Hereditary Stomatocytosis: Membrane and Metabolism Studies

By William C. Mentzer, Jr., W. Byron Smith, Jerry Goldstone, and Stephen B. Shohet

A defect in the protein kinase-mediated phosphorylation of erythrocyte membrane proteins, previously unrecognized in stomatocytosis, was discovered in a boy with hereditary stomatocytosis and severe hemolytic anemia. The high-sodium, low-potassium erythrocytes of this patient were remarkably permeable to both sodium and potassium. The rate of ouabain-inhibitable active cation transport was more than ten times normal and was sustained by an increase of similar magnitude in glycolysis. The deformability in vitro of fresh stomatocytes was reduced and deteriorated further after a brief period of incubation with glucose. Ferrokinetic studies showed that these rigid cells were sequestered by the spleen. When stomatocytes were deprived of glucose in vitro, ATP depletion and ATPase cation pump failure rapidly ensued. Because of their permeability defect, such depleted cells rapidly became swollen and lysed. Prolonged entrapment in acidic, hypoglycemic regions of the spleen would recapitulate these unfavorable events in vivo. In this regard, splenectomy was followed by an improvement in erythrocyte survival, although evidence of continuing hemolysis was obtained.

Since the initial report by Locke et al. in 1961,1 a number of instances of hereditary stomatocytosis associated with chronic hemolysis of varying severity have been identified.2-7 These disorders share with hereditary spherocytosis an autosomal dominant mode of transmission and abnormal erythrocyte osmotic fragility but differ in displaying, at times, striking abnormalities of erythrocyte cation composition and in failing to improve completely after splenectomy as do patients with spherocytosis.

In this article, we describe a boy in whom severe hemolytic anemia was associated with stomatocytosis. Erythrocyte cation composition was grossly deranged despite extraordinarily high levels of cation transport. An abnormality in the phosphorylation of spectrin, a membrane protein, was discovered similar to, but more severe than, that recently described in hereditary spherocytosis.8 Erythrokinetic studies suggested that the spleen played an important role in the
hemolytic process. When stomatocytic red cells were incubated in vitro under hypoglycemic conditions chosen to resemble the splenic environment in vivo, rapid metabolic depletion ensued, accompanied by swelling, increased rigidity, and eventually hemolysis. That similar events occurred in vivo was suggested by the patient’s subsequent favorable response to splenectomy.

MATERIALS AND METHODS

Routine hematologic counts, osmotic fragility, hemoglobin electrophoresis, hemoglobin heat stability, ferrokinetics, and DF32P autologous red cell survival were done by the methods cited.

Erythrocyte enzymes, including hexokinase, phosphoglucoisomerase, phosphofructokinase, aldolase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, pyruvate kinase, lactate dehydrogenase, adenylate kinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, glutathione reductase, glutathione peroxidase, and transaminase, were assayed spectrophotometrically. Mg2+-dependent RBC membrane ATPase was measured in RBC ghosts by the method of Nakao et al. RBC metabolic intermediates were determined in perchloric acid extracts. Glucose-6-phosphate, fructose-6-phosphate, fructose 1, 6 diphosphate, glyceraldehyde-3-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, 2-phosphoglycerate, phospho-enol-pyruvate, and pyruvate were assayed fluorometrically. RBC ATP, 2, 3-DPG, and glutathione were measured spectrophotometrically. Oxygen affinity was measured at 37°C following equilibration of whole-blood samples with gases of known oxygen tension. The results were corrected to a pH of 7.4. Red cell stromal lipids were fractionated and quantitated by thin-layer chromatography and determination of lipid phosphorus. The extent of phosphorylation of spectrin as described in detail elsewhere was determined postsplenectomy by incubating erythrocyte membrane ghosts prepared by the method of Dodge and his co-workers with ATP in the presence of Mg2+ for 1 hr at 37°C. The ghosts were then solubilized in 1% SDS, and spectrin was isolated by electrophoresis in 5% polyacrylamide gel. Frozen gels were sliced in 1- or 2-mm segments, transferred to scintillation vials, dissolved in 30% H2O2, and counted by Cerenkov radiation in a scintillation counter.

RBC cation concentrations were measured by flame photometry after washing three times in isosmotic Tris-MgCl2 (pH 7.4). Cell water was measured by dessication at 70°C to constant weight (approximately 48 hr).

