Hereditary Spherocytosis: Observations on Hemolytic Mechanisms and Iron Metabolism

By William H. Crosby and Marcel E. Conrad

The hemolytic disease associated with hereditary spherocytosis (HS) is a result of an incompatibility between the patient's abnormal red cells and the spleen. Although there is an intrinsic fault of the red cells which permits their premature destruction, the spleen is essential to the hemolytic process; after splenectomy the hemolytic disease disappears because the red cells, although still spherocytic, survive a normal length of time. This antagonism is not a peculiarity of the patient's spleen. If his red cells are transfused into a normal person they are quickly destroyed by the spleen of the recipient.

The spleen in HS possesses certain characteristics which may indicate the manner in which the red cells are destroyed. The spleen is enlarged, the cords are congested with erythrocytes and the sinusoids are empty. The phagocytic cells which comprise the walls of the sinusoids are hypertrophied. This suggests that spheroidal red cells traverse the pulp cords slowly and when they reach the sinusoids they are destroyed by the littoral phagocytes. It has seemed reasonable to assume that because HS cells are misshapen, the spleen may trap them, and because they are especially susceptible to the effects of stagnation, they are prematurely destroyed.

The HS spherocyte demonstrates several abnormalities:
1. First of all is the abnormality of shape. The cell, however, is not a true sphere. Like a normal red cell the HS spherocyte is a biconcave disc, but thicker than normal and less in diameter.
2. Related to the abnormality of shape there is a disproportion between surface area and volume. The volume is normal but the surface area is less.
3. Increased osmotic and mechanical fragility.
4. A tendency to excessive hemolysis during sterile incubation at 37°C, especially when oxalate is used as the anticoagulant.
5. Increased amounts of nonhemoglobin iron. Many HS red cells are de-
livered from the marrow with iron granules in them, so-called siderocytes.4,5

6. An abnormally high mean corpuscular hemoglobin concentration.13

7. An abnormality of glucose metabolism. This has not been defined, but it manifests itself, for example, by impaired incorporation of plasma phosphate into adenosine triphosphate in the spheroocyte.27,30 It has also been reported that fluoride interferes with uptake of phosphate by HS cells* but not by normal erythrocytes.35

It is not known which of these abnormalities, if any, predisposes the spheroocyte to destruction by the spleen. In the present investigation we attempted to learn if the abnormal shape was responsible for the trapping of spherocytes by the spleen. "Whereas normal discoidal cells probably could circulate through the spleen without difficulty, spherocytes because of their shape might find it difficult to traverse the slitlike stomata leading from the pulp into the splenic sinuses. This hypothesis is attractive," says Dacie,13 "but difficult to confirm or refute." We believe that our experiment has tested this hypothesis and that the results refute it. In two patients with HS we were able to modify the shape of the spherocytes, making them thinner by deliberately inducing iron deficiency. Other characteristic red cell abnormalities also disappeared. The cells became hypochromic and in one case normally resistant to osmotic and mechanical injury and to the effects of incubation. But their ability to survive in the circulation with an intact spleen was not changed.

MATERIALS AND METHODS

Subjects of the experiment were the two young men in whom the diagnosis of spherocytosis was made and its hereditary etiology was established by finding other cases in each patient's immediate family. The Coombs test was negative.

Phlebotomy was performed, 500 ml. each time, two or three times weekly until the iron stores were depleted, the serum iron was low, moderate anemia was established, and the reticulocyte count fell. The loss of hemoglobin was computed at each phlebotomy by multiplying volume of blood by the hemoglobin concentration. The loss of iron was also calculated (0.338 Gm. Fe/100 Gm. Hb).

