulocyte staining fluid8 and sealed at both ends. These pipets were sent to physicians or laboratories near the family's home. A drop of blood was obtained from the person to be examined, mixed with the reticulocyte stain and left to stand for 10 minutes. Films were prepared and sent to our laboratory to be counted.

Red Cell Survival Time

Red cell survival time studies were performed by the method of differential agglutination (Ashby). A modification of the method described by Mollison9 was used. Whole blood was transfused within a few days after venesection. Hundred per cent post-transfusion values refer to the highest unagglutinable count which was obtained twenty-four to forty-eight hours after transfusion.

Red cell survival in case four was estimated by tagging the patient's red cells with radioactive chromium.10 Two hundred μc. of chromium-51 (1.165 mc./mg., 0.79 mg./ml.) were incubated at room temperature for 60 minutes with 40 ml. of whole heparinized blood; 50 to 60 per cent uptake of chromium can be expected under these conditions. After incubation the plasma was removed and the cells were carefully washed three times with isotonic saline. The radioactivity of the red cells 20 minutes after injection was considered to be the 100 per cent reference value for the survival time study. Serial blood specimens were collected during the next twenty-one days and red cell radioactivity determined with a scintillation counter. Actual values obtained were corrected for radioactive decay and for elution of chromium-51 from red cells. Chromium elutes from red cells with a half-life of 77 days.13

Iron Metabolism

Serum iron was determined by the method of Hamilton, Gubler, Cartwright, and Wintroub.14 Plasma iron disappearance was determined by injecting a tracer dose of iron-59 intravenously and sampling the patient's blood during the ensuing three to five hours.15 Iron-59-chloride in sodium citrate was given in case 3, and iron-59 bound to pure iron-binding globulin (provided by Cutter and Company) in case 4. Biologic half-life of the injected iron was determined using the method described by Huff, et al.16 Plasma iron turnover was calculated by the following formula15:

\[
\frac{0.673 \times 24 \text{ (hrs. per day)} \times \text{gamma of Fe/1 ml. of plasma}}{\text{plasma volume}} \times \text{biol. \ life Fe 59 (hrs.)} \times 1000 \text{(gamma of Fe/mg.)}
\]

Siderocyte stains were performed as previously described.17

Fragility Tests

A. Osmotic fragility tests were done by the method of Dacie.18 Twenty cu. mm. of whole blood were placed in 10 ml. of the various saline dilutions. The supernatant hemoglobin from hemolyzed red cells was determined colorimetrically. Osmotic fragility tests were also performed on sterile defibrinated blood after incubation for 24 hours at 37 C.

B. Mechanical fragility tests were performed using a rotating device fashioned after Shen, Castle, and Fleming.19 Defibrinated blood, 0.5 ml. in a 50 ml. Erlenmeyer flask with ten glass beads, was rotated on an 8 inch radius for 90 minutes at 30 rpm. The amount of hemoglobin liberated was compared with unrotated controls. Results were expressed in per cent of cells hemolyzed. Mechanical fragility tests were also performed on sterile defibrinated blood after 24 hours' incubation.

C. Lysis of erythrocytes during incubation was determined by incubating 2 ml. of blood under oil for 24 hours at 37 C. These specimens were initially collected in potassium oxalate, (0.1 ml. of a 2 per cent solution) heparin, (0.1 ml. of a 1 per cent solution) sodium citrate (0.1 ml. of a 4 per cent solution) and without anticoagulant (serum). Supernatant
hemoglobin was determined colorimetrically after incubation using a quantitative benzidine method.¹⁸

**Pigment Studies**

Plasma hemoglobin was determined by a quantitative method.¹⁸ Fecal urobilinogen was determined on daily specimens using Watson’s method.¹⁹ Feces were collected for four days in cases 1 and 2. Many more specimens (described in Results) were examined in the work-up of cases 3 and 4.

**Abnormal Hemoglobins**

Fetal hemoglobin was sought as an alkaline-resistant component.²⁰ Electrophoretic examinations of hemoglobin solutions were performed by paper electrophoresis. The method was worked out in this laboratory using the Durrum electrophoresis cell.²¹ One hundredth ml. of a 3 per cent solution of oxyhemoglobin solution was streaked at the apex of filter paper strips (Whatman No. 3MM) which were suspended in veronal buffer (pH 8.6, ionic strength .05). A current of 0.8 to 2 milliamperes per paper strip at 260 to 280 volts was delivered for 4 hours from a power supply unit of regulated voltage. This technic allowed clear-cut quantitative separations of sickle (S), normal (A), and C hemoglobins.

**Case Reports**

**Case 1**

A 2 day old white, male infant was transferred to Walter Reed Army Hospital with the diagnosis of erythroblastosis fetalis. At delivery it had been noted that amniotic fluid and cord were greemish. The child was pale (Hb 9 Gm./100 ml.) and icteric (bilirubin 9.8 mg./100 ml.). Liver and spleen were grossly enlarged. No ABO or Rh incompatibility between mother and infant could be demonstrated. The blood type of both was A Rh positive (CDe). Nevertheless it was felt by the Pediatric Service that the infant was suffering from erythroblastosis due to an undefined blood-group incompatibility. An exchange transfusion of group O Rh-negative blood was administered shortly after admission with good immediate results. When the infant was re-examined at the age of 3 months, a moderately severe hemolytic process was found still to be present. The hematologic status of the child was closely followed during subsequent months (table 1). When no improvement was apparent at the age of 10 months, splenectomy was performed. The child recovered from the operation without difficulty, but the intensity of the hemolytic process did not decrease during 18 months following the operation.

**Case 2**

A 23 year old woman of English descent, the mother of the infant (case 1), had always considered herself a healthy person. Except for a severe attack of jaundice at the age of 7 years there had been no significant illness. Physical examination was negative except for mild splenomegaly (2 cm. below costal margin). Hematologic data are summarized in table 1.

