Hereditary Spherocytosis

I. Clinical, Hematologic and Genetic Features in 28 Cases, with Particular Reference to the Osmotic and Mechanical Fragility of Incubated Erythrocytes

By Lawrence E. Young, M.D., Mary Jane Izzo, M.S. and Richard F. Platter, M.D.

Clinical, hematologic and genetic data on 28 cases of hereditary spherocytosis are presented in this report for the purpose of characterizing this disorder as completely as possible with available methods. Abnormalities of the red corpuscles in affected persons are described in an effort to formulate diagnostic criteria that may be particularly helpful in evaluating patients having no demonstrably affected relatives. The hereditary aspects of the disease are discussed, especially from the standpoints of gene mutation and variations in gene penetrance or expression.

Determinations of osmotic and mechanical fragility of the erythrocytes were made in every case using both freshly drawn defibrinated blood and blood incubated at body temperature for 24 hours. Increased mechanical fragility of freshly drawn cells and greater than normal increase in osmotic fragility (and usually of mechanical fragility) of incubated cells were demonstrated in each patient. These abnormalities were repeatedly found in all cases, even after splenectomy or when the hemolytic process was relatively inactive, in contrast to the findings in patients representing other types of hemolytic disease encountered in this clinic. Determinations of fragility of incubated red cells not only proved useful in detecting abnormalities of the red corpuscle but also yielded results of interest in relation to theories of red cell destruction, as first shown by Emerson, Shen, Ham and Castle.15, 16

The condition best known by the terms congenital hemolytic jaundice, familial hemolytic jaundice, acholic jaundice, congenital hemolytic anemia, familial hemolytic anemia and spherocytic or globe-cell anemia is most appropriately called hereditary spherocytosis. This designation is preferred because the disorder is thought to be inherited as a Mendelian dominant and is characterized by the presence of abnormally thick red blood cells called spherocytes, while either anemia or jaundice or both may be lacking.

The exact nature of the abnormality of the red cells in this disease is not yet clear.5, 6, 9, 10, 17, 30, 41 Some observers believe that erythropoiesis is defective and that the cells are abnormal when released from the marrow into the circulation. Others have contended that the spheroidal cells may be produced by the action of lytic agents.9, 10 Although this controversy is not settled, certain characteris-

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ties of the cells are well established and others will be discussed in this paper and in subsequent reports. Observations on the role of the spleen in this disease are presented separately. It should be emphasized here, however, that clearly demonstrable abnormalities of the erythrocyte persist after splenectomy even though anemia and jaundice are regularly relieved.

METHODS

Certified hemocytometers and diluting pipets were used in enumerating red blood cells. Counts used in computing mean corpuscular volume and thickness were the average of 4 conventional counts, each made from a separate pipet. Reticulocytes were stained with brilliant cresyl blue and counter-stained with Wright's stain. The proportion of red cells containing reticulum was determined by examining 1,000 cells.

The hemoglobin content of whole blood was measured with either the Evelyn or Kromatol photometer employing tubes containing 10 ml. of hemoglobin solution. The solutions were prepared by delivering either 0.02 ml. or 0.05 ml. of whole blood with calibrated pipets into distilled water. One drop of concentrated ammonium hydroxide was added to each tube just before the colorimetric reading was made. The concentration of bilirubin in serum or plasma was determined by Dueci and Watson's modification of the method of Malloy and Evelyn.

Mean diameter of red blood cells was computed from measurements made with a calibrated micrometer or was determined by use of the Haden-Hausser erythrocytometer. The latter method actually measures the mode of cell diameters, but in most instances the figures read on the erythrocytometer did not differ significantly from the mean values determined with the micrometer. Mean corpuscular thickness was calculated by use of the formula,

\[ MCT = \frac{\text{mean corpuscular volume}}{\left(\frac{\text{mean diameter}}{2}\right)^2} \]

Osmotic fragility of erythrocytes was determined quantitatively by the method of Shen, Ham and Fleming and mechanical fragility by the method of Shen, Castle and Fleming, using both freshly drawn defibrinated blood and defibrinated blood that had been incubated for 24 hours at 37 C. under sterile conditions. The pH of the blood used in measurements of osmotic fragility was adjusted to the range 7.35 to 7.45 for fresh blood and 7.30 to 7.40 for incubated blood by equilibration with a mixture of 10 per cent CO\textsubscript{2} and 90 per cent O\textsubscript{2}. The concentration of sodium chloride in the series of tubes used in measuring osmotic fragility decreased by decrements of 0.5 per cent in the range from 0.90 to 0.70 and from 0.20 to 0.05 per cent salt, and by decrements of 0.02 per cent in the range from 0.70 to 0.20 per cent salt. Tonocities of the hypotonic solutions of sodium chloride were corrected to allow for the addition of plasma, since one part whole blood was mixed with 10 parts salt solution in the tests of osmotic fragility. The corrections took into account the hematocrit of the blood being examined and were based upon the assumption that the tonicity of plasma is equivalent to 0.90 per cent sodium chloride. The buffering action of the blood was sufficient to maintain the pH of the blood-saline mixtures at 7.35 to 7.45 despite the fact that the salt solutions did not contain buffer.

The hematocrit was adjusted to 35 per cent for determinations of mechanical fragility, while the hematocrit of samples used for studies on osmotic fragility was left unaltered. A photograph of the rotator used in this laboratory for subjecting red cells to the trauma of rolling glass heads is shown in figure 1. The distance from the center of the flask to the center of the rotating wheel is 8.0 cm. Duplicate samples of 0.5 ml. of blood were rotated for 90 minutes at 28 r.p.m. in 50 ml. Erlenmeyer flasks containing 10 spherical glass beads of uniform size (4.0 mm. diameter) and shape. Rotation was routinely performed at room temperatures of 22 to 28 C. in the tests of mechanical fragility.

A simpler "qualitative" test of osmotic fragility was carried out in some instances in conjunction with the more elaborate procedure. Five ml. of freshly drawn venous blood were
placed in a cylindrical bottle containing 4 mg. of potassium oxalate and 6 mg. of ammonium oxalate. The diameter of the bottle was 2.5 cm. and the height of the bottle was 5.0 cm. After agitating the blood in the bottle by rotation in the hand for 3 minutes, the pH was usually 7.4 to 7.5 but was occasionally as high as 7.7. One drop (approximately 0.05 ml.) of the blood was added to each of a series of tubes containing 1.0 ml. of hypotonic solution of sodium chloride; the salt concentration decreased in the series by decrements of 0.02 per cent. The blood and saline were thoroughly mixed by inversion and the tubes were then placed in a refrigerator at 4 C. for 24 hours; in more recent tests refrigeration for only 4 hours proved to be adequate for determining the point of initial hemolysis without centrifugation of the tubes. At the end of this period the tubes were inspected to determine the highest concentration of salt at which hemolysis could be detected with the naked eye, and the highest concentration at which the cells appeared to be completely hemolyzed. The concentrations recorded were not corrected for the tonicity of the small amounts of plasma added to the NaCl in performing the tests. The simplified test of osmotic fragility was also carried out with defibrinated venous blood that had been incubated at 37 C. for 24 hours. The pH of freshly

![Rotator used in determinations of mechanical fragility by the method of Shen, Castle and Fleming.](image)

Fig. 1.—Rotator used in determinations of mechanical fragility by the method of Shen, Castle and Fleming.14

drawn defibrinated blood was usually in the range 7.8 to 8.1 but after incubation without adjustment the pH was usually in the range 7.3 to 7.5.