Red cell indexes were computed from counts of at least 2000 cells,14 high-speed microhematocrits,25 and carefully calibrated hemoglobinometry.11 Red cell life span was measured by transfusion of Cr31-labeled cells (normal values of Cr31 t½ = 27 to 35 days); blood volume was measured at the same time.21 The average life span of transfused red cells was calculated by the method of Dornhorst.19 This method cannot be used with curves derived with Cr31 on long-lived red cells because of the phenomenon of "elution." But with very short-lived red cells the daily loss of radioactivity by elution is an inconsiderable proportion of that lost because of hemolysis. The rates of hemolysis and red cell production were computed from life span and red cell volume as described in earlier publications.6,8 Reticulocytes were stained by the method of Brecher1; siderocytes by a Prussian-blue reaction.5 Erythrocyte dimensions were determined by photomicrography and measurement of rouleaux26; the mean surface area of the red cells was computed from the diameter and thickness (normal value 135 sq. μ). Hemoglobin type was established by paper electrophoresis28; fetal hemoglobin was measured as an alkali-resistant fraction34; osmotic fragility was determined photometrically, immediately and again after 24 hours sterile incubation at 37 C.14 Mechanical fragility was measured by the method of Young et al.40; with normal blood less than four per cent of the red cells are hemolyzed. "Autohemolysis" was measured

*We were unable to confirm this with red cells from our patients and affected members of their families.
before whole blood was incubated 24 hours under oil at 37 C. using oxalate, citrate, heparin and no anticoagulant; the concentration of hemoglobin in the plasma was measured before and after this incubation by means of a quantitative benzidine reaction; after incubation of normal blood there is less than 40 mg./100 ml. of hemoglobin in the plasma. Serum iron was measured by the method of Ramsey; unsaturated iron-binding capacity by the method of Ventura; and bilirubin by the method of Malley and Evelyn.

RESULTS

Case 1.—The patient, an 18 year old white male, was admitted to Walter Reed General Hospital on July 12, 1958 with a diagnosis of HS. Although he was in good health, he was lightly jaundiced and his spleen was enlarged 4 cm. below the costal margin. The marrow showed a moderate normoblastic hyperplasia and a slight increase of visible iron stores. Hemoglobin concentration was 14.8 Gm./100 ml., and red cell mass was 33 ml./per Kilogram of body weight. Reticulocytes were 4 per cent. The Cr\textsuperscript{51} t\textsuperscript{1/2} of the patient’s autotransfused red cells was 15 days (fig. 1); in another recipient it was also 15 days. It was estimated that the rate of hemoglobin production was about 55 Gm. per day, which is eight times the normal. The MCV was 83 cu.\mu, the MCH 31 \mu g. the MCHC 37 per cent. The cellular dimensions were characteristic of HS (table 3). Osmotic and mechanical fragility tests were abnormal, and autohemolysis was greatly increased during incubation (table 1 and fig. 2). The patient had a well compensated hemolytic disease without

*We are indebted to Dr. Spencer Baab of Salt Lake County General Hospital for examination of the patient's family. None of his siblings had the disease, but his mother had been splenectomized for HS in 1947.
anemia. While the preliminary studies were being completed, the nature and the purpose of the procedure were discussed with the patient, and he consented. The series of phlebotomies was begun, and between September 15 and October 27 a total of 6 L. of blood was withdrawn on 12 occasions. This was a loss of 61 Gm. of hemoglobin and 2.07 Gm. of iron. The serum iron fell. Maximum reticulocyte response was noted on October 3, after the eighth phlebotomy. Thereafter, the red cell indexes began to decrease as iron deficiency developed. Anemia was most severe on October 31 after the last phlebotomy (Hb. 7.5 Gm./100 ml.). During the course of bleeding the patient's bilirubin was often normal, but as soon as the phlebotomies were stopped it became elevated again.

The patient was now iron deficient. After six weeks had elapsed to permit the formation of a new population of hypochromic red cells, a re-evaluation was begun on December 8 (table 1). At this time, there was no perceptible change in the size of the spleen. Hemoglobin concentration was 8.2 Gm./100 ml.; the red cell mass was 17.5 ml./Kg. Serum iron was 60 jg./100 ml. MCV was 64 cu.μ, MCH 19 μg, and MCHC 29 per cent. Cellular dimensions were also diminished (table 3). Osmotic fragility, mechanical fragility and the tests for autoheinolysis were normal. The microcytic hypochromic anemia was characterized by thin red cells; they appeared so under the microscope (fig. 3) and they behaved so in the fragility tests (figure 2 and table 1). However, when red cells were tagged with Cr51 and transfused into the patient himself and into the same recipient as before, there was no significant difference between the Cr51 t ½ of the hypochromic cells and what it had been earlier (fig. 1). Iron deficiency had corrected the spherocytosis but not the hemolytic disease.