**Case 3**

A 23 year old soldier of English-Pennsylvania Dutch descent was transferred to Walter Reed Army Hospital for investigation of jaundice and splenomegaly. The patient had been told as a child that his spleen was enlarged. Since the age of 16 years the patient had suffered from mild bouts of jaundice associated with passage of dark urine. These episodes lasted several days and occurred every three to four months. At the age of 18 years the patient had been hospitalized for a severe attack of jaundice associated with malaise, anorexia, vomiting, and dark urine. During the year prior to admission the patient had

* A detailed case report of this patient will be published separately.
vague upper abdominal pains not related to meals but relieved by amphojel. Mild bronchial asthma had been present for several years. He had never received a transfusion.

Physical examination revealed mild scleral icterus. The spleen was palpated 7 cm. below the costal margin. The liver was 3 cm. below the costal margin. Significant hematologic data are summarized in table 1. Urinalysis was negative. Blood urea nitrogen, blood glucose, serum proteins, and liver function tests, including BSP excretion, were normal. X-ray studies of the upper gastrointestinal tract showed spasm and some deformity of the duodenal bulb. Gall bladder films and skeletal survey were negative. Esophagoscopy on two occasions revealed "minimal submucosal veins in the lower third of the esophagus."

In view of normal portal pressure (135 mm. of saline as determined at laparotomy in both splenic and omental veins), the significance of this finding is obscure. PPD, histoplasmin, and coccidioidin tests were negative. Red cell volume by the chromium-51 technic was 28.2 ml. of red cells per Kg. of body weight. The normal value is 29 to 31 ml. per Kg.

Splenectomy was performed after all studies were complete. Apart from atelectasis of the lower lobe of the left lung, the patient made an uneventful recovery. Temporary reticulocytosis to 40 per cent developed after splenectomy. Fecal urobilinogen decreased somewhat postoperatively but in comparison with normal values remained greatly elevated (see Results). The blood picture otherwise was essentially unchanged (table 1).

**Case 4**

A 23 year old white soldier of American ancestry was transferred to Walter Reed Army Hospital because of jaundice and anemia. The patient stated that he probably had been slightly jaundiced most of his life. During the months prior to admission, weakness and mild right upper quadrant pain developed and he became nauseated and more severely jaundiced. His urine never changed in color. He was admitted to a field hospital in Germany where liver function studies, including BSP excretion, were negative. It was discovered that the patient was anemic. A diagnosis of hemolytic anemia was made and he was transferred to the United States.

### Table 1. Hematologic Data of Patients with Hereditary Nonspherocytic Hemolytic Disease

<table>
<thead>
<tr>
<th></th>
<th>Case 1 before splenectomy</th>
<th>Case 1 11 yrs. after splenectomy</th>
<th>Case 2 before splenectomy</th>
<th>Case 2 24 mos after splenectomy</th>
<th>Case 4 before splenectomy</th>
<th>Case 4 24 mos after splenectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10⁶)</td>
<td>2.6</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Hb (Gm./100 ml.)</td>
<td>8.6</td>
<td>8.2</td>
<td>11.2</td>
<td>12.4</td>
<td>13.5</td>
<td>11.7</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>—</td>
<td>34</td>
<td>33</td>
<td>36</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>MCH</td>
<td>33</td>
<td>27</td>
<td>28</td>
<td>31</td>
<td>34</td>
<td>31</td>
</tr>
<tr>
<td>MCV</td>
<td>110</td>
<td>84</td>
<td>90</td>
<td>94</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>24</td>
<td>34</td>
<td>34</td>
<td>35</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>8450</td>
<td>9050</td>
<td>4150</td>
<td>8350</td>
<td>20,600</td>
<td>4800</td>
</tr>
<tr>
<td>Platelets</td>
<td>Adequate</td>
<td>750,000</td>
<td>315,000</td>
<td>352,000</td>
<td>1,100,000</td>
<td>160,000</td>
</tr>
<tr>
<td>Retics</td>
<td>18%</td>
<td>17%</td>
<td>6.5%</td>
<td>9.5%</td>
<td>5%</td>
<td>15%</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>—</td>
<td>Normo-blastic hyperplasia</td>
<td>—</td>
<td>Normo-blastic hyperplasia</td>
<td>—</td>
<td>Normo-blastic hyperplasia</td>
</tr>
<tr>
<td>Bilirubin, total</td>
<td>0.9</td>
<td>1.8</td>
<td>2.1</td>
<td>—</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>direct</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
<td>—</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Fecal urobilinogen per 24 hrs (mg.)</td>
<td>42.2</td>
<td>200.3</td>
<td>1290</td>
<td>736</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td>Hemolytic index (normal 10-20)</td>
<td>100</td>
<td>—</td>
<td>39</td>
<td>215</td>
<td>120</td>
<td>86</td>
</tr>
</tbody>
</table>
Physical examination revealed icterus of sclerae and skin. The spleen was palpable 5 cm. below the costal margin. All other physical findings were negative. A radiologic survey of bones and gall bladder was negative. Plasma proteins and cholesterol and the liver function tests continued to be normal. Hematologic findings are summarized in table 1. The patient refused splenectomy and was discharged from the service.

Results

Family Studies

Cases 1 and 2. A 5 year old sibling and the father of the infant (case 1) were studied in our laboratory and found to be entirely normal. In case 2, the parents of the patient, as well as three of her siblings and four nephews and nieces were examined by the reticulocyte screening test described above. No other cases of hemolytic disease were detected (fig. 1).

Fig. 1.—Family tree of cases 1 and 2. No further cases of hereditary nonspherocytic hemolytic disease were discovered. Black indicates hereditary nonspherocytic hemolytic disease.

Case 3. No direct family study could be performed since the family refused to cooperate. A sister was known to have an enlarged spleen and episodes of jaundice. The mother was said to be mildly anemic.

Case 4. The patient's mother was studied in our laboratory and was found to be normal (including electrophoretic studies of hemoglobin). The patient's father, two sisters, a paternal uncle, and both paternal and maternal grandmothers were studied by the reticulocyte screening method. One sister (17 years old) was found to have 15 per cent reticulocytes and some anisocytosis. This sister had previously been known to be anemic with bouts of jaundice (fig. 2).