Defibrination was routinely performed throughout this study by using sterile Erlenmeyer flasks of 125 ml. capacity containing one glass bead of 4.0 mm. diameter per ml. of blood. The flasks were gently, but continuously, swirled by hand until all of the beads had been trapped in the fibrin clot, usually for an additional period of approximately 2 minutes. Blood defibrinated in this way showed little or no hemolysis. Immediately after defibrination the blood was transferred to another sterile Erlenmeyer flask (containing no beads) from which it was subsequently removed for the various tests.

Antiglobulin (Coombs) tests15 were made on the erythrocytes of each patient by mixing 0.1 ml. of a 2 per cent suspension of thrice washed cells in 0.85 per cent solution of sodium chloride with an equal volume of a 1:10 dilution of anti-human-serum rabbit serum in an 8 X 60 mm. tube. The mixture was allowed to stand at room temperature for 2 hours (15 minutes in recent tests proved adequate), after which the tube was centrifuged at 1,000 r.p.m. for 1 minute before macroscopic and microscopic examinations were made to detect the presence or absence of agglutination. The same procedure was carried out on normal cells as a negative control. The rabbit sera employed were stored in small tubes at -18 C. and were tested at frequent intervals against Rh positive human cells sensitized in vitro with human serum containing incomplete anti-Rho (anti-D) antibodies and against cells
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from patients with acquired hemolytic anemia. The rabbit sera in dilutions of from 1:320 to 1:2,560 regularly agglutinated such sensitized corpuscles.

Lysolecin fragility was determined by comparing the hemolytic action of lysolecin on the cells of patients having hereditary spherocytosis with its action on cells of normal individuals. The lysolecin was obtained by incubating cotton mouth mucosai venon with egg yolks, adding 95 per cent alcohol, drying, extracting with ether and redrying.

Observations

Laboratory Data

The principal laboratory findings in 28 individuals with hereditary spherocytosis and in the controversial case M.S., are summarized in table 1. Diagnosis in Cases 1 to 18 and 23 to 25 is considered well established since one or more affected relatives were encountered in each of the 9 families involved, as will be shown in figure 7. In Cases 19 to 22 affected relatives were not found but the diagnosis was supported by persistence of spherocytosis after splenectomy, in association with other laboratory findings which will be discussed later. Diagnosis is less certain in Cases 26 to 28 because both affected relatives and post-splenectomy observations are lacking. The laboratory findings in these cases are, however, entirely in accord with a diagnosis of hereditary spherocytosis, and these individuals have therefore been included in the numbered series with some reservation. When more than one examination was made prior to splenectomy the findings at the time of the lowest pre-splenectomy red cell count were recorded in the table. The post-splenectomy figures recorded were obtained at the time of the first examination 4 or more months after operation. Findings at the time of additional examinations are given in table 1 for Cases 22, 28 and M.S. since these cases will be the subject of special comment.

Most of the 79 persons examined during this study were patients or relatives of patients in the Strong Memorial Hospital and the Clifton Springs Sanitarium and Clinic. Examinations prior to splenectomy in Cases 2, 7, 12, 13, 19 to 22 and 24 were made by observers other than the authors. In most of these instances the hemoglobin determinations were carried out by the acid hematin method employing a standardized Sahli apparatus, and osmotic fragility of the red cells was estimated by the "qualitative" method previously described. Satisfactory comparison of pre- and post-splenectomy findings in individual patients can therefore be made only in Cases 3, 10, 14, 17 and M.S. Comparison of pre- and post-splenectomy observations on the groups of patients concerned can also be made with profit, however, as will be pointed out subsequently. Soon after splenectomy Patients 16 (C. D.) and No. 26 (L. E.) moved to distant cities. Since large numbers of normal donated erythrocytes were still present in the circulation of these patients at the time of discharge from the hospital, incomplete post-splenectomy observations on Case 26 are recorded in table 1 and no post-operative data are given for Case 16.

Antiglobulin (Coombs) tests were carried out on erythrocytes from each of

* Case 27 was examined at the St. Mary's Hospital of Rochester through the courtesy of Dr. Jacob Adler. Members of the Ash family were examined at the St. Joseph's Hospital, Elmira, N. Y., through the courtesy of Dr. C. M. Hower.

† The authors are indebted to Dr. Charles A. Doan and Dr. Claude-Starr Wright of Columbus, Ohio, for a follow-up report on Case 26.
Table 1.—Laboratory Data on Cases Included in Study

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<tr>
<th>Patient</th>
<th>Pre- or Post-Splenectomy</th>
<th>Age yrs.</th>
<th>Red cells per mm.</th>
<th>Reticulocytes</th>
<th>Total serum bilirubin mg.</th>
<th>MCV u</th>
<th>MCD u</th>
<th>MCT u</th>
<th>Osm. fragility NaCl causing 2% hemolysis</th>
<th>Mechanical fragility NaCl hemolysis</th>
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<td>7.0</td>
<td>2.3</td>
<td>5.4</td>
<td>neg.</td>
</tr>
</tbody>
</table>

* Mean corpuscular diameter measured with Haden-Hauser erythrocytometer; unmeasured measurements were made with calibrated ocular micrometer.
† Highest concentration of sodium chloride causing grossly detectable hemolysis in "qualitative" test of osmotic fragility made by observers other than authors.
‡ Normal values for osmotic and mechanical fragilities of fresh and incubated red cells are given in tables 5 and 6.
§ Cold agglutinin titers recorded as "negative" are actually "less than 1:10" (final dilution of serum after addition of cell suspension).

Figures for mechanical fragility of Case 2 are not tabulated because of technical difficulties encountered in making the determinations in this case.

†† Observations on Case 24 were made only 10 weeks after operation at which time transfused red cells were present in the circulation in unknown numbers.
** Iterus index.
the patients included in this study and were negative in all instances. In only 19 cases were the tests made prior to splenectomy. The negative antiglobulin

