Nonspherocytic Congenital Hemolytic Anemia

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THE GREAT majority of cases of hereditary hemolytic disease fall into one of four groups: hereditary spherocytosis, thalassemia (Mediterranean anemia), sickle cell disease and allied syndromes associated with an abnormal hemoglobin type, and hereditary elliptocytosis with hemolytic anemia. However, in recent years a number of cases of congenital hemolytic disease which differ from the above disorders have been reported. They were first discussed in detail in 1953 by Dacie, Mollison, Richardson, Selwyn and Shapiro1 who reported twelve cases and reviewed the literature. Perusal of the literature now reveals that over 60 such cases of anemia have been reported, most of which have appeared under the title of Nonspherocytic or Atypical Congenital Hemolytic Anemia. These anemias form a heterogeneous group which vary in red cell morphology, osmotic fragility, clinical severity and mode of inheritance. Nevertheless as a group they have certain features in common: (1) spherocytosis is lacking and the osmotic fragility of fresh blood is not increased, (2) no abnormal type of hemoglobin has been demonstrated in the red cells, (3) although congenital, a history of familial involvement is not uncommonly absent, (4) most cases have occurred in persons of British or Northern European stock, and (5) splenectomy usually has no beneficial effect.

In 1954 Selwyn and Dacie2 made a further study of four cases, which they found, on the basis of in vitro tests, could be separated into two types (Type I and Type II). The most outstanding difference between these types was in autohemolysis and the correction of autohemolysis by the addition of glucose. In Type I they found that the autohemolysis of whole blood alone was normal, and that the addition of glucose decreased the degree of autohemolysis, although by a lesser amount than in normal blood. In Type II the autohemolysis of whole blood alone was greatly increased and was not decreased by the addition of glucose.

This paper reports observations on the clinical and hematological features of seven cases of nonspherocytic congenital hemolytic disease, occurring in four different families, together with results of family studies. All patients were of either British or Northern European descent, and in none had any abnormal type of hemoglobin been demonstrated. Four of the cases occurring...
in one family showed an autohemolysis test similar to that of Selwyn and Dacie's Type I and the other three occurring in three different families presented an autohemolysis test similar to that of Type II.

The cases were characterized by observations on the following features:

1. Hematological features
   A. Red cell morphology
   B. Red cell osmotic fragility (a) of fresh blood, (b) of blood after incubation at 37 C. for 24 hours
   C. Red cell mechanical fragility
   D. Red cell autohemolysis (a) of blood alone, (b) of blood with added glucose, (c) of blood with added adenosine and (d) of blood with adenosine triphosphate (ATP)

2. Clinical features
   A. Family history
   B. Age of onset
   C. Effect of splenectomy
   D. Mechanism of hemolysis

The cases in the literature with similar clinical and hematological features are reviewed. The classification of the nonspherocytic hemolytic anemias is briefly discussed.

MATERIALS AND METHODS

Hematological Studies

Hemoglobin and hematocrit estimations and cell counts were carried out on oxalated venous blood by standard methods. Blood and marrow films were prepared and stained as described by Dacie. Siderocyte stains were performed by the method of Douglas and Dacie. The direct antiglobulin (Coombs) test and Ham's acid serum test were performed by standard technics. The technic used for the demonstration of the sickle cell phenomenon was that described by Ham using sodium metabisulphite as a reducing agent.

Fragility Tests

Osmotic fragility was measured by the modification of the method of Parpart and co-workers, in which heparinized blood is used, and the pH and temperature of the solutions are carefully controlled. The osmotic fragility was determined on fresh blood and on blood incubated at 37 C. for 24 hours. The normal range in our laboratory is shown by the shaded areas in the osmotic fragility charts (fig. 2).

Mechanical fragility of the red cells was determined by a modification of the method of Shen, Castle and Fleming, using 0.5 ml. samples of blood after adjustment of the packed cell volume to 35 per cent. The blood was placed in a 50 ml. Erlenmeyer flask containing 10 steel balls of 4 mm. diameter, and the flask was then rotated vertically on a wheel, for 90 minutes at room temperature. The center of the flask was 8 cm. from the axis of the wheel. Normal values by this technic range from 3 per cent to 8 per cent lysis (mean 5.5 per cent).

The rate of autohemolysis of red cells was determined by a modification of the method described by Dacie, in which the amount of hemolysis occurring in sterile defibrinated blood after incubation at 37 C. is measured. Blood collected with sterile precautions was defibrinated in 100 ml conical flasks. One ml. of blood was transferred into each of eight sterile screw-capped 5 ml. bottles. In each of the first two bottles blood alone was placed; to each of the second two bottles had previously been added 0.05 ml. of a 10 per cent...
solution of glucose in sterile 0.85 per cent sodium chloride; to each of the third two bottles had previously been added 0.05 ml. of a solution of adenosine in sterile 0.85 per cent sodium chloride to give a final concentration of 0.02 M. in the whole blood; and to each of the fourth two bottles had been added 0.05 ml. of a solution of adenosine triphosphate (ATP) in sterile 0.85 per cent sodium chloride to give a final concentration of 0.02 M. in the whole blood. The eight bottles were then incubated at 37 C. At the end of 24 hours they were gently rotated to mix their contents, and at the end of 48 hours the amount of hemolysis was measured as described by Dacie. The result is expressed as the mean of the values obtained in the duplicate bottles. The normal range of lysis after 48 hours incubation was: (a) blood alone 1.0–3.5 per cent (mean 2.4 per cent), (b) blood with added glucose 0–0.7 per cent (mean 0.34 per cent), (c) blood with added adenosine 0–0.8 per cent (mean 0.35 per cent), (d) blood with added adenosine triphosphate 0–0.8 per cent (mean 0.35 per cent).

Biochemical Studies

**Serum bilirubin** was estimated by the method of Ducci and Watson, using pure bilirubin as the standard.

**Plasma hemoglobin** was estimated by