Blood Morphology

Rouleaux formation and cell thickness were studied on fresh blood specimens under cover slips. Normal rouleaux were seen in all four cases of hereditary nonspherocytic hemolytic disease. This was in contrast to hereditary spheroctosis where the thick spherocytes form irregular and uneven rouleaux (fig. 3). It has been our experience that this simple test is one of the best means of differentiating hereditary nonspherocytic hemolytic disease from hereditary
Fig. 2.—Family tree of case 4. A sister of the patient suffers from a similar disease. All other family members indicated (except those dead) were studied and found to be normal. Black indicates hereditary nonspherocytic hemolytic disease.

Fig. 3.—Fresh whole blood under coverslips to show the shape of rouleaux. A. Normal. B. Hereditary nonspherocytic hemolytic disease. C. Hereditary spherocytosis. Note that the long rouleaux in C are deformed.
spherocytosis. There was no stippling, sickling, fragmentation, nor any other significant morphologic abnormality of the red cells. Mild anisocytosis and poikilocytosis were noticed in all patients. In case 1 and case 3 a few target cells appeared after splenectomy. Some oval cells but no cigar-shaped cells were seen in case 4. This was interpreted as symptomatic ovalocytosis since neither parent had oval or elliptic cells. A few nucleated red cells were seen in case 1 and case 3 after splenectomy.

Red Cell Survival Time Studies

Figures 4 and 5 show the results of the red cell survival time studies of cases 3 and 4. Normal cells transfused into these patients had a normal life span. When

![Diagram of red cell survival time studies](image)

**Fig. 4.—Red cell life span determinations in case 3.** (For explanation of the curves see the text.)
the cells from patient 3 were transfused into a normal recipient, the mean life span was thirteen days. Cells from patient 4 were tagged with chromium-51 and injected into the patient himself. Their mean life span proved to be seventeen days. These red cells were washed with saline before injection. Had they not been washed the life span might have been slightly longer.

Since there is good evidence that the life span of red cells in cases of hereditary hemolytic anemia may vary within a given red cell population, a "pseudoexponential" (concave) rather than linear (straight) survival time is obtained. Some cells are destroyed rapidly while other more hardy erythrocytes survive much longer. The device of extending the most rapidly falling slope of the survival
time curve to the horizontal time axis arrives at a value for mean red cell life span.\textsuperscript{9, 10} A simple arithmetic explanation of mean red cell life span is given in Mollison's book.\textsuperscript{9} More rigidly, mean red cell life span (L) is defined by the equation \( L = \frac{N}{d} \) where \( N \) is the number of red cells in the circulation and \( d \) is their rate of destruction per day. Our results with cross-determination of erythrocyte survival time are characteristic of those found in all hemolytic anemias associated with intrinsic defects of the red cell\textsuperscript{23-26} and rule out an extracorporeal hemolytic mechanism as a cause of the anemia.

\textit{Fragility Studies}

\textit{Osmotic fragility.} In some exceptional cases of hereditary spherocytosis the blood may exhibit normal osmotic fragility.\textsuperscript{27} After such spherocytic cells are incubated at 37 C. for 24 hours, a marked increase in osmotic fragility can be demonstrated.\textsuperscript{28, 29} This increase exceeds considerably the slightly augmented osmotic fragility of normal cells after incubation. Figure 6 demonstrates the osmotic fragility findings in our patients compared with those in normal controls and in a mild case of hereditary spherocytosis. Osmotic fragility in all patients was

\begin{figure}[h]
\centering
\includegraphics[width=\columnwidth]{osmotic_fragility.png}
\caption{Osmotic fragility tests before (1) and after (2) incubation.}
\end{figure}
HEREDITARY NONSPHEROCYTIC HEMOLYTIC DISEASE

Table 2.—Mechanical Fragility of Red Cells Before and After Incubation for 24 Hours at 37 C.

<table>
<thead>
<tr>
<th></th>
<th>Before incubation</th>
<th>After incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>2.4%</td>
<td>23.3%*</td>
</tr>
<tr>
<td>Case 2</td>
<td>3.0%</td>
<td>14.2%</td>
</tr>
<tr>
<td>Case 3</td>
<td>3.4%†</td>
<td>17.5%†</td>
</tr>
<tr>
<td>Case 4</td>
<td>2.2%</td>
<td>18.9%</td>
</tr>
<tr>
<td>Normal</td>
<td>2-6.5%; mean 4.1% (19 det.)</td>
<td>6.3-15.6%; mean 11.5% (17 det.)</td>
</tr>
<tr>
<td>Hered. spherocytosis (3 cases)</td>
<td>11.6%; 18%; 22.6%</td>
<td>33%; 33%; 35%</td>
</tr>
</tbody>
</table>

* After splenectomy.
† After splenectomy. Presplenectomy values essentially similar.

within normal limits or diminished when tested after splenectomy (especially case 3). Questionably increased fragility of the blood before incubation should be noted in case 2. On incubation, a mild increase of osmotic fragility over normal values was observed in cases 1, 2 and 4. This procedure, however, failed to bring out the striking increase of osmotic fragility of incubated red cells which is found in hereditary spherocytosis and which is usually much greater than that shown in the mild case of hereditary spherocytosis in figure 6.

In 0.65 per cent sodium chloride solution, less than 15 per cent hemolysis was obtained with all bloods of cases of hereditary nonspherocytic hemolytic disease after incubation for 24 hours. More than 18 per cent (usually more than 50 per cent) hemolysis at 0.65 per cent saline after incubation has been observed in all our cases of hereditary spherocytosis and in those studied by others. For practical purposes a single screening fragility test after incubation at 0.65 per cent saline appears sufficient. Failure to show hemolysis of more than 18 per cent at this concentration of saline helps to rule out hereditary spherocytosis in most instances. It should be noted, however, that in two of four cases of hereditary nonspherocytic hemolytic disease studied by Dacie, et al.7 the increase of hemolysis on incubated osmotic fragility fell within the range seen in hereditary spherocytosis.

Mechanical fragility. Mechanical fragility is increased in hereditary spherocytosis29 and some other hemolytic anemias.30 With spherocytosis and sickleemia it is greatly increased after incubation.29 Table 2 summarizes the mechanical fragility findings in our cases. It will be noted that mechanical fragility was normal before incubation in all four cases. Mechanical fragility after incubation was normal in case 2, very mildly increased in cases 3 and 4, and moderately increased in case 1. None showed the marked increase of incubation mechanical fragility seen in hereditary spherocytosis. Mechanical fragility tests before and after incubation appeared to be a good means of differential diagnosis between these two diseases.