Table 2.—Determinations of Serum Bilirubin Prior to Splenectomy in Patients with Hereditary Spherocytosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Date of Bilirubin Determination</th>
<th>Serum Bilirubin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>D1'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mg./100 ml.</td>
<td>mg./100 ml.</td>
</tr>
<tr>
<td>1. B. S.</td>
<td>3/27/47</td>
<td>1.9</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>4/16/48</td>
<td>2.2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>4/17/48</td>
<td>5.3*</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>4/18/48</td>
<td>5.4*</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>4/19/48</td>
<td>3.6*</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>4/20/48</td>
<td>3.9*</td>
<td>0.2</td>
</tr>
<tr>
<td>3. S. D.</td>
<td>6/12/47</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>7/ 7/49</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>4. D. D.</td>
<td>5/22/47</td>
<td>1.3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>3/26/49</td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>5. R. S.</td>
<td>3/27/47</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>6/22/48</td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>6. H. S.</td>
<td>9/11/47</td>
<td>2.3</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>12/10/46</td>
<td>1.4</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>6/22/48</td>
<td>4.6</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>9/ 7/49</td>
<td>1.2</td>
<td>—</td>
</tr>
<tr>
<td>11. J. D.</td>
<td>9/28/46</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>12/ 5/46</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>12/10/46</td>
<td>1.7*</td>
<td>0.3</td>
</tr>
<tr>
<td>14. J. N.</td>
<td>12/19/46</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>3/ 8/47</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>6/ 7/47</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>15. R. N.</td>
<td>1/3/47</td>
<td>1.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>12/28/50</td>
<td>1.1</td>
<td>—</td>
</tr>
<tr>
<td>17. F. C.</td>
<td>3/8/50</td>
<td>1.9</td>
<td>—</td>
</tr>
<tr>
<td>18. G. C.</td>
<td>12/28/50</td>
<td>2.1</td>
<td>0.1</td>
</tr>
<tr>
<td>23. J. A.</td>
<td>1.0</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>25. M. A.</td>
<td>3.0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>26. L. E.</td>
<td>1.5</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>
reactions in the post-splenectomy group, like those in the pre-splenectomy group, are nevertheless to be contrasted with the consistently positive results obtained in certain patients with “acquired” hemolytic disease both before and for long periods after splenectomy.1, 18, 30, 45, 50, 53

Cold agglutinin titers against autologous cells were determined by a previously described31 technic and are recorded in table 1. Further details on the titers in relation to splenectomy in Cases 2, 7, 13 and M.S. have been reported separately.31 The only additional patient having cold agglutinins in a titer of 1:128 or above was Case 26 and in this patient the titer was determined only once after splenectomy (at one month) and was found to be 1:640. In no case was an elevated cold agglutinin titer associated with symptoms of viral infection, and in our experience it did not appear that the cold agglutinin titer was related to the rate of red cell destruction.

Nine of the individuals studied had little or no anemia when examined prior to splenectomy. None of the patients had a “crisis” during the period of observation and consequently there were no opportunities to observe abnormalities of the marrow such as recently reported by Owren32 and Dameshek and Bloom.9 Figures for total serum bilirubin and the direct one minute (D1’) fraction in pre-splenectomy cases are recorded in table 2. The relatively low (mostly normal) D1’ values in this group of patients with hemolytic disease are in keeping with the experience of Ducie and Watson.14 Interpretation of the D1’ readings is difficult, however, in view of the recent observations of Klatskin and Drill.28 The low values for total bilirubin and the normal red cell counts in some persons with spheroidal red cells support the argument for using the generally applicable term “hereditary spherocytosis” in place of “acholuric jaundice,” “congenital hemolytic anemia” and other designations that are often inappropriate in describing this disorder.

Mildly affected individuals having little or no jaundice are sometimes referred to as having only “spherocytic trait.” Whether or not such persons may at some time during their lives develop anemia and icterus cannot be predicted on the basis of our experience or the reported observations of others. Repeated examinations over long periods of time are needed in order to acquire a better understanding of the natural history of this disease. Observations on the nonsplenectomized patients listed in table 1 have been made over periods of from one to five years and have thus far revealed only minor fluctuations. An effort should be made to repeat these examinations on the unoperated patients at appropriate
intervals in the future, and especially during periods when these individuals are suffering from infectious diseases.

Spherocytes were observed in fixed smears in each case included in this study. Patients with little or no anemia usually had relatively few cells that were considered spheroidal and examination of many well-stained microscopic fields was necessary for adequate appraisal. Since cells that have been over stained may look like spherocytes, it is essential that impressions be based only on fields in which there are many cells showing the normal central area of relative transluency. It is admittedly difficult in some instances to state whether or not the spheroidal appearance of a few of the red cells in fixed smears and in wet preparations is significantly different from that seen in normal blood. There is an obvious need for more objective methods of estimating degrees of spherocytosis.

Estimates of mean corpuscular thickness, calculated from the figures for mean corpuscular volume and diameter, are subject to considerable error, i.e., errors in red cell counting and in measuring diameters with either a hemometer or ocular micrometer. The average values for MCV, MCD and MCT recorded in table 3 are based on small numbers of patients and it should be noted in table 3 that only 6 of the 14 patients whose MCT was computed prior to splenectomy had a red cell count below 4 M. at the time of examination. The differences among the groups listed in table 3 are not striking. Mean corpuscular thickness is generally increased and mean corpuscular diameter decreased significantly in the severely affected cases; in the same cases spherocytosis is quite apparent in smears. In cases with little or no anemia, however, and with minimal spherocytosis evident in smears, the MCD and the calculated MCT are usually not sufficiently abnormal to be of aid in substantiating a diagnosis of spherocytosis.

**Clinical Features**

Certain clinical features encountered in this disease are summarized in table 4. In 6 of the 28 numbered cases there were no symptoms referable to spherocytosis, while 22 had complaints due to anemia or had noted recurrent, mild icterus.

---

**Table 3.**—Mean Corpuscular Volume, Diameter and Thickness in Normal Individuals and in Patients with Hereditary Spherocytosis Before and After Splenectomy

<table>
<thead>
<tr>
<th></th>
<th>MCV</th>
<th>MCD</th>
<th>MCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 normal donors</td>
<td>87e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 patients pre-splenectomy</td>
<td>84r</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 patients post-splenectomy</td>
<td>83r</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Only 6 of the 14 patients whose MCT was computed prior to splenectomy had a red cell count below 4 M. at the time of examination. The differences among the groups listed in table 3 are not striking. Mean corpuscular thickness is generally increased and mean corpuscular diameter decreased significantly in the severely affected cases; in the same cases spherocytosis is quite apparent in smears. In cases with little or no anemia, however, and with minimal spherocytosis evident in smears, the MCD and the calculated MCT are usually not sufficiently abnormal to be of aid in substantiating a diagnosis of spherocytosis.

---

* Through the courtesy of Dr. Stacy R. Mettler of San Francisco we have been informed that T. D., Case 10, developed anemia (RBC 2.72 M., reticulocytes 10 per cent) during a bout of infectious mononucleosis (sheep cell agglutination titer of 1:806). This illness occurred in September 1949, about two years after he was examined in our laboratory. Splenectomy was performed a month later and a month after operation the red cell count was 4.66 M.
frequently associated with malaise. In 14 cases, anemia or jaundice was first
detectable at age 5 or younger, and in 8 patients the age at onset of symptoms
ranged from 10 to 45 years. In cases with minimal symptoms the date of onset
could not be given with accuracy because in many instances the patients were
not aware that they had been "under par" until after splenectomy when they
first learned what it is like to feel "normal."