Bidirectional cation flux was measured with 42K and 24Na. RBC were washed three times, suspended in Krebs-Henseleit buffer, pH 7.4, then incubated at 37°C in a shaking waterbath. pH was maintained at 7.4 ± 0.05 by a gasometric pH stat. For control studies, samples were removed hourly for determination of cell supernatant Na+ and K+ concentrations and for measurement of radioactivity. Duplicate aliquots of either washed cells or supernatant were counted in a well-type gamma counter.

Due to their unusually rapid flux rates, it was necessary to obtain samples every 5–10 min when hereditary stomatocytosis were studied. In some instances, net fluxes were determined without isotopes by serial measurement of changes in intracellular K+ and Na+ concentrations. In these studies, changes in intracellular K+ and Na+ were also directly measured using MgCl2 washed cell suspensions.

For in vitro studies of cell metabolism, washed erythrocytes were incubated in Krebs-Henseleit buffer, pH 7.4, as previously described, except that pH control was achieved with a gasometric pH stat device. At intervals, samples were removed and perchloric acid extracts prepared for analysis of intermediates. Glucose and lactate were assayed spectrophotometrically.

The portion of glycolysis devoted to cation transport was determined by measurement of ouabain-inhibitable lactate production. Work involved in active cation transport was calculated as previously described. Efficiency of pumping was determined by dividing the actual pump work per millimole lactate by 18 (18 Cal. is the theoretical yield of energy from the glycolytic generation of 1 mM of lactate).

The rate of passage of dilute RBC suspensions through 8-μm Millipore filters was determined at 37°C using 15 cm water pressure as previously described. Informed consent was obtained from the parents prior to all procedures involving the patient.
CASE REPORT

W.D. required exchange transfusion for hyperbilirubinemia as a newborn and soon thereafter developed chronic anemia and splenomegaly. During infancy and early childhood, the hemoglobin ranged from 3.6 to 9.7 g/100 ml and the reticulocyte count from 20% to 47%. Transfusions were required twice during infancy and on four subsequent occasions from age 3 yr 7 mo to 4 yr 7 mo for exacerbations of anemia, usually associated with infections.

When first seen by us at 3 yr 7 mo of age, he was pale, icteric, and small in stature (height, third percentile; weight, thirty-fifth percentile). Both spleen and liver were palpably enlarged, and there was clinical and radiographic evidence of extreme marrow hyperplasia, resembling that seen in thalassemia major. Other abnormalities included extreme myopia, nystagmoid eye movement, a 4-5-cm hemangioma near the right buttock, and minor structural deformities of both third toes. At 4 yr 4 mo of age, the hemoglobin was 8.0 g/100 ml, the hematocrit was 25.7%, the red count was 2.12 x 10^6/cu mm, the MCV was 122 μl, the MCH was 28.4 g, the MCHC was 31.3%, and the reticulocyte count was 34.6%. The haptoglobin was 0 mg/100 ml, the indirect bilirubin was 2.9 g/100 ml, and the plasma hemoglobin was 9.2 mg/100 ml. A bone marrow aspirate showed extreme erythroid hyperplasia. Serum iron was 110 μg/100 ml, and iron-binding capacity was 279 μg/100 ml. Electrophoresis revealed no abnormal hemoglobin, and hemoglobin heat stability at 50°C was normal. The concentration of hemoglobin F was 5.8%. Whole-blood oxygen affinity was slightly decreased (p50 = 27.8 mm Hg; nI = 25-26). All erythrocyte glycolytic intermediates and all assayed enzymes of the glycolytic pathway were normal or increased in activity, consistent with the extremely young, reticulocyte-rich population of cells surveyed. The ascorbate-cyanide test was normal, as were red cell glutathione content and assayed enzymes of the pentose phosphate pathway. Ferrokinetic studies (Table 1) showed an extremely rapid plasma iron turnover and a remarkable increase in daily hemoglobin synthesis. Mean erythron life span, calculated from 59Fe turnover, was 3.7 days, whereas mean circulating red cell life span (DF32P) was 7.2 days. Organ scanning disclosed rapid sequestration of newly 59Fe-labeled erythrocytes in the spleen, the spleen:heart ratio reaching a maximum of 2.2:1 at 3 days.