Lysis of Erythrocytes During Incubation (Autohemolysis)29, 30

The differential incubation test with serum and heparinized, citrated, and oxalated plasma (see Methods) has proved a valuable tool in the study of hemolytic anemias. Extreme degrees of hemolysis are seen in oxalated blood from patients
with spherocytosis. The phenomenon is practically limited to this abnormality. Less hemolysis has been seen with the blood of patients with hemolytic anemia without spherocytosis. Table 3 summarizes the differential incubation findings of our patients as compared with normal subjects and those with hereditary spherocytosis. On the basis of this test, the condition in case 4 could not be distinguished from hereditary spherocytosis. The results of the tests in case 3 are only slightly above normal and returned to normal after splenectomy.

For practical purposes, an incubation test with oxalated blood is sufficient. Absence of intense hemolysis in such a blood specimen incubated for 24 hours rules out hereditary spherocytosis, since marked hemolysis on incubation has been observed in all patients with this disease (except one small child), tested so far by us and others. The test is not specific for hereditary spherocytosis. It is also positive in spherocytosis associated with acquired hemolytic anemia. A similar test with defibrinated blood left for 24 hours at 37 C revealed increased hemolysis in two out of five of Dacie's cases of hereditary non-spherocytic hemolytic disease.

Iron Metabolism

Iron-59 tracer studies. The turnover of plasma iron was studied in cases 3 and 4 (fig. 7). The concentration of plasma iron in patient 3 was 143 μg. per 100 ml. The biologic half life of an intravenous tracer dose of iron-59 was approximately twelve minutes. (Normal is eighty-five to one hundred minutes.) On the basis of the clearance rate the plasma iron turnover was computed to be 5 mg. per Kg. of body weight per day. This value is about 10 times the normal rate and may be artificially high: the biologic half-life curve was extrapolated from points derived from samples of low specific activity. The concentration of plasma iron in patient 4 was 108 mg. per 100 ml. The biologic half life of intravenous iron-59 tracer was forty-two minutes. Appropriate calculations revealed plasma iron turnover of 1.1 μg. per Kg. of body weight per day. This value agrees moderately well with iron turnover calculated from hemoglobin mass and red cell life span determinations.

Table 3.—Differential Incubation Test with Various Anticoagulants

<table>
<thead>
<tr>
<th>Normal (mg.)</th>
<th>Case 1</th>
<th>Case 2 (mg.)</th>
<th>Case 3 before</th>
<th>Case 3 after</th>
<th>Hered. spherocytosis (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalate</td>
<td>&lt;20</td>
<td>12</td>
<td>76</td>
<td>43</td>
<td>275</td>
</tr>
<tr>
<td>Citrate</td>
<td>&lt;10</td>
<td>3.3</td>
<td>24</td>
<td>2.9</td>
<td>13.5</td>
</tr>
<tr>
<td>Heparin</td>
<td>&lt;10</td>
<td>5.1</td>
<td>32</td>
<td>5.7</td>
<td>57</td>
</tr>
<tr>
<td>Serum</td>
<td>&lt;10</td>
<td>3.8</td>
<td>58</td>
<td>12.5</td>
<td>53</td>
</tr>
</tbody>
</table>

Hemoglobin 11.7 Gm. per 100 ml. Hemoglobin mass = 0.117 × 5740 (blood volume) = 640 Gm. Hemoglobin destroyed per day = total hemoglobin mass / mean life span = 640 / 17 = 37.6 Gm. of hemoglobin. 1 Gm. of hemoglobin contain 3.33 mg. of iron, 37.6 × 3.33 = 112.6 mg. of iron turned over per day. Weight 76 Kg. 112.6 / 76 = 1.65 mg. Fe/Kg /day.
In case 3 large amounts of hemosiderin were demonstrated in parenchymal and Kupffer cells in a liver biopsy specimen by Gomori's modification of the Prussian blue method. In case 4 hemosiderin was present in smaller amounts. Neither patient had received transfusions nor medicinal iron. The increased iron stores may, therefore, be related to increased iron absorption due to anemia.

*Siderocytes.* Red cells with iron-staining inclusion bodies could be demonstrated in significantly increased numbers after splenectomy in both case 1 (24 per cent) and case 3 (12 per cent). Studies of survival time of siderocytes in association with the Ashby technic in one case have suggested that the spleen removes...
these inclusion bodies from erythrocytes, allowing the red cell which previously contained siderocytes to live out its expected life span. The exact role of siderocytes in iron metabolism is unknown. Their presence in large numbers signifies some defect in erythropoiesis as well as failure of the splenic mechanism for removal of particulate red cell inclusions.

**Pigment Metabolism**

*Plasma hemoglobin.* Plasma hemoglobin was found to be normal (below 5 mg./100 ml.) in all cases (case 1 not tested). This confirmed similar observations of plasma hemoglobin in six patients of another sibship of this disease and in hereditary spherocytosis. Plasma hemoglobin in acquired hemolytic disease is frequently increased over normal values.

*Fecal urobilinogen.* Fecal urobilinogen was determined on a four day feces collection in case 1 before splenectomy. Urobilinogen excretion was 42 mg. per day which was distinctly elevated for a child of 9 months. The patient's mother (case 2) had only a slightly elevated fecal urobilinogen (223 mg. for 24 hours). Her hemolytic index, however, was clearly increased (table 1).