| Patient | Age when first examined years | Age at onset of symptoms years | Cholecysto-
|---------|-------------------------------|-------------------------------|asis present
|         |                               |                               | Palpable spleen
|         |                               |                               | Spleen removed
|         |                               |                               | Weight of spleen Gm.
|         |                               |                               | Spher-
|         |                               |                               | ocytosis in relatives
| 1. B. S. | 40                           | infancy                        | yes     | yes    | no    | —    | yes
| 2. J. D. | 23                           | 12                             | yes     | yes    | yes   | 844  | yes
| 3. S. D. | 3                            | 3                              | no      | yes    | yes   | 170  | yes
| 4. D. D. | 1                            | no symptoms                    | NE      | no     | no    | —    | yes
| 5. R. S. | 47                           | no symptoms                    | NE      | yes    | no    | —    | yes
| 6. H. D. | 54                           | 44                             | yes     | yes    | no    | —    | yes
| 7. R. S. | 6                            | 5                              | no      | yes    | yes   | 145  | yes
| 8. C. D. | 53                           | no symptoms                    | NE      | no     | no    | —    | yes
| 9. P. D. | 21                           | 21                             | yes     | yes    | yes   | 1018 | yes
| 10. T. D.* | 24                        | 18                             | yes     | yes    | yes   | ?    | yes
| 11. J. D. | 26                           | infancy                        | NE      | yes    | no    | —    | yes
| 12. M. F. | 38                           | 10                             | yes     | yes    | yes   | ?    | yes
| 13. W. T. | 23                           | infancy                        | no      | yes    | yes   | 875  | yes
| 14. J. X. | 48                           | 45                             | no      | yes    | yes   | 1190 | yes
| 15. R. N. | 11                           | no symptoms                    | NE      | no     | no    | —    | yes
| 16. C. D. | 42                           | 26                             | yes     | no     | yes   | 500  | yes
| 17. F. C. | 37                           | 36                             | yes     | yes    | yes   | 730  | yes
| 18. G. C. | 11                           | no symptoms                    | NE      | yes    | no    | —    | yes
| 19. H. R. | 20                           | infancy                        | yes     | yes    | yes   | 1190 | yes
| 20. J. L. | 25                           | 5                              | yes     | yes    | yes   | 648  | yes
| 21. B. W. | 8                            | infancy                        | no      | yes    | yes   | 560  | yes
| 22. J. V. | 2                            | infancy                        | no      | no     | yes   | 130  | yes
| 23. J. A. | 54                           | no symptoms                    | NE      | yes    | no    | —    | yes
| 24. J. A. | 25                           | 3-5                            | yes     | yes    | yes   | 900  | yes
| 25. M. A. | 28                           | 3-5                            | yes     | yes    | no    | —    | yes
| 26. L. E. | 7                            | infancy                        | no      | yes    | yes   | 230  | yes
| 27. A. V. | 2                            | 2                              | NE      | yes    | no    | —    | yes
| 28. H. E. | 4                            | 4                              | NE      | yes    | no    | —    | yes
| M. S.    | 20                           | 17                             | no      | no     | yes   | 295  | yes

* See footnote, p. 1080
† One or both parents not available for examination. See figure 7.
‡ See discussion regarding lack of spherocytosis in relatives.
NE: Not adequately examined.

The spleen was readily palpated in 23 of the 28 patients; in only 1 of the
anemic adults (Case 16) with spherocytosis it could not be felt. Cholecystosis
was demonstrated either by operation or by cholecystogram in 12 of the 14
numbered patients who were more than 11 years of age and were adequately
examined. This experience is in accord with that of others,19, 4 and serves to
emphasize the fact that when a person inherits the spherocytic anomaly, his
chances of developing gall stones are great unless excessive red cell destruction is terminated by splenectomy.

Skeletal deformities, leg ulcers and abnormal pigmentation, described in some cases of hereditary spherocytosis,19, 24 were not observed in the group under consideration. Roentgenographic examinations of the bones were made with negative results in Cases 3, 7, 21, 26 and 28.

Table 5—Data on Osmotic Fragilities of Fresh and Incubated Red Cells from Normal Individuals and from Patients with Hereditary Spherocytosis Before and After Splenectomy

<table>
<thead>
<tr>
<th></th>
<th>Fresh Blood</th>
<th>Blood Incubated 24 Hours at 37 degrees C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per Cent Hemolysis</td>
<td>Per Cent Hemolysis</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>5%</td>
</tr>
<tr>
<td>26 Normal Individuals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % NaCl</td>
<td>.45</td>
<td>.44</td>
</tr>
<tr>
<td>σ % NaCl</td>
<td>.016</td>
<td>.013</td>
</tr>
<tr>
<td>Coeff. Var. %</td>
<td>3.5</td>
<td>2.9</td>
</tr>
<tr>
<td>17 Patients with Hereditary Spherocytosis Before Splenectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % NaCl</td>
<td>.59</td>
<td>.55</td>
</tr>
<tr>
<td>σ % NaCl</td>
<td>.082</td>
<td>.068</td>
</tr>
<tr>
<td>Coeff. Var. %</td>
<td>13.9</td>
<td>12.4</td>
</tr>
<tr>
<td>12 Patients with Hereditary Spherocytosis After Splenectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % NaCl</td>
<td>.54</td>
<td>.52</td>
</tr>
<tr>
<td>σ % NaCl</td>
<td>.019</td>
<td>.021</td>
</tr>
<tr>
<td>Coeff. Var. %</td>
<td>3.6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

The spleen was removed from 16 of the 28 numbered patients included in this study and in every case the red cell count and serum bilirubin concentration returned to normal following operation. There were no recurrences of anemia or icterus during the post-operative periods which ranged from five months to seventeen years. The spleens showed varying degrees of enlargement as indicated in table 4, but the principal finding in each case was marked engorgement of the pulp with red cells as emphasized in the accompanying paper.34

Osmotic Fragility of Erythrocytes

The mean values for concentration of NaCl causing 2, 5, 10, 25, 50 and 75 per cent hemolysis of freshly drawn and incubated red cells from normal donors
and from patients with hereditary spherocytosis are listed in table 5 and plotted graphically in figure 2. It is evident that the increase in osmotic fragility of spheroidal cells after incubation is distinctly greater than that of normal corpuscles. In the splenectomized cases fewer freshly drawn cells showed marked increase in osmotic fragility than in the pre-splenectomy group; that is, the “diagonal” and “humped” curves for fresh blood were not seen after removal of the spleen and “tailed” curves were encountered much less frequently after operation. Mean curves for incubated spherocytic blood, on the other hand, reveal slightly greater osmotic fragility in the postsplenectomy cases. One interpretation of this finding is that the spleen, when present, may eliminate

those cells which after incubation would show greatest increase in susceptibility to lysis in hypotonic saline.

Typical curves of osmotic fragility of red cells from both splenectomized and nonsplenectomized patients with hereditary spherocytosis are shown in figure 3. Consideration of these curves will aid in interpreting the data presented in table 5. Curves of various shapes similar to those reported by others were observed in tests on freshly drawn blood from pre-splenectomy patients while in the splenectomized group most of the curves were of the upright sigmoid type, with a small standard deviation and coefficient of variation. It seems likely that in the nonsplenectomized patients the cells showing greatest susceptibility to lysis in hypotonic saline are those which have somehow escaped from the spleen after having undergone considerable change within the “splenic incubator.” The curves for incubated blood from both pre- and post-splenectomy

---

**Figure 2.**—Plot of mean concentrations of NaCl causing 2, 5, 10, 25, 50 and 75 per cent hemolysis of freshly drawn and incubated red cells from normal donors and from patients with hereditary spherocytosis. Data on which this figure is based are given in table 5.
cases showed a variety of patterns, and in this small series there was slightly greater variation in the splenectomized group. This is in contrast with the relatively uniform results obtained with freshly drawn blood from the splenectomized patients and suggests that incubation brings to light the varied cell population that is permitted to circulate in patients from whom the spleen has been removed.