Now the patient was prepared for splenectomy. His anemia was treated by giving him iron by mouth 0.9 Gm. of ferrous sulfate per day. On February 25, the spleen was removed. It weighed 385 Gm. Histologic examination revealed severe congestion of the pulp with mild reactive hyperplasia of the reticuloendothelial cells of the sinuses. At the same time he was still iron deficient, and his red cells were hypochromic (table 1). In July 1959, he was re-examined. At this time his red cells were once again spherocytic and abnormal in the fragility tests, but survival was normal when the red cells were tagged with Cr51.
Fig. 2.—Results of the osmotic fragility tests performed in both cases. Solid lines represent results before blood was incubated 24 hours at 37 C.; broken lines after incubation. Incubation for 24 hours caused the fragility of the cells to become greatly increased. But after phlebotomy the incubation caused a great proportion of the cells to become intensely resistant. This “paradoxic effect” of incubation is evidently a characteristic of hypochromic red cells. Cross hatching defines the normal range. Case 1 at top, case 2 at bottom.
HEREDITARY SPHEROCYTOSIS

Fig. 3.—The microscopic appearance of hereditary spherocytes before and after induction of iron deficiency by repeated phlebotomy. "Iron deficiency will flatten the spherocyte."22

A. (× 895; reduced) Case 2. March 13, 1959. Red cell rouleaux before phlebotomy showing deformity of rouleaux characteristic of spherocytosis.18


and transfused into the patient (fig. 1 and table 1). Splenectomy had cured the hemolytic disease but not the spherocytosis.

Case 2.—The patient was a 24 year old white soldier admitted to Walter Reed General Hospital in March 1959 with a diagnosis of HS. He had a history of anemia in childhood, for which he had received one transfusion at the age of eight. His mother and sister also proved to have HS. Physical examination was unremarkable except that the patient's spleen was 4 cm. below the costal margin. Hematologic studies are shown in table 2 and figure 4. They are noteworthy in that they reflect an exceptionally rapid turnover of hemoglobin. The patient was not anemic, and yet the Cr²⁺⁺⁺⁺⁺⁺ of his tagged red cells was only seven days; in a normal recipient the t½ was only 45 hours. reticulocyte count was 11 per cent, and there were seven per cent siderocytes, an exceptional finding for a patient with an intact spleen. The fragility tests were severely abnormal. Other evidence of abnormal iron me-
Table 2.—Laboratory Studies in Case 2: Phlebotomy of 9.5 L. of Blood Done between April 8 and June 5 (Splenectomy Done on July 18)

<table>
<thead>
<tr>
<th></th>
<th>Mar. 31</th>
<th>Apr. 27</th>
<th>June 2</th>
<th>July 7</th>
<th>Aug. 27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cells (millions)</td>
<td>4.5</td>
<td>2.7</td>
<td>2.4</td>
<td>3.8</td>
<td>6.0</td>
</tr>
<tr>
<td>Hemoglobin (Gm.)</td>
<td>14.9</td>
<td>9.2</td>
<td>7.1</td>
<td>9.1</td>
<td>13.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.5</td>
<td>25.5</td>
<td>21.5</td>
<td>29.8</td>
<td>45.0</td>
</tr>
<tr>
<td>MCV (cu.μ)</td>
<td>88</td>
<td>95</td>
<td>90</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>MCH (μg.)</td>
<td>33</td>
<td>33</td>
<td>29.5</td>
<td>24.0</td>
<td>22.5</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>38</td>
<td>36</td>
<td>33</td>
<td>31.0</td>
<td>29.5</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>11</td>
<td>30</td>
<td>17</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Siderocytes (%)</td>
<td>7</td>
<td>6</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Bilirubin (mg.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.8</td>
<td>1.0</td>
<td>2.6</td>
<td>1.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Direct</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Plasma iron (μg.)</td>
<td>238</td>
<td>166</td>
<td>95</td>
<td>60</td>
<td>23</td>
</tr>
<tr>
<td>Unsaturated iron-binding capacity (μg.)</td>
<td>0</td>
<td>28</td>
<td>193</td>
<td>267</td>
<td>393</td>
</tr>
<tr>
<td>Red cell survival Cr51 t ½ (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self</td>
<td>8</td>
<td>10</td>
<td>27</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Recipient</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Red cell volume (ml./Kg.)</td>
<td>25.5</td>
<td>18</td>
<td>24.5</td>
<td>25.5</td>
<td>18</td>
</tr>
<tr>
<td>Blood volume (ml./Kg.)</td>
<td>73.5</td>
<td>82.5</td>
<td>63.0</td>
<td>73.5</td>
<td>82.5</td>
</tr>
<tr>
<td>Mechanical fragility (%)</td>
<td>16</td>
<td>5</td>
<td>6</td>
<td>16</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2.—Laboratory Studies in Case 2: Phlebotomy of 9.5 L. of Blood Done between April 8 and June 5 (Splenectomy Done on July 18)