Since a cyclical pattern of excretion of fecal urobilinogen has been reported in one case of hereditary nonspherocytic hemolytic disease, an attempt was made to collect daily specimens of feces of patients 3 and 4 for prolonged periods. Due to the difficulty of feces collection, specimens could not be obtained every single day. No cyclical pattern of fecal urobilinogen excretion, however, could be recognized in either case. The mean value of twenty-three determinations in case 3 before splenectomy averaged 1230 mg. per 24 hours. This value comes rather close to the calculated amount of fecal urobilinogen expected from blood destruction (1600 mg.), assuming complete conversion of all hemoglobin to urobilinogen with full recovery of the pigment. In case 4 only one third (430 mg./24 hours) of the calculated amount (1300 mg./24 hours) of fecal urobilinogen was recovered. After splenectomy fecal urobilinogen excretion decreased significantly in case 3 (720 mg./24 hours in thirty-three determinations). This amount of fecal urobilinogen was still markedly elevated, however, and was almost twice as high as in the nonsplenectomized case 4. The diminished pigment excretion may be interpreted as a slight decrease of the rate of hemolysis. The mechanism of such improvement is obscure. It cannot be related to previously existing hemolytic hypersplenism superimposed on the intrinsic red cell defect since the survival time of normal red cells was normal before splenectomy in this patient.

*Porphobilinogen.* Since porphobilinogen had been found in the urine of a patient

<table>
<thead>
<tr>
<th>Table 4.—Abnormal Hemoglobin Studies</th>
</tr>
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<tbody>
<tr>
<td>Case</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
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<td>4</td>
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<td>5</td>
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</tbody>
</table>

Hb-A = normal adult hemoglobin
FIG. 8.—A. Case 1. Spleen, hereditary nonspherocytic hemolytic disease. The splenic cords are widened and cellular but not congested. The sinuses contain moderate numbers of red cells. AFIP Acc. 496888. Hematoxylin and eosin (× 225).

B. Spleen, hereditary spherocytosis (for comparison). The splenic cords are heavily congested, the sinuses are almost empty and the lining cells are prominent. AFIP Acc. 545885. Hematoxylin and eosin (× 225).


D. Case 6. Spleen, hereditary nonspherocytic hemolytic disease. Prussian blue method (Gomori). Note the abundance and distribution of the iron-containing pigment with sparing of the lymphatic follicles. AFIP Acc. 195916 (× 120).

E. Spleen, hereditary spherocytosis (for comparison). Prussian blue method (Gomori). Deposition of iron-containing pigment not nearly as heavy as in hereditary nonspherocytic hemolytic disease. AFIP Acc. 195607 (× 120).

F. Spleen, without evidence of intrinsic disease, traumatic death (for comparison). Prussian blue method (Gomori) shows no evidence of iron-containing pigment. AFIP Acc. 180924 (× 120).
with hereditary nonspherocytic hemolytic disease, this pigment was searched for in the urines of all four patients. No porphobilinogen was found.

Search for abnormal hemoglobin. Advances in the understanding of sickle-cell disease and other hereditary hemolytic anemias have resulted from studies of abnormal hemoglobin in such states. Table 4 summarizes the results of abnormal hemoglobin studies in all four cases and in an additional case of hereditary nonspherocytic hemolytic disease from another institution (case 5). No abnormal hemoglobin could be demonstrated electrophoretically. A slight elevation of fetal hemoglobin was most significant in the adult patient (case 2). It was of doubtful significance in cases 1 and 5 because of the young age of these patients. Mild elevations of fetal hemoglobin have also been seen in some cases of hereditary spherocytosis. This may be interpreted as a reactivation of fetal hemapoiesis due to bone marrow stress.

Histology of the Spleen (Cases 1, 3, and 6)*

Sections of the spleen were similar in all three cases and are described together. In hematoxylin and eosin stained sections the pulp cords appeared increased in cellularity and moderately thickened. The increased cellularity was partly due to an increase in the number of lymphocytes and monocytes, partly to the presence of numerous pigment-laden macrophages. The pulp cords were not congested. Most of the sinuses were distinct and contained red cells in varying amounts. No dilatation or congestion of the sinuses was present. The cells lining the sinuses were not prominent. Lymphatic nodules were well defined and had well developed reaction centers with little evidence of phagocytosis. Marginal zones of lymphatic nodules were well demarcated and showed no significant deviations from normal. The capsule, trabeculae, arteries, and veins showed no abnormalities. Large amounts of hemosiderin were demonstrated in the macrophages of the red pulp and lining cells of the sinuses by the prussian blue method. Lymphatic nodules were devoid of iron-containing pigment. The fibrillar network as demonstrated by reticulum stains was more prominent than usual. There were irregularly shaped and thickened reticulum fibers which did not form part of the normal delicate fibrillar reticulum. Masson stains showed no increase in the amount of connective tissue.

The most striking single microscopic feature which differentiated hereditary nonspherocytic hemolytic disease from hereditary spherocytosis was the absence of the severe congestion of the red pulp cords in the former (fig. 8a). A section from the spleen of a patient with hereditary spherocytosis (fig. 8b) is shown for comparison. Another fairly distinctive change was the abundance of iron-containing pigment which was consistently found in the spleen of our patients with hereditary nonspherocytic hemolytic disease (fig. 8d). In contrast, the amount of iron-containing pigment found in spleens of patients with hereditary spherocytosis varied but was not nearly as abundant (fig. 8e).

Discussion

It is expected that further study of hemoglobin and erythrocytic stromal and enzymatic abnormalities will more clearly define the red cell defect or defects in
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hereditary nonspherocytic hemolytic disease. Until this is accomplished, any inherited hemolytic syndrome not associated with a characteristic anomaly of red cell shape must necessarily be classified as hereditary nonspherocytic hemolytic disease. Such a classification does not rule out the likely possibility that this diagnosis includes a variety of hemolytic diseases which have in common the lack of any anomaly of red cell shape. These diseases might be caused by a variety of mechanisms that shorten the life span of the affected erythrocytes.

With this reservation in mind we may compare our cases with those of other authors. In all patients, the disease was due to an intrinsic red cell defect. It was not limited to any ethnic group. Splenomegaly was usually present. Red cell shape was essentially normal although there were minor differences in shape and size, and some ovalocytosis was observed in several instances. After splenectomy, siderocytes, target cells, and Howell-Jolly bodies appeared in most patients. One of Haden's families and the patient described by Feinberg and Watson very definitely appeared to form a separate category, since in these patients basophilic stippling of the red cells was outstanding. Haden's first family differed from patients in other kindreds in that the blood showed a marked tendency to autohemolysis on standing. Hemoglobinemia was also present. Otherwise in hereditary nonspherocytic hemolytic disease there was usually normochromic, normocytic, or slightly macrocytic anemia with significant reticulocytosis. The bone marrow showed normoblastic erythroid hyperplasia. Serum bilirubin sometimes was normal but indirect bilirubin was always elevated. Fecal urobilinogen was increased.