The shape of the curve obtained with incubated cells has in our experience not been distinctive. Upright and diagonal curves and curves of rather intermediate shape have been observed in cases of hereditary spherocytosis and there has been no apparent relation to the degree of anemia, icterus or reticulocytosis.

![Graphs](image)

**FIG. 3.—Typical curves of osmotic fragility of freshly drawn and incubated red cells from splenectomized and nonsplenectomized patients with hereditary spherocytosis.**

The tonieities plotted in the graphs of this figure were not corrected for addition of plasma to the hypotonic solutions of NaCl.

This figure is reproduced from Medical Clinics of North America 35: 574, 1951, through the courtesy of W. B. Saunders Co.

Perhaps additional experience will bring to light certain patterns in the osmotic fragility curves for incubated cells from individuals with spherocytosis, but the shape of the curves thus far obtained in such cases has been variable and has been not unlike that observed in some normal subjects and in patients suffering from a variety of hematologic disorders. The shape of the curve for incubated cells is rather constant in repeated tests on cells from any given individual, but no single shape seems to be typical of hereditary spherocytosis.

The usefulness of the "incubation test" in detecting subtle abnormalities of the erythrocytes is illustrated in figure 4. In these 7 cases the osmotic fragility curves for fresh blood were within or near the normal range. After incubation, however, the cells from all of these patients yielded curves well outside the range for incubated normal corpuscles. All of the curves shown in the graphs
on the left side of this figure represent results obtained with the 4 affected members of the “Dry” family, and two of the curves in the graphs on the right side of this figure show results from the affected members (Cases 14 and 15) of the “Na” family.

All of the curves shown in figure 4 represent results obtained with pre-splenectomy samples of blood. It is apparent from examination of this figure that abnormalities of osmotic fragility may be manifest in some cases only after the cells have been incubated. This appears to be particularly true in mildly affected cases, in asymptomatic relatives and in splenectomized patients. When anemia and icterus are lacking and spherocytosis is minimal or questionable in smears and wet preparations, the incubation test may be of considerable aid in detecting abnormalities of the red cells.

“Qualitative” determinations of osmotic fragility were made in a number of cases on freshly drawn oxalated blood and on incubated defibrinated blood in an effort to demonstrate the usefulness of a simplified incubation test which could be carried out in laboratories lacking special equipment. The highest concentrations of NaCl at which hemolysis was visible in these tests are plotted in figure 5. Limited experience with the simplified incubation test indicates that this procedure may be used to advantage when facilities are not available for adjusting the pH of blood and for determining quantitatively the per cent hemo-
lysis in each tube. In all cases thus far examined in this manner, the 
tonicity causing grossly detectable lysis of incubated cells from patients with hereditary 
spherocytosis was significantly greater than that hemolyzing incubated normal 
cells. This was true even in the cases in which the tonicity causing lysis of freshly 
drawn cells was only slightly above that capable of lysing normal red corpuscles. 
The maximal concentrations of salt causing detectable hemolysis in the qualitative 
tests did not differ by more than one tube (±0.02 per cent salt) from those 
determined in the quantitative test carried out simultaneously.

Mechanical Fragility of Erythrocytes

Results of determinations of mechanical fragility (MF) of freshly drawn and 
ineubated red cells from normal donors and from patients with hereditary

spherocytosis are summarized in table 6 and figure 6. In every case the MF of 
freshly drawn red cells from affected persons was significantly greater than that 
encountered in normal individuals. This was true even in cases showing no anemia 
and little or no increase in osmotic fragility of freshly drawn cells. The increase 
in MF of spheroidal cells after incubation is of additional interest, despite the 
fact that the MF of incubated cells from several normal subjects was in or near 
the lower range for incubated cells from patients with spherocytosis.

It is noteworthy that the mean MF of both freshly drawn and incubated cells 
like the mean figures for osmotic fragility was somewhat lower in the splenec-
tomized group, and that the coefficient of variation was also smaller in this 
group. The differences between the pre- and post-splenectomy groups might 
have been more striking if the former had included a larger proportion of severe 
cases.
Lysis of Erythrocytes During in Vitro Incubation (Autohemolysis)

Autohemolysis of sterile, defibrinated blood from Cases 9, 11, 13, 14, 17, 18, 22 to 25, 27 and 28 was measured by Dacie's procedure after incubation at 37 C. for periods of 24 and 48 hours. Hemolysis of incubated cells from the 12 patients

Table 6—Data on Mechanical Fragilities of Fresh and Incubated Red Cells from Normal Individuals and from Patients with Hereditary Spherocytosis Before and After Splenectomy

<table>
<thead>
<tr>
<th></th>
<th>Fresh Blood</th>
<th>Incubated Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 Normal Individuals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % hemolysis</td>
<td>2.6</td>
<td>8.8</td>
</tr>
<tr>
<td>σ % hemolysis</td>
<td>0.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Coeff. Var. %</td>
<td>32.0</td>
<td>36.9</td>
</tr>
<tr>
<td>18 Patients with Hereditary Spherocytosis before Splenectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % hemolysis</td>
<td>12.3</td>
<td>29.2</td>
</tr>
<tr>
<td>σ % hemolysis</td>
<td>4.6</td>
<td>9.2</td>
</tr>
<tr>
<td>Coeff. Var. %</td>
<td>37.2</td>
<td>31.5</td>
</tr>
<tr>
<td>13 Patients with Hereditary Spherocytosis after Splenectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean % hemolysis</td>
<td>8.5</td>
<td>21.9</td>
</tr>
<tr>
<td>σ % hemolysis</td>
<td>2.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Coeff. Var. %</td>
<td>28.2</td>
<td>24.8</td>
</tr>
</tbody>
</table>

Fig. 6.—Results of mechanical fragility tests on red cells from 26 normal donors, 18 nonsplenectomized and 13 splenectomized patients with hereditary spherocytosis. Case numbers are adjacent to the symbols.

(5 splenectomized and 7 nonsplenectomized) with hereditary spherocytosis greatly exceeded that of cells from normal individuals, the differences being most marked after incubation for 48 hours. Hemolysis of incubated cells from the patients with hereditary spherocytosis was significantly greater than that of cells from 8 of 10 patients suffering from chronic hemolytic disease with erythrocyte-
bound antibody ("acquired" hemolytic anemia). During severe hemolytic crises the red cells from 2 of the patients in the latter group underwent very rapid in vitro hemolysis, while in more quiescent phases of the disease in vitro hemolysis took place nearly as slowly as in normal red corpuscles. These observations are to be extended and reported separately. Defibrinated blood from the mothers of Patients 22 and 28 showed no more hemolysis after incubation than did normal blood.