Splenectomy was performed without incident at 4 yr 7 mo of age. The spleen weighed 430 g and was congested with erythrocytes. Examination of scrapings of the cut surface of the spleen under phase microscopy revealed only occasional stomatocytes. The majority of erythrocytes had the appearance of macrospherocytes. During 18 mo of observation after splenectomy, no further transfusions were required. The hemoglobin rose to 10.4–11.6 g/100 ml, and the mean of numerous absolute reticulocyte counts fell from 8.57 x 10^4/μl presplenectomy to 5.16 x 10^4/μl postsplenectomy. Sixteen months postsplenectomy the MCV was 115 μl, the MCH was 36 μg, and the MCHC was 29.3%. Evaluation of erythrokinetics at this time (Table 1) demonstrated little improvement in the survival of DF32P-labeled circulating red cells but a nearly threefold increase in the mean erythron life span and a corresponding reduction in plasma iron turnover and daily hemoglobin synthesis.

RESULTS

Nature of the Erythrocyte Abnormality

Prior to splenectomy, 10%-15% of erythrocytes examined on a Wright-stained blood smear were stomatocytes; postsplenectomy, this percentage in-

<table>
<thead>
<tr>
<th>Table 1. Erythrokinetics in Hereditary Stomatocytosis</th>
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<td>Plasma iron turnover (mg/hr/liter blood)</td>
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<td>Maximum net incorporation of 59Fe into circulating erythrocytes (%)</td>
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<tr>
<td>Hemoglobin synthesis (g/liter day)</td>
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<tr>
<td>59Fe mean erythron lifespan (days)</td>
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<td>DF32P circulating erythrocyte life span</td>
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Table 2. Cation Flux in Fresh Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>K⁺ Influx (meq/liter cells/hr)</th>
<th>Na⁺ Efflux (meq/liter cells/hr)</th>
<th>K⁺/Na⁺ Ratio</th>
<th>K⁺ Concentration (meq/liter cells)</th>
<th>Na⁺ Concentration (meq/liter cells)</th>
<th>Water Content (ml/liter cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.D. Presplenectomy</td>
<td>27.9</td>
<td>27.9</td>
<td>1.0</td>
<td>54.8</td>
<td>61.0</td>
<td>692</td>
</tr>
<tr>
<td>Postsplenectomy</td>
<td>21.6</td>
<td>30.9</td>
<td>1.43</td>
<td>37.0</td>
<td>73.0</td>
<td>704</td>
</tr>
<tr>
<td>Normal range†</td>
<td>1.05–1.68</td>
<td>1.50–2.31</td>
<td>1.1–1.8</td>
<td>90–103</td>
<td>6–12</td>
<td>670–690</td>
</tr>
<tr>
<td>Reticulocytosis</td>
<td>1.8</td>
<td>2.2</td>
<td>1.22</td>
<td>86.1</td>
<td>7.5</td>
<td>—</td>
</tr>
</tbody>
</table>

*Active transport refers to that portion of total cation flux which is ouabain inhibitable. The figures presented represent net fluxes, obtained by measurement of changes in intracellular cation concentration during incubation. Simultaneous measurement of ⁴²K influx and ³⁴Na efflux postsplenectomy yielded values similar to those obtained by measurement of net fluxes (⁴²K influx, 18.9 meq/liter cells/hr; ³⁴Na efflux, 24.2 meq/liter cells/hr).

†Values presented are for mature erythrocytes. Active transport may be increased as much as twofold in reticulocyte-rich blood, and cell water is slightly higher (up to 71%). For comparative purposes, data obtained from a patient with hemolytic elliptocytosis whose reticulocyte count was 9.6% are also shown.

The changes in osmotic fragility reflected profound abnormalities in membrane cation permeability as shown in Table 2. Active transport of both Na⁺ (Na⁺ efflux) and K⁺ (K⁺ influx) against concentration gradients were more than tenfold normal. However, even these remarkable increases in active transport were insufficient to prevent major changes in cation composition. Presplenectomy, the red cell potassium concentration was only half normal, while Na⁺ concentration was increased sixfold. The changes in cation composition were further accentuated following splenectomy. Erythrocyte water content was normal to slightly increased in fresh cells, but following metabolic depletion, cells rapidly became “water logged.” The permeability abnormalities appeared to be limited to erythrocytes, as the cation concentration and water content of fresh specimens of liver, spleen, muscle, and platelets from W.D. resembled values obtained in patients with other hemolytic anemias.