Red cell survival studies performed in five cases, including two of our own, showed diminished mean life span of the affected erythrocytes ranging from twelve to seventeen days. Normal red cells transfused into the patients had a normal life span, although one of the patients in the second family of Kaplan and Zuelzer had a transient superimposed extracorpuscular hemolytic mechanism with premature destruction of the normal cells. The Coombs test was negative in all cases. Sickle cell preparations and acid and heat fragility tests were negative. Osmotic and mechanical fragility tests were normal.

A rather constant mean red cell life of twelve to seventeen days in these patients should be contrasted with the varying severity of anemia. The degree of anemia in any hemolytic disease depends upon the mean red cell life span and the extent of bone marrow compensation. It has been shown that an uninhibited bone marrow may increase its output 7 to 8 times. With maximum hemoglobin output of such magnitude, anemia will occur only when the mean red cell life span is less than fifteen to twenty days. The bone marrow in case 3 compensated to a maximal degree since with a life span of thirteen days anemia was mild.

In case 4 there was moderate anemia with a mean red cell life span of seventeen days. 

\[ \text{Hb}: 12.4 \text{ Gm./100 ml.; blood volume: 4600 ml.} \]
\[ \text{mean life span of R.B.C.: 13 days;} \]
\[ \text{weight of patient: 61 Kg.} \]
\[ \text{Daily destruction of Hb = Hb/1 ml. x bl. vol. \times \text{mean life sp. of R.B.C.}} \]
\[ \text{= 43.8 Gm. Hb.} \]
\[ \text{= 718 mg. Hb/Kg./day.} \]
\[ \text{normal hemoglobin production is 90 mg. per Kg. per day. The patient, therefore, produced 7.9 times the normal amount of hemoglobin.} \]
days. Daily production of hemoglobin was computed to be 500 mg. per Kg. or 5.5 times the normal. Similarly patient L. L. of Kaplan and Zuelzer had severe anemia with a mean life span of thirteen days. In this case and in our case 4 a relative bone marrow inhibition may be assumed to exist.

**Mechanism of Inheritance.**

The available data suggest that these diseases are transmitted as Mendelian dominant characteristics. Members of both sexes are affected. In some instances a diagnosis can be made only if the disease can be demonstrated in blood relatives. When the family study is negative, the diagnosis may be very difficult. Sometimes no hemolysis can be recognized in the parents of the affected patients. (Cf. cases 2 and 4 of our series, Kaplan and Zuelzer, and cases 1 and 3 of Dacie.) Several possible explanations may be invoked for this phenomenon. Doubtful paternity is a most unlikely explanation since hemolytic stigmata were absent in the parents of five different families. Presence of the disease in siblings of the propositus (our case 4, Kaplan and Zuelzer, case 1 of Dacie) rules out mutation as an acceptable explanation. Another possibility is that a recessive abnormal gene is inherited from both parents to produce a homozygous state that is manifested by hemolytic anemia. This mechanism is similar to the genetic pattern of sickleemia. It is incompatible with the simple Mendelian dominant pattern that has been demonstrated in families with nonspherocytic hemolytic disease. Finally, the gene responsible for the disease may be present with such low penetrance in one of the parents that it cannot be demonstrated by available methods. This latter well-known genetic phenomenon seems the most likely explanation. It has been encountered in families with hereditary spherocytosis. Since hereditary nonspherocytic hemolytic disease is probably not one disease, different genetic mechanisms may be operating in various families. More data are required for definitive genetic analysis.

**Differential Diagnosis**

Case 1 and the children of Kaplan and Zuelzer's second family illustrate that hereditary nonspherocytic hemolytic disease should be considered in the differential diagnosis of erythroblastosis fetalis.* A positive Coombs test will be found in the great majority of cases of Rh and the rarer blood factor incompatibilities, but will be negative in instances of hereditary hemolytic disease at birth. Hereditary spherocytosis and Cooley's anemia rarely are full blown at birth. Hemolytic disease of the newborn due to ABO incompatibility may give diagnostic difficulties since the Coombs test is often negative in such cases. An exchange transfusion with O Rh-negative (cde) blood, cross-matched by means of an indirect Coombs test, should be administered when the definite cause of hemolytic disease in the newborn cannot readily be demonstrated. This procedure could not be harmful in the hereditary hemolytic diseases. It would benefit any isoimmune hemolytic disease of the newborn, except hemolytic disease due to anti-c which can be recognized by appropriate tests.

Hereditary spherocytosis may sometimes be difficult to rule out. Ordinarily the

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* An additional case of the condition present at birth has recently come to our attention.
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presence of microspherocytes, the characteristic deformed rouleaux pattern, increased osmotic, mechanical and oxalate fragility clearly differentiate hereditary spheroctysis from hereditary nonspherocytic hemolytic disease. Some cases of hereditary spheroctysis fail to show significant spheroctysis and exhibit normal osmotic fragility. Incubation of the blood of such patients for 24 hours at 37°C will usually unmask the characteristic defect and reveal significantly increased osmotic fragility. There are two cases of hereditary nonspherocytic hemolytic disease in the literature (cases 1 and 4 of Dacie) in which a marked increase of osmotic fragility was observed after incubation. Conversely, patients with undoubted hereditary spheroctysis, (cases 7 to 9 of Dacie) failed to show the expected increase of osmotic fragility on incubation. Definite diagnosis in such cases may be possible only by study of other affected members of the family or by the response to splenectomy. Removal of the spleen cures the anemia of hereditary spheroctysis—even when mild—but the patient with hereditary nonspherocytic hemolytic disease derives little or no benefit from splenectomy. In most cases the microscopic examination of rouleaux patterns in fresh blood, an osmotic fragility test, and the osmotic fragility in 0.65 per cent sodium chloride after 24 hours' incubation will demonstrate the presence of spheroctysis.