A word of explanation should be added to make it clear that the term "auto-hemolysis" used in connection with these experiments refers merely to release of hemoglobin from cells by mechanisms that are poorly understood and that may differ from patient to patient. After limited experience with the procedure, we are in accord with Dacie who states that a positive result should be regarded "solely as a pointer to a hemolytic process."

Lysolecithin Fragility of Erythrocytes

The lysolecithin fragility test was performed on the red cells of 4 patients before splenectomy and 9 patients after splenectomy. The fragility of the erythrocytes to lysolecithin was in all cases slightly greater than that of normal control cells, but the differences were considered insufficient for diagnostic purposes.

Observations on Heredity of Spherocytosis

The pedigrees of the 17 families included in this study are shown in figure 7. Cases 2, 7, 9, 12, 13, 14, 16, 17 and 24 were the propositi of their respective families. The only case assigned a number in each of the other families was the propositus. The 10 affected individuals to whom numbers are not assigned were examined in other clinics and all were reported to show increased osmotic fragility together with varying degrees of anemia, icterus and splenomegaly. The 5 cross hatched symbols represent unexamined relatives who were reported by the patients to have chronic anemia and/or recurrent jaundice.

The symbols containing open circles represent the 51 relatives who were examined by the authors and found to have no hematologic abnormality and, with one exception to be cited, no palpable organs or masses in the abdomen. Studies carried out on the relatives included complete blood count, reticulocyte count, examination of blood smears for spherocytes, determinations of serum bilirubin and osmotic and mechanical fragility of freshly drawn and incubated red cells. One brother of Patient 17 had a readily palpable spleen and a second brother had a serum bilirubin concentration of 1.5 mg. per cent. One of the sisters of Patient 28 had a serum bilirubin concentration of 2.4 mg. per cent. Since no other pertinent abnormalities were detected in these siblings, they have with some reservation been assigned "normal" symbols in figure 7.

Erythrocytes of all patients and relatives examined were tested with anti-A, -B, -M, -N, -D (Rho) and absorbed B sera. Most of the examinations were made before a larger battery of antisera became available. No individual could be ex-
cluded from the family group on the basis of these tests. The ancestors of the
propositi were predominantly English in 8 families, German in 5, and Italian in
2 families and French-Canadian in 1 family.

Data concerning the incidence of spherocytosis in siblings and offspring of
propositi are presented here for possible inclusion in subsequent tabulations of
the inheritance of this disease. All available relatives were examined without
respect to their medical history. Of the 17 examined siblings of the propositi in
the “Dra,” “Dry,” “Na,” “Co,” “Ro,” “As” and “Eh” families, only 4 were
affected. Individuals not examined by the authors cannot properly be included
in computing the incidence of spherocytosis among siblings of propositi, although
their status is indicated in figure 7. Information concerning miscarriages and
neonatal deaths in these families is not sufficiently accurate for tabulation. The

shortage of affected siblings, notably in the “Co” and “Eh” families, will be dis-
 talked later. Of the 8 examined offspring of propositi in the “Dra,” “Na,” “Co,”
“La” and “As” families, 4 were affected.

If spherocytosis is a dominant characteristic,2, 20, 26, 22, 33, 39 one of the parents
of every patient would be expected to show some evidence of the disease. Families
“Vo” and “Eh” are of particular interest in this regard in that both parents of
the propositi were examined either two or three times and found to be normal.
Their spleens were not palpable and they had no anemia, reticulocytosis, hyper-
bilirubinemia or increase in osmotic or mechanical fragility of freshly drawn or
incubated red cells. The only exception was the mother of Case 22 whose freshly
drawn red cells showed mechanical “fragility” of 4.9, 4.3, 6.9 and 3.7 per cent
respectively on four occasions over a period of three years. The figures for me-
chanical hemolysis of incubated erythrocytes from this mother were 23.2, 13.7, 8.2

Fig. 7.—Pedigrees of 17 families included in study.
and 9.1 per cent respectively. These irregularly abnormal results are difficult to interpret in the light of normal figures for all other laboratory tests on this parent. With this exception, normal findings were encountered not only in both parents of Patients 22 and 28 but also in other relatives who might have been expected to show evidence of spherocytosis. The significance of these observations will be discussed subsequently.

Persistence of Abnormalities of the Erythrocyte after Splenectomy

Slight spherocytosis and the previously described abnormalities of erythrocyte fragility were detected at the time of each postoperative examination in all of the splenectomized cases. Autohemolysis in blood specimens incubated 48 hours was much greater than normal in each of 5 splenectomized patients. Hematologic studies were repeated several times in most cases, and at least one examination was made one or two years after operation in every case except numbers 16, 17, 24 and 26.

Previous observers,6 19, 22, 24, 32, 34, 48 have reported varying degrees of change toward normal following splenectomy in patients with hereditary spherocytosis. In our experience, spherocytosis has been much less marked after splenectomy, but abnormalities in osmotic and mechanical fragility have persisted in all of the 11 cases followed one or more years after operation. Abnormalities of fragility remained especially apparent in tests with incubated red corpuscles. These findings strongly support the view that, although splenectomy terminates excessive red cell destruction, the spleen is not solely responsible for the defects observed in the erythrocytes in this disease.

Case M. S. (table 1 and figure 7) is of unusual interest. The hematologic findings were indistinguishable from those of hereditary spherocytosis for a period of over two years prior to splenectomy. Sections of the spleen revealed the presence of numerous red cells in the pulp. Five months after operation slight spherocytosis was still evident in the blood smears and the osmotic and mechanical fragility of both freshly drawn and incubated red cells was significantly greater than normal. Subsequent examinations, however, including tests for autohemolysis and osmotic and mechanical fragility over a period of three and one-half years have revealed no hematologic abnormality whatsoever. The most recent examination of this patient was made during the fifth month of pregnancy and included measurements of osmotic and mechanical fragility of red cells after incubation for 48 hours.

Since both parents, as well as a sibling and a daughter, were repeatedly found to be free from hematologic abnormality, and since the patient’s blood picture became entirely normal after splenectomy, this case cannot properly be labeled one of hereditary spherocytosis in the light of our present knowledge. The laboratory data on this patient are presented in table 1 for comparison with the unequivocal cases of hereditary spherocytosis, but M. S. has been excluded from the numbered series and from the other tables and figures of this report. More complete understanding of the M. S. case can be reached only by further observations on this patient and on any other patients who may present similar findings.
DISCUSSION

The results of the customary clinical and hematologic examinations on the patients included in this study are for the most part similar to those reported by others.13, 20, 33, 47 and need not be discussed further.