The course of phlebotomies was begun and between April 8 and June 5 the patient was bled 9500 ml. in 19 increments of 500 ml. This was a loss of 99 Gm. of hemoglobin and 3.36 Gm. of iron. On July 1, the patient was moderately anemic (Hb 9.1 Gm./100 ml.; red cell mass 18 ml. per Kilogram), his serum iron was 60 μg./100 ml. His reticulocyte count, which had gone as high as 30 per cent during the bleeding, was now 4 per cent; siderocytes were no longer present in the blood. MCV had diminished from 88 to 76 cu.μ and the red cell dimensions were also diminished (table 3 and figure 3). Osmotic fragility was greatly improved, but not completely normal (fig. 2). The hypochromic red cells tagged with Cr51 showed no improvement in survival in his own circulation as compared with survival of his cells before phlebotomy (fig. 4).

Splenectomy was performed on July 18 without correcting the patient's iron deficiency. Six weeks later the survival of these hypochromic cells was measured once again. The Cr51 t ½ in the patient's circulation was 27 days, a value within the normal range. The spleen weighed 635 Gm. Microscopic examination revealed the congested pulp, empty sinuoids and hypertrophied littoral cells which characterize the spleen in HS.

**Comment on Iron Metabolism**

For several reasons the second patient is regarded as an exceptional case of HS. The diagnosis is not questioned: his mother and sister have the same disease characterized by spherocytosis, splenomegaly, reticulocytosis and jaundice. However, the life span of his red cells was exceptionally brief. The half life of Cr51 in his cells and in his own circulation was seven days, and the average life span of the cells was five days. One unit (500 ml.) of his blood was transfused into a normal recipient. The large amount was used in order to study decay of the transfused cells by means of differential agglutina-
Fig. 4.—Decay of Cr$^{15}$ radioactivity of autotransfused erythrocytes from the second patient.

tion as well as by Cr$^{51}$. The Ashby procedure failed for technical reasons, but the half-life of the Cr$^{51}$ was 45 hours. There was other evidence to indicate that the patient's red cells were being turned over extremely rapidly. He was not anemic, yet the reticulocytic count was 12 per cent. In most patients with HS who are not anemic before splenectomy, the reticulocyte count is less than 5 per cent$^{13}$ (case 1). This patient also had 6 per cent siderocytes in his blood before splenectomy. The life span of the iron granules in siderocytes is very brief before splenectomy,$^9$ and their presence in the blood in this case suggests that the output of red cells was high. The amount of red cell production required to compensate for this severe hemolytic disease has been computed on the basis of survival of his red cells in his own circulation. The average life span of his red cells was five days, and his hemoglobin mass was 9.6 Gm. per kilogram. The daily output of hemoglobin by his marrow was 1925 mg. per kilogram which is 20 times the normal rate (90 mg. per kilogram per day). His total Hb. production was 135 Gm. per day, as compared with a normal value of 6 Gm. for a man of his weight. During the time of bleeding the output of the marrow was increased by an additional 1.6 Gm. of Hb. per day to compensate for the blood loss. To accomplish the synthesis of 136 Gm. of Hb. the patient must each day have used 460 mg. of iron, this in addition to the iron which was wasted in siderocyte granules.$^7$
Table 3.—Red Cell Dimensions from Photomicrographic Measurements