Sickle cell anemia and its variants are excluded by the sickling test. Mediterranea anemia is a hypochromic anemia with the characteristic peripheral blood picture of target cells, fragmented cells, and decreased osmotic fragility. Normoblasts are usually seen in the most severe manifestation of Mediterranean disease (Cooley's anemia).

Hereditary hemolytic elliptocytosis may be recognized by the typical elliptic and cigar-shaped erythrocytes and by similar findings in other members of the family. In some cases of hemolytic elliptocytosis marked red cell fragmentation is also seen. Splenectomy benefits patients with hemolytic elliptocytosis.

A new inherited hemoglobin abnormality—hemoglobin C—has been described in American Negroes. It can be distinguished from normal and sickle cell hemoglobin by electrophoresis. When combined with normal hemoglobin (C-A or C-trait) its presence is indicated by many target cells. The homozygous condition in offspring of two parents who are carriers of the C trait is associated with a mild hemolytic anemia and splenomegaly (homozygous hemoglobin C disease). Many target cells and fragments are also seen. Simple paper-electrophoretic study of hemoglobin will differentiate homozygous hemoglobin C disease from hereditary nonspherocytic hemolytic disease since hemoglobin in the latter condition has the electrophoretic mobility of normal adult (A) hemoglobin.

Paroxysmal nocturnal hemoglobinuria is differentiated by positive acid and heat fragility tests, a positive thrombin test and the almost invariably presence of hemoglobinemia. Acquired hemolytic anemia is ruled out by positive family findings and a negative Coombs test. Cases of acquired hemolytic anemia with a negative Coombs test may cause great diagnostic difficulties which sometimes can be resolved only by red cell survival time study. Normal red cells transfused into patients with acquired hemolytic anemia are rapidly destroyed while normal survival of normal red cells occurs in hereditary nonspherocytic hemolytic disease. We have faced this diagnostic problem in two cases during the past year. Both were shown to be acquired hemolytic anemia associated with sarcoidosis.
Patients with hyperbilirubinemia—frequently familial—with no apparent hematologic or hepatic abnormalities (familial nonhemolytic jaundice, constitutional hepatic dysfunction) may present diagnostic difficulties. Recent work as well as our own observations of such cases point to several mechanisms causing hyperbilirubinemia. Careful history, multiple liver function tests, and liver biopsy may reveal the hyperbilirubinemia to be a residual of subclinical hepatitis, which may not have been adequately treated. In other cases an accumulation of a peculiar brownish non-iron pigment has been noted in liver biopsies. Mild hemolysis has been postulated in some of these patients because of increased mechanical fragility after incubation. Increased fecal urobilinogen actually has been demonstrated in other cases. Even if mild hemolysis occasionally exists, an additional inability of the liver to clear bilirubin must be assumed, for the normal liver is quite able to clear at least 2 to 3 times the normal output of bilirubin. Failure of the liver to clear bilirubin in the absence of hepatic or hemolytic disease has been the conventional explanation for constitutional hepatic dysfunction. It is not unlikely that some cases that have been diagnosed as constitutional hyperbilirubinemia may in reality have been mild cases of hereditary nonspherocytic hemolytic disease.

Reports exist in the literature of familial anemia which may superficially be confused with hereditary nonspherocytic hemolytic disease. The abnormality in these families was characterized by hereditary sex-linked elliptocytic hypochromic anemia. The hemolytic component of these anemias is usually mild. The disorder in such cases appears to be primarily one of hemoglobin synthesis. The characteristic genetic pattern of sex linkage, the hypochromia, and the mild hemolytic disease clearly differentiate these patients from those with hereditary nonspherocytic hemolytic disease. Stransky and Regala reported familial macrocytic or normocytic hypochromic anemia with peripheral erythroblastosis and normal osmotic fragility in Filipino children. These cases resemble Cooley's anemia. Erythroblastosis is not characteristic of hereditary nonspherocytic hemolytic disease except at birth or immediately after splenectomy. Debler described familial hypochromic anemia in children that responded to splenectomy.

_Treatment_

The practice of giving iron to patients with poorly understood anemias is condemned. The ability to absorb iron is increased in patients with anemia, and administration of iron for long periods only increases the siderosis of liver, pancreas, and other organs. The use of iron is not indicated in hereditary anemias unless iron deficiency is clearly demonstrated.

Splenectomy in hereditary nonspherocytic hemolytic disease has been of little or no value. Occasionally hemolysis may have diminished slightly (case 3) but still has continued briskly in all patients on whom the operation was performed. The failure of improvement may be contrasted with the striking response to splenectomy in hereditary spherocytosis. Though spherocytes persist after splenectomy in hereditary spherocytosis, the rate of hemolysis becomes normal. While it is usually recommended that even patients with mild hereditary spherocytosis should be splenectomized, patients with uncomplicated hereditary non-spherocytic hemolytic disease probably should not be operated on.
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A comparison of the splenic histologic findings in the two conditions suggests an explanation for the variation in response to splenectomy. The splenic pulp in hereditary spherocytosis is markedly congested with spherocytes. The spleen and no other organ selectively retains and destroys spherocytes. Removal of the spleen allows spherocytes to live a normal life span and the hemolytic disease is cured. Failure to respond to splenectomy in hereditary nonspherocytic hemolytic disease may be related to the lack of congestion which can be explained by the normal shape of the affected red cells. No selective trapping occurs. After splenectomy the defective erythrocytes continue to be destroyed prematurely even in the absence of the spleen.

Even though splenectomy does not affect the basic hemolytic process of hereditary nonspherocytic hemolytic disease, the operation may be indicated in severe cases. The rate of hemolysis may become somewhat less. A very large spleen may be predisposed to rupture in patients who perform physical labor. Thrombocytopenia, leukopenia, and extracorpuscular hemolytic disease superimposed on the already existing intrinsic red cell defect, are complications that may develop in the presence of a markedly enlarged spleen due to any cause. If several children of a family suffer from the condition, splenectomy may be attempted in the most severely afflicted patient. If no improvement occurs, the operation should not be performed in the siblings.

SUMMARY

Extensive studies were performed on four cases from three unrelated kindreds with a familial hemolytic syndrome not associated with any significant red cell anomaly (hereditary nonspherocytic hemolytic disease). These cases were compared with similar ones already reported in the literature.