The need for precise characterization of the disease, hereditary spherocytosis, is apparent. Jaundice, splenomegaly, anemia, reticulocytosis, normoblastic hyperplasia of the marrow and increased excretion of urobilinogen in the feces are encountered in a variety of hemolytic disorders and are clearly nonspecific. The mere demonstration of spherocytes likewise does not permit an exact diagnosis, because spherocytes can be produced by a number of mechanisms. It is especially important to bear in mind that spherocytes may be present in patients with a form of "acquired" hemolytic anemia that might appropriately be termed "chronic hemolytic disease with erythrocyte-bound antibody." This is a chronic disorder in which the red corpuscles are consistently agglutinated in the antiglobulin test and recent experience indicates that this disease may be readily confused with hereditary spherocytosis.53 It must also be appreciated that not all hereditary hemolytic anemias are associated with spherocytosis. In addition to sickle cell anemia and Mediterranean anemia, several other types of inherited hemolytic disease have been described in recent years.4

An unequivocal diagnosis of hereditary spherocytosis can be made, with current knowledge, only when spherocytes are demonstrated in the patient and in one or more of the patient's relatives. Further studies of the characteristics of the red corpuscles in this disorder may ultimately enable the clinician to make a firm diagnosis even when relatives cannot be examined. It was partly with this goal in mind that the various laboratory tests described in this paper were studied. The characteristics of the erythrocyte in this disease and the observations on inheritance of spherocytosis deserve further comment, especially as these findings pertain to the problem of clinical diagnosis.

Characteristics of the Red Corpuscles in Hereditary Spherocytosis

Shape

Abnormal thickness of the red cells is readily detected in smears and wet preparations and by calculation of the MCT when spherocytosis is marked, i.e., (usually) when the disease is relatively active. Painstaking examination of many satisfactory microscopic fields is necessary for estimation of cellular thickness when the disease is relatively quiescent and only a few cells show significant increase in thickness. The MCT in such cases may be normal. Decision as to the presence or absence of spherocytosis may at times be so difficult on the basis of microscopic examination of the blood that diagnosis must rest chiefly on demonstration of increased fragility of the red cells.

Osmotic Fragility

Osmotic fragility of freshly drawn red corpuscles was usually, but not always, greater than that of normal red cells, while in all cases included in this report there was abnormally great susceptibility of the erythrocytes to lysis in hypotonic saline after sterile incubation of the blood for 24 hours. Normal osmotic
fragility of freshly drawn red cells from persons with hereditary spherocytosis has been noted and commented upon by a number of other observers.¹ ¹⁹ 20 33 49 The changes in fragility occurring with incubation are not only of value in detecting abnormalities of the erythrocyte but are also of theoretical interest because of the likelihood that cellular alterations occurring during in vitro incubation are similar to those taking place in cells stagnating within the spleen as first suggested by Emerson, Shen, Ham and Castle.¹⁹ The manner in which thick cells may be trapped in the splenic pulp—there to undergo swelling and subsequent lysis—is discussed in the accompanying paper.⁴⁴

The limitations of the simplified “qualitative” test of osmotic fragility are acknowledged. This procedure nevertheless reveals abnormal fragility in a portion of the cell population, especially after incubation of the blood. Although quantitative determination of the proportions of the cells having lesser degrees of fragility may be of considerable theoretical interest, demonstration of the presence of a few cells with marked increase in fragility after incubation appears to be sufficient from the standpoint of clinical diagnosis.

**Mechanical Fragility**

In our experience the MF of freshly drawn red cells from every affected person has been significantly greater than that encountered in normal individuals. The MF of incubated erythrocytes has usually, but not always, been greater than that of incubated normal red cells. The method of estimating MF is necessarily crude because the cells must be subjected to trauma unlike the wear and tear of circulation in order to produce measurable hemolysis in a relatively short period of time (90 minutes). The results are nevertheless reproducible and correlate well with tests measuring in vivo survival of the red cells in question, as shown by Shen, Castle and Fleming⁴⁰, ⁴¹ and Ham, Shen, Fleming and Castle.²³ It is of interest that dog erythrocytes of approximately known age tagged with radioactive iron have been shown to have increasing mechanical fragility as they near the end of their life span.⁴⁶ These observations suggest that mechanical factors may play a role in destroying normal cells as well as spheroidal corpuscles. The extent to which patients with hereditary spherocytosis may destroy their red cells by the mechanical trauma of circulation, in addition to lysis within the spleen, remains to be determined.

**Lysis during in Vitro Incubation at Body Temperature**

Lysis of incubated red cells, as first measured by Ham and Castle²² and Dacie⁵ and more recently by Crosby,¹ deserves further investigation as a diagnostic procedure. Limited experience reveals that incubated red cells from patients with hereditary spherocytosis, either before or after splenectomy, undergo lysis more readily than cells from normal individuals and from most patients with “acquired” hemolytic anemia (chronic hemolytic disease with erythrocyte-bound antibody). Red cells from patients with the “acquired” disorder may, however, hemolyze rapidly in vitro when drawn during periods of very active in vivo hemolysis.
**Reaction in Antiglobulin (Coombs) Test**

Singer and Motulsky have reported a positive antiglobulin reaction in a severely anemic patient whose relatives showed evidence of spherocytosis and Wright, Dodd and Bouroncle have obtained positive tests with red cells from 4 of 21 patients with “congenital hemolytic icterus.” Boorman, Dodd and Loutit, on the other hand, have obtained negative antiglobulin reactions in all of 23 hereditary cases. It is noteworthy that the red cells from all of the 28 cases included in this report gave negative reactions with potent antiglobulin rabbit serum which never failed to give strong reactions in high titer with red cells from 12 patients with “acquired” hemolytic disease. All available relatives (15 in 6 families) of the patients in the latter group were hematologically normal and their red cells were not agglutinable by antiglobulin serum.

Elimination of positive reactions due to cold hemagglutinins and to the use of inadequately absorbed rabbit serum, and adequate characterization of all cases tested, will be necessary for proper evaluation of the antiglobulin test in the study of hemolytic disorders.

**Persistence of Abnormalities**

Spherocytosis (albeit minimal in some cases) and increased osmotic and mechanical fragility of the red cells, especially after incubation, have persisted in all of the 11 cases of hereditary spherocytosis followed in this clinic for one or more years after splenectomy. These abnormalities were also readily demonstrated at the time of every preoperative examination in each of the 28 cases. It should be stressed again, however, that these findings may be encountered in other hemolytic disorders during periods of very rapid hemolysis, especially in patients suffering from “chronic hemolytic disease with erythrocyte-bound antibody.” It has been our experience that in the latter disease, spherocytosis and increased fragility cannot be detected during relatively quiescent periods, particularly after splenectomy, even though the antiglobulin test remains strongly positive. Persistence of these abnormalities is an important characteristic of hereditary spherocytosis, on the other hand, and pending further observation should be regarded as an essential feature.

Case M. S. has been excluded from the present series because abnormalities of the red cells can no longer be demonstrated, and also because both parents of this patient are normal from the hematologic standpoint. We have no knowledge of any patient, with an affected parent, sibling or offspring and with red cells giving negative antiglobulin reactions, whose incubated erythrocytes have failed to show increased osmotic fragility following splenectomy. Search for such cases will be continued, however, and should be urged in other clinics.

**Inheritance of Spherocytosis**

If this relatively uncommon disorder is inherited as a Mendelian dominant, as is generally believed, one parent of each patient should be affected and abnormal erythrocytes should be found in one-half of the siblings and offspring. Debré and associates have reported 1 case and Race 39 3 cases in offspring of parents who showed no evidence of the disease, but the hematologic examinations carried
out on these families have not been described. Meulengracht\textsuperscript{32} was also unable
to detect abnormalities in the parents of an affected patient (Hy family), but
one of the offspring of the patient was clearly affected. Meulengracht\textsuperscript{32} first sug-
gested that disease in the propositus had developed through mutation. Later,\textsuperscript{33}
however, he offered the explanation that the parents in this case and some of the
relatives in 6 other families examined by him might have been so mildly affected
that the laboratory tests used were inadequate for demonstration of abnor-
malities.