Membrane Structure and Biochemistry

Total erythrocyte membrane lipids, as well as phospholipid composition, analyzed on three occasions both pre- and postsplenectomy, were normal. Disc gel electrophoresis of solubilized membrane proteins showed a normal pattern with normal amounts of spectrin. However, protein kinase-mediated incorporation of ³²P from labeled ATP into the spectrin of membrane ghosts was only 20% of normal (W.D. = 80 pmoles P, per mg protein; control, 458 pmoles P, per mg protein) and resembled values obtained in hereditary spherocytosis (119–210 pmoles P, per mg protein).

Studies of Cell Metabolism

Lactate production in vitro (Table 3) was approximately six times normal for mature erythrocytes and two to three times the value usually obtained in
Table 3. Energy Requirements for Cation Transport*

<table>
<thead>
<tr>
<th></th>
<th>Total Lactate Production (mM/liter cells/hr)</th>
<th>Ouabain-inhibitable Lactate Production (mM/liter cells/hr)</th>
<th>Pump Work (Cal/hr/liter cells)</th>
<th>Pump Work/mM Lactate (Cal/mM)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient W.D.</td>
<td>19.4</td>
<td>11.5</td>
<td>45.5</td>
<td>3.96</td>
<td>22</td>
</tr>
<tr>
<td>Normal control</td>
<td>1.8</td>
<td>0.33</td>
<td>5.12</td>
<td>15.52</td>
<td>86</td>
</tr>
<tr>
<td>High-reticulocyte</td>
<td>4.2</td>
<td>0.45</td>
<td>7.12</td>
<td>15.82</td>
<td>88</td>
</tr>
</tbody>
</table>

*Values shown are for cells suspended in autologous plasma. Virtually identical results were obtained when cells were incubated in Krebs-Henseleit buffer.
†Hemolytic elliptocytosis, see Table 2.

reticulocyte-rich blood. More than half of the observed lactate production was ouabain inhibitable and, therefore, devoted to cation transport. Addition of ethacrynic acid, thought by some to be an inhibitor of a membrane cation pump distinct from that inhibited by ouabain, led to further inhibition of glycolysis. Sixty-five to seventy-one per cent of lactate production was inhibited by the combination of ouabain and ethacrynic acid. Cell ATP levels remained stable during incubation with glucose for 4 hr and actually rose 10%–20% if ouabain or ouabain and ethacrynic acid were present. The amount of pump work accomplished per millimole of lactate produced was estimated by simultaneous measurement of net flux of Na⁺ and K⁺ and the gradients against which these fluxes operated together with the lactate production in the presence or absence of ouabain. As seen in the last column, red cells from patient W.D. appeared to utilize glycolysis for cation transport less efficiently than normal. Consistent with their remarkable energy requirements, red cells from patient W.D. deteriorated rapidly when deprived of glucose. Figure 1 compares the effects of glucose deprivation of normal erythrocytes and of post-splenectomy erythrocytes from patient W.D. Although only freshly drawn blood from patient W.D. was used, the preparation of cells for incubation by washing three times in glucose-free buffer was a sufficient stress to lower cell ATP from 2.21 (normal, 1.59 ± 0.16) to 1.16 mmoles per liter cells and cell 2,3-DPG from 4.62 (normal, 4.54 ± 0.57) to 2.93 mmoles per liter cells. Normal cells were virtually unchanged during an equivalent period of preparation. During subsequent incubation without glucose, ATP levels fell swiftly in stomatocytes and more slowly in normal cells. Incubation with glucose prevented loss of ATP in both normal cells and in those of patient W.D.

There was appreciable swelling of glucose-deprived stomatocytes. Within 2½ hr the MCV rose from 120 to 140, and the cell water increased from 68.5% to 74.6%. During incubation, glucose-deprived cells lost 1.09 mM of ATP and 2 mM of 2,3-DPG. Sodium gain eventually exceeded potassium loss, as shown in Fig. 1, increasing the intracellular osmotic pressure. Within 2½ hr, the swollen, metabolically depleted stomatocytes began to hemolyze. No hemolysis of control erythrocytes was noted.

Cell Deformability

The deformability of hereditary stomatocytes was estimated by measurement of the flow rate of dilute solutions of cells through 8-μm pore size Millipore filters...
Fig. 1. Effects of glucose deprivation on hereditary stomatocytes. Erythrocytes from W.D. (---), from a normal control (-----), or from a high-reticulocyte (10%) control (---) with anemia responding to folic acid therapy were incubated at 37°C in glucose-free medium at a pH of 7.4.