1. Hereditary nonspherocytic hemolytic disease appears to be transmitted as a Mendelian dominant. Frequently the gene responsible for the condition seems to have low expressivity. In some cases, the hereditary mechanism may be due to inheritance of a recessive gene from each parent. The basic erythrocytic defect responsible for the condition is unknown. In view of various clinical and hematologic findings, it is likely that hereditary nonspherocytic hemolytic disease may be a group of diseases involving more than one mechanism.

2. All criteria of hemolytic anemia (erythroid hyperplasia of the bone marrow, reticulocytosis, hyperbilirubinemia, increased fecal urobilinogen, rapid turnover of tracer iron in the plasma) were satisfied.

3. Red cell survival time studies revealed an intraerythrocytic defect with a mean life span of twelve to seventeen days. Normal red cells transfused into the patients under study survived normally. Anemia was normochromic and normocytic or macrocytic; it varied from mild to severe.

4. Osmotic and mechanical fragility of the red cells was normal. Osmotic and mechanical fragility tests after incubation at 37°C for 24 hours in some showed a mild increase compared with normal controls. Autohemolysis of incubated oxalated blood was not marked and varied from case to case.

5. The electrophoretic mobility of hemoglobin from the patients was that of normal adult hemoglobin. Small increases of fetal hemoglobin were seen in several cases.
6. In contrast to the histologic findings in hereditary spherocytosis the splenic pulp was not congested, but hemosiderin deposits were heavy. Liver biopsy specimens showed deposits of hemosiderin in parenchymal and Kupffer cells.

7. Splenectomy did not arrest the hemolytic process. Mild improvement was seen in one case. In most cases the operation is of no value.

8. Diagnostic difficulties may be encountered with mild cases of hereditary spherocytosis. Examination of rouleaux in fresh blood and an osmotic fragility test in 0.65 per cent sodium chloride after incubation usually establishes the differential diagnosis. The condition may present clinically as hemolytic disease of the newborn and must be differentiated from erythroblastosis due to Rh or other blood group incompatibilities. Other hereditary hemolytic diseases such as sickle cell anemia, Cooley’s anemia, hereditary spherocytosis, and hereditary hemolytic elliptocytosis are easily ruled out by their typical clinical and hematologic manifestations. When a family study is negative or cannot be done, a red cell survival time determination may be necessary to rule out acquired hemolytic anemia with a negative Coombs test. Some cases that have been diagnosed as constitutional hyperbilirubinemia (familial nonhemolytic jaundice) may actually represent mild hereditary nonspherocytic hemolytic disease.

**SUMMARIO IN INTERLINGUA**

Studios extensive esseva facite super quatro casos—ex tres familias non-affin—de un syndrome de hereditari morbo hemolytic nonspherocytic, i.e. un hemolytic syndrome familial non associate con significative anomalias del erythrocytas. Iste casos es comparat.e con simile casos previamente reportate in le litteratura.

1. Il pare que hereditari morbo hemolytic nonspherocytic es transmittite como un dominante mendelian. Frequentemente le gen responsable pare haber un basse expressivitate. In alicun casos le mechanismo hereditari implica forsan un gen recessive ab cata parente. Le basic defecto erythrocytic del qual le condition resulta non es cognoscite. Varie constatationes clinico e hematologic rende probable que hereditari morbo hemolytic nonspherocytic es un gruppo de morbos que involve plus que un singule mechanismo.

2. Omne criterios de anemia hemolytic esseva presente: erythroide hyperplasia del medulla ossee, reticulocytosis, hyperbilirubinemia, augmentate urobilinogeno fecal, e un rapide excambio de ferro traciator in le plasma.

3. Le tempore de superviventa del erythrocytas que esseva determinate como inter dece-duo e dece-septe dies revelava un defecto intraerythrocytic. Erythrocytas normal, que esseva transfundite a in le patientes sub observation, superviveva normalmente. Le anemia esseva normochromic e normocytic o macrocytic; illo variava ab benigna a sever.

4. Le fragilitate osmotic e mechanic del erythrocytas esseva normal. Post incubation pro 24 horas a 37 C. teste de fragilitate osmotic e mechanic monstava in alicun casos un moderate augmento in comparation con normal specimens de controle. Le autohemolyse de incubate sanguine oxalate non esseva marcate, e variava de caso in caso.

5. Le mobilitate electrophoretic del hemoglobina del patientes esseva idente con lo del hemoglobina de adultos normal. Parve augmentationes de hemoglobina fetal esseva observe in alicun casos.
In contrasto con le constatazioni histologic in spherocytosis hereditari le pulpa splenic non esseva congestionate, sed le depositos de hemosiderina esseva considerabile. Specimens de biopsia hepatic monstrava depositos de hemosiderina in cellulas parenchymal e cellulas Kupffer.

Splenectomia non poteva arrestar le processo hemolytic. In un caso iste operation produceva un leve melioramento. In le majonitate del casos illo ha nulle valor.

Difficultates diagnostic pote esser incontrate its casos de leve spherocytosis hereditani. Le examination de rouleaux in sanguine fresc e un test de fragilitate osmotic 0,65 pro cento de chlorido de natrium post incubation suffice generalmente a establir un diagnose differential. Le condition pote presentar se clinica mente como morbo hemolytic del neonato, e in iste caso illo debe esser differentiate de erythroblastosis debite a incompatabilitate con Rh o altere gruppos sanguine. Altere hemolytic morbos hereditari—anemia a cellulas falciforme, anemia Cooley, spherocytosis hereditari, hereditari elliptocytosis hemolytic, etc.—es facile a eliminar per medio de lor typic manifestationes clinic e hematologic. Quando un studio familial non es facibile o da resultatos negative, un determination del tempore de superviventia del erythrocytas pote devenir necessari pro excluder un diagnose de acquiret anemia hemolytic con un negative test Coombs. Alicun casos diagnosticate como hyperbilirubinemia constitutional (familial jalnessa nonhemolytic) pote in realitate representar leve hereditari morbo hemolytic nonspherocytic.

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Hereditary Nonspherocytic Hemolytic Disease: A Study of a Singular Familial Hemolytic Syndrome

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