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Diameter (μ)</th>
<th>Thickness (μ)</th>
<th>Surface area (μ²)</th>
<th>Volume (μ³)</th>
<th>Surface/Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before phlebotomy (July 21)</td>
<td>8.15</td>
<td>1.73</td>
<td>130.7</td>
<td>80.6</td>
<td>1.62</td>
</tr>
<tr>
<td>After phlebotomy (Jan. 30)</td>
<td>7.89</td>
<td>1.65</td>
<td>121.6</td>
<td>71.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before phlebotomy* (March 13)</td>
<td>7.8</td>
<td>2.03</td>
<td>125.3</td>
<td>86.6</td>
<td>1.45</td>
</tr>
<tr>
<td>After phlebotomy* (July 7)</td>
<td>7.3</td>
<td>1.94</td>
<td>110.3</td>
<td>72.5</td>
<td>1.52</td>
</tr>
</tbody>
</table>

*Examples of the cells photographed for these measurements are shown in figure 3.

Table 4.—Autohemolysis during Sterile Incubation

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 3</td>
<td>Dec. 8</td>
</tr>
<tr>
<td>Oxalate</td>
<td>320</td>
</tr>
<tr>
<td>Citrate</td>
<td>100</td>
</tr>
<tr>
<td>Heparin</td>
<td>100</td>
</tr>
</tbody>
</table>

*Blood placed in test tubes under mineral oil with various anticoagulants and incubated 24 hours at 37°C. The cells were then resuspended, the tubes were centrifuged and the hemoglobin in the plasma was measured. The figures are mg. of Hb./100 ml.

There were signs of abnormal iron metabolism. The plasma iron, for example, was 238 μg./100 ml. and the iron-binding capacity was completely saturated. Furthermore, although the patient had never been given therapeutic iron and had received only one transfusion (250 mg. Fe), his body iron stores were greatly in excess of normal. We removed 3.6 Gm., which is 2 to 3 times the normal amount of storage iron. He had accumulated this much iron in excess of his requirements, and he had done it with a saturated iron-binding globulin and in the absence of anemia. HS is not usually an iron-loading disease, but in this case it appears to be.

An additional insight into iron metabolism was provided by this patient’s reticulocyte response. During the period of phlebotomy the combination of hemolysis, hemorrhage and anemia caused an increase of reticulocytes to 30 per cent. Thereafter as the bleedings continued and iron deficiency developed, the reticulocyte count fell. On July 1 it was only 4 per cent, although, he was anemic and the rate of hemolysis was demonstrated to be extremely fast. His production of red cells continued at a rapid rate in order to counterbalance hemolysis, but only a few of the new red cells had any reticulum. In iron deficiency there is evidently a disturbance of erythroid maturation as there is in pernicious anemia. The cytoplasm of the erythroblast matures and loses its reticulum before the nucleus is lost, so that the new cells coming into the blood are not reticulocytes.

In both patients there appeared to be some resistance to the development of hypochromia of the red cells, even in the presence of iron deficiency. In normal people who develop iron deficiency the MCHC is the last of the indexes
to fall, but when the MCV has gone as low as 65 cu.μ and the MCH to 20 μg., the MCHC is definitely less than normal. But in the first patient, whose indexes became that low, the MCHC was 30 per cent, which is normal. Several factors may be involved. In iron-deficient dogs with hypochromic red cells, the sudden induction of a hemolytic reaction with phenylhydrazine gives rise to a generation of normochromic red cells. A large amount of red cell iron is released at one time, and for a few days the iron supplies in the marrow are normal. In these patients with HS, the rapid destruction of red cells and recycling of hemoglobin iron may have affected the quality of erythropoiesis. However, the nature of the disease HS seems a better reason for the presence of normal MCHC in the presence of iron deficiency. In HS the MCHC is higher than normal. The first patient, for example, had an MCHC of 37 per cent before he became iron deficient. Therefore, an MCHC of 30 per cent was extremely low for him.

After this patient was started on therapeutic iron in December, his hemoglobin concentration increased by the end of January from 8.2 Gm./100 ml. to 10 Gm., but the indexes did not improve. He continued to turn over his red cells very rapidly, and he did not use the increased amount of iron to improve the quality of his red cells but rather to produce an increased number of hypochromic cells.