(Fig. 2). The results indicated that even fresh stomatocytes were less deformable than normal. Furthermore, the rigidity of these cells rapidly increased during brief periods of incubation in vitro at 4°C, 22°C, or 37°C, presumably as a consequence of metabolic depletion which, as mentioned previously, accompanied even the briefest in vitro procedure at normal or even at reduced temperature.

Family Studies

Patient W.D., of Portuguese ancestry, was the sole member of his family known to have anemia or jaundice. His parents, his two brothers, and his two sisters were clinically and hematologically normal. Red cell Na+ and K+ concentrations were also normal in both parents and siblings. His mother had aborted two pregnancies, one at 4½ mo and the other at 6½ mo. The first had an oddly shaped face and the second had a shortening of one arm. Complete blood grouping of the patient and both his parents did not exclude paternity. The Rh null blood type was not present in the patient or his parents.

DISCUSSION

The heterogeneous group of disorders in which stomatocytes appear on the peripheral blood smear has recently been reviewed.7 In many instances, stomatocytes are not associated with reduced erythrocyte survival. However, occasionally, as in patient W.D., stomatocytes are the consequence of a major abnormality in membrane permeability and result in serious hemolytic anemia.
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The pathophysiology of hemolysis in these disorders has not been defined. Mild G6PD deficiency\(^1\) and inherited deficiencies in erythrocyte glutathione\(^2\)\(^5\) have been described in several pedigrees. In most instances, however, attention has focused upon the dramatic changes in membrane integrity and cation composition. Although these changes imply an abnormality in membrane composition or structure, the precise nature of this abnormality is still obscure. Membrane lipid composition, normal in W.D., has also been unremarkable in other patients.\(^3\)\(^4\)\(^7\) Membrane-associated enzymes, notably Mg\(^{2+}\)-dependent ATPase, have also exhibited normal activity.

A defect in membrane glycoproteins has been suggested by the association of the Rh null blood group with hereditary hemolytic anemia in several cases of stomatocytosis.\(^27\) However, the normal Rh type of W.D. and his parents eliminates such a possibility here. Changes in membrane contractile proteins have been previously described in hereditary spherocytosis\(^28\) and have been implicated in the vinblastine-induced conversion of biconcave disc-shaped erythrocytes into stomato-spherocytes.\(^29\) The diminished phosphorylation of spectrin by ATP noted in stomatocytes from W.D. is the first direct evidence for abnormality of a membrane protein in hereditary stomatocytosis. If this defect is parallel to that recently described in hereditary spherocytosis,\(^8\) the abnormality would appear to be in a defective membrane protein kinase rather
than in the spectrin itself. Although the precise function of protein kinase in membrane physiology is not known, studies in muscle and brain indicate a relationship to permeability. Greenquist and Shohet have also proposed a role for protein kinase in the maintenance of erythrocyte shape and deformability. Their studies suggested that the changes in membrane contractile proteins noted by Jacob in hereditary spherocytes might be mediated through a conformational change in the substrate proteins of the kinase secondary to deficient phosphorylation. Since protein kinase activity in the erythrocytes of W.D. is as low or lower than that characteristic of hereditary spherocytosis, defective phosphorylation of membrane protein may play an equally important role in the induction of the shape changes as well as the permeability and pump efficiency defects seen in hereditary stomatocytosis.

Their remarkable energy requirements for cation transport render hereditary stomatocytes unusually vulnerable to the effects of metabolic depletion. In an attempt to simulate the hypoglycemic splenic environment, stomatocytes were deprived of glucose during incubation in vitro. Metabolic depletion was rapid and was followed by intracellular accumulation of sodium, cell swelling, and hemolysis. Similar changes occurred much more gradually in normal cells.

Although changes in K permeability accompany ATP depletion of stomatocytes, they are overshadowed by the even greater abnormalities of Na permeability which characterize these cells and rapidly result in intracellular Na accumulation, requisite gain of water, cell swelling, and the production of a bloated “hydrocyte.” In part, this Na influx is due to loss of the ATP requiring cation transport mechanism. Furthermore, there is depletion of normal intracellular anions, notably 2,3-DPG, in stomatocytes. In contrast, in erythrocyte pyruvate kinase deficiency, due to the location of the enzymatic abnormality, 2,3-DPG levels are elevated or normal even after a considerable period of metabolic depletion. As shown by Parker, 2,3-DPG depletion leads to a net increase in osmotically active intracellular anions as monovalent anions (usually Cl) enter the cell to maintain electrochemical neutrality. Thus, following metabolic depletion there is an increase in intracellular osmotic pressure in stomatocytosis associated with Na gain. In pyruvate kinase deficiency, metabolic depletion results in net cation (K) loss, decreased intracellular osmotic pressure, and cellular dehydration.