The normal findings in both parents of Patients 22 and 28 could be explained
in four ways. (1) One of the parents of each propositus may have abnormal red
cells but the anomaly may not be detectable by methods at hand. Minor degrees
of penetrance or expression could explain the existence of such carriers who may
be asymptomatic and also have normal blood by current standards. The slight
and variable increases in mechanical fragility of the cells from the mother of
Case 22 suggest that she may have minimal abnormality of the erythrocytes
and that still more sensitive tests might make the abnormality more apparent.

(2) One or both of these patients may be suffering from spherocytosis as a
result of gene mutation. The similarity of the osmotic fragility curves among
the individual members of the “Dry” and “Na” families of this report and in the
families described by Discombe\textsuperscript{12} and Wiedemann\textsuperscript{49} is also suggestive of gene
mutation.

(3) These individuals may be suffering from an atypical or acquired form of
hemolytic anemia associated with positive antiglobulin reactions, despite the
prompt and lasting response to splenectomy in Case 22 and the persistence of
abnormal erythrocytes in both cases. Experience to date, however, with the
battery of laboratory tests used in this study would prejudice us against ready
acceptance of this explanation of the findings in these families.

(4) Illegitimacy is a possibility that can be neither substantiated nor excluded,
but is considered very unlikely in the cases in question.

Further observations will be necessary in order to determine which of these
explanations is the most plausible.

In Race’s\textsuperscript{39} study of 183 members of the 26 families affected with spherocytosis,
the affected members (11) of sibships were significantly outnumbered by the
apparently normal members (35). Race attributed the shortage of affected siblings
to (1) high infant mortality rate among spherocytic children, (2) limitation of
penetrance and (3) existence of acquired hemolytic disease in some individuals
thought to have the inherited disorder. The extensive studies of Gänsslen, Zipper-
len and Schütz\textsuperscript{49} and Hansen and Klein,\textsuperscript{24} while including many individuals, do
not lend themselves to genetic analysis because the hematologic data are in-
complete and the method of selection of individuals studied is not clear.\textsuperscript{36}

In table 7 the figures on affected sibs and children of propositi in this study
are combined with those of Race (considering only individuals actually
examined). Although the numbers involved are small, it is noteworthy that
while the shortage of affected sibs (15 of 63) is significant, there is no shortage
of affected offspring (15 of 31). The higher incidence among children as com-
pared with sibs might be most satisfactorily explained on the basis of mutation
in some of the propositi or on the assumption of low expressivity in some of the
sibs. Since extensive laboratory tests were carried out on the blood of each person examined in the Rochester group, it seems unlikely, but not impossible, that complete lack of gene expression could account for negative findings in a substantial proportion of sibs.

There are wide variations in the severity of the hemolytic process in non-splenectomized patients with hereditary spherocytosis, and it is reasonable to believe that variations in gene penetrance may be at least partly responsible (in addition to environmental factors). Incubated red cells from mildly affected persons appear to be nearly as susceptible to osmotic and mechanical lysis as are incubated cells from the more severe cases. Although the "incubation" tests thus far employed are materially in detecting otherwise inapparent abnormalities of the erythrocyte, still more sensitive tests may be required for demonstration of abnormalities in persons whose expressivity for spherocytosis is very low. If such tests could be devised, it might be possible to explain in part the apparent shortage of affected siblings and parents. The previously described minimal findings in the mother of Patient 22, in the brothers of Patient 17 and the sister of Patient 28 lend support to the concept of limited penetrance and further sub-

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<th>Table 7—Distribution of Affected and Unaffected Siblings and Offspring of Propositi in London (Data of Race39) and Rochester</th>
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<td>Number of Persons Affected</td>
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stantiate the need for additional laboratory procedures to aid in the diagnosis of this disease. The relative importance of mutation and low expressivity in explaining the hereditary pattern of this disease can be determined only by further study of certain families such as those cited in this report and in the publications of Race39, Meulengracht32 and other observers.

Race39 has reported the only instance in which 2 persons (first cousins) with spherocytosis were mated. Three children of this mating were shown to be affected, 1 was normal and there were 2 miscarriages, which may have been the expected homozygotes.

**Summary**

Clinical, hematologic and genetic data on 28 cases of hereditary spherocytosis are presented for the purpose of characterizing this disorder as completely as possible. On the basis of this experience it is recommended that the following typical laboratory findings be sought in establishing a diagnosis in suspected cases: (1) Presence of spherocytes or abnormally thick red cells in peripheral blood; (2) greater than normal osmotic fragility of the red cells; in cases in which the fragility of fresh cells is not significantly increased, determinations should be made after sterile incubation of the blood at body temperature for 24 hours;
(3) greater than normal mechanical fragility of freshly drawn red cells; (4) negative antiglobulin (Coombs) test; (5) greater than normal lysis of the red cells during sterile incubation at body temperature for 48 hours; and (6) presence of similar abnormalities in relatives.

Abnormality of the erythrocyte persisted in all of the 11 patients in this series followed one or more years after splenectomy. An unusual case of chronic hemolytic anemia is described but not included in the numbered series because (1) both parents were hematologically normal and (2) spherocytosis and abnormally great osmotic and mechanical fragility and autohemolysis could not be demonstrated after the fifth postoperative month. Classification of this case is deferred pending further experience.

Demonstration in a parent, sibling or offspring of red cells showing the aforementioned abnormalities is necessary for an unequivocal diagnosis, but this requirement cannot always be met because relatives may not be available for examination. Moreover, when parents and/or several siblings are examined without positive findings, low gene expressivity, gene mutation and illegitimacy may be considered as explanations. Evidence is cited to suggest the possibility of a low degree of penetrance or expression in some cases and to illustrate the need for still more sensitive laboratory tests that might aid in diagnosis of the mildest forms of this disease. The lower incidence of spherocytosis in siblings of propositi than in offspring of propositi is cited as evidence bearing on the theory of gene mutation in some propositi.

A simplified “qualitative” test of osmotic fragility of incubated red cells is described.

ADDENDUM

Since this manuscript was prepared we have studied an additional case in which no hematologic abnormalities could be detected in either of the parents despite the fact that the findings in the patient were typical of hereditary spherocytosis.

Antiglobulin tests on red cells from patients with hemolytic disorders are currently made in our laboratory by mixing 3 per cent suspensions of red cells in saline with equal volumes of antiglobulin serum in serial dilutions ranging from 1:10 to 1:5,120. At least 3 different antiglobulin sera are used in testing the red cells from each patient since antiglobulin sera vary widely in their capacity to agglutinate antibody-coated erythrocytes from patients with auto-immune hemolytic disease. Prozones have been observed with some antiglobulin sera when tested with cells from such patients.

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Hereditary Spherocytosis: I. Clinical, Hematologic and Genetic Features in 28 Cases, with Particular Reference to the Osmotic and Mechanical Fragility of Incubated Erythrocytes

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