Comment on Hemolytic Mechanism

The abnormal thickness of the HS red cell, the fragility and its tendency to hemolyze when stagnating apparently have little to do with its premature destruction by the spleen.

Life-span was not increased by iron deficiency and hypochromia. The cells became smaller and thinner, the resistance in tests of osmotic and mechanical fragility improved, and autohemolysis during incubation was greatly diminished. But modification of these characteristics was not reflected by improved survival in the presence of the spleen. On the basis of these results we suspect that it is not the abnormal shape of the HS red cell which leads to its trapping by the spleen; and the phenomenon of autohemolysis during stagnation does not appear to be responsible for its premature destruction.

The cells of HS differ from normal red cells in several other ways, and it is conceivable that one of these is the means whereby the spleen identifies them. The experiment eliminated two: (1) The abnormally high concentration of hemoglobin (MCHC); (2) the presence of iron granules in mature red cells: siderocytosis, a condition which is characteristic of HS as well as other varieties of hemolytic disease. However, iron deficiency reduced the MCHC to normal and stopped the formation of siderocytes.

Faults have been demonstrated in the carbohydrate metabolism of hereditary spherocytes, and it is possible that such a lesion is responsible for the cells being trapped and destroyed by the spleen. Since this metabolic fault has not yet been defined, it is impossible to say how it might mediate premature hemolysis when the spleen is present and yet permit normal survival of the spherocytes after the spleen has been removed.

The problem of splenic hemolysis is not peculiar to the spherocyte. With
other abnormalities the red cells are sequestered by the spleen and the characteristic picture of splenic pathology develops: the congested pulp cords and empty sinusoids with hypertrophy of the sinusoidal cells.

**Summary**

1. Two healthy patients with hereditary spherocytosis were phlebotomized until they developed iron deficiency and the erythrocytes became hypochromic. The hereditary spherocytes were no longer spheroidal: they became thin, and the fragility tests improved. However, the life span of the cells in the circulation was not improved. Later, splenectomy corrected the hemolytic disease.

2. In both patients, prior to the experiment, the hemolytic disease was compensated. There was no anemia despite the rapid turnover of red cells. In one of the patients, whose average red cell life span was only five days, the output of hemoglobin must have been exceedingly high. It was computed to be 135 Gm. per day, or 20 times the normal rate.

3. Some aspects of iron metabolism in hereditary spherocytosis are discussed.

4. The shape of the red cell in HS does not appear to be responsible for its premature destruction by the spleen. Iron deficiency corrects the spherocytosis, but it does not correct the hemolytic disease. Splenectomy corrects the hemolytic disease, but it does not correct the spherocytosis.

**Summario in Interlingua**

1. Duo patientes con spherocytosis, ambes in bon stato de sanitate, esseva subjicite a phlebotomia usque illes disveloppava carentia de ferro e monistrava erythrocytos que esseva hypochromic. Le spherocytos hereditari habeva cessate esser spheroide. Illos esseva tenue, e le tests de fragilitate esseva plus favorabile. Tamen, le longevitate del cellulas in le circulation non esseva meliorate. Plus tarde le morbo hemolytic esseva corrigite per splenectomia.

2. Ante le experimento le morbo hemolytic in ambe patientes esseva compensate. Illes monistrava nulle anemia in despecto del rapide catabiose del erythrocytos. In un del patientes, in qui le longevitate medie del erythrocytos esseva solmente cinque dies, le production de hemoglobina esseva sin dubita excessivemente alte. Esseva calculate pro illo un magnitude de 135 g per die, i.e. 20 vices le nivello normal.

3. Es discutite certe aspectos del metabolismo de ferro in spherocytosis hereditari.

4. Il pare que le conformation del erythrocytos in spherocytosis hereditari non es responsabile pro lor destruction prematur per le splen. Carentia de ferro corrig le spherocytosis sed non le hemolyse. Splenectomy corrig le hemolyse sed non le spherocytosis.

**References**

3. Crosby, W. H.: The pathogenesis of spherocytes and leptocytes (target
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