Reported cases of stomatocytosis with ion transport abnormalities may be separated easily into two groups, as shown in Table 4: one resembling pyruvate kinase deficiency or the family recently described by Glader and his co-workers, in which dessicytosis is the predominant lesion, and the other in which hydrocytosis is the predominant lesion. In contrast, in hereditary stomatocytosis, the opposite seems to be the case. Furthermore, when hydrocytosis is the predominant lesion in stomatocytosis, there is greater derangement of cation composition (see Table 4).

As would be expected following ATP depletion, incubated stomatocytes were less deformable than normal. However, in contrast to previous studies, even fresh, metabolically replete, stomatocytes exhibited reduced deformability. In view of the extreme metabolic lability of such cells in vitro, even during brief
Table 4. Reported Cases of Hereditary Stomatocytosis With Abnormalities of Ion and Water Transport

<table>
<thead>
<tr>
<th></th>
<th>Osmotic Fragility (Unincubated)</th>
<th>MCHC (%)</th>
<th>RBC Cations</th>
<th>Results of Splenectomy</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Na⁺ (meq/liter cells)</td>
<td>K⁺ (meq/liter cells)</td>
</tr>
<tr>
<td>Dessicytosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miller et al.²</td>
<td>Decreased</td>
<td>33.5-34</td>
<td>20-21.5</td>
<td>85-87</td>
</tr>
<tr>
<td>Honig et al.³</td>
<td>Decreased</td>
<td>35.9</td>
<td>6.8</td>
<td>94.6</td>
</tr>
<tr>
<td>Miller.*</td>
<td>Decreased, fragile tail</td>
<td>39.40</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hydrocytosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oski et al.⁴</td>
<td>Increased</td>
<td>28.2-29.3</td>
<td>39-53</td>
<td>73-86</td>
</tr>
<tr>
<td>Lo et al.⁵</td>
<td>Increased</td>
<td>28.9</td>
<td>65.2</td>
<td>38.6</td>
</tr>
<tr>
<td>Zarkowsky et al.²</td>
<td>Increased</td>
<td>23-24</td>
<td>91-108</td>
<td>35-47</td>
</tr>
<tr>
<td>Patient W.D.</td>
<td>Increased</td>
<td>29.3-31.7</td>
<td>61-73</td>
<td>37-55</td>
</tr>
<tr>
<td>Normal</td>
<td>—</td>
<td>32-36</td>
<td>6-12</td>
<td>90-103</td>
</tr>
</tbody>
</table>

*Unlike other cases of dessicytosis, incubation of erythrocytes from this patient results in greatly increased osmotic fragility. Since no RBC cation data are available, the inclusion of this case in the table is tentative.

†Splenectomy not done.

preparative procedures, it is probable that even “fresh” stomatocytes are partially metabolically depleted. Nevertheless, it is clear that only a brief exposure to conditions unfavorable for glycolysis will render stomatocytes rigid. Within the splenic pulp, deformability is an essential requirement in order that erythrocytes may traverse pores with a maximum diameter of 3 μ. Because of their rigidity, stomatocytes would be expected to be detained within the splenic pulp. The acidic, hypoglycemic splenic environment, unfavorable for glycolysis, should generate the same sequence of events observed in glucose-deprived stomatocytes in vitro. Indeed, ferrokinetic studies in W.D. indicated significant sequestration and hemolysis of erythrocytes in the spleen, and splenectomy reduced the intensity of hemolysis, as evidenced by a threefold improvement in the mean erythron life span, a reduction in the absolute reticulocyte count, and amelioration of anemia. Unlike hereditary spherocytes, the metabolic requirements of stomatocytes from patients such as W.D. are so extreme as to be compromised even in the absence of the spleen, and significant hemolysis persists postsplenectomy. Nonetheless, based on the favorable response of W.D. and other similar patients¹³ (see Table 4), it seems appropriate to consider splenectomy if isotopic studies indicate splenic sequestration.

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REFERENCES


27. Sturgeon P: Hematological observations
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on the anemia associated with blood type Rh null. Blood 36:310, 1970


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WC Jr Mentzer, WB Smith, J Goldstone and SB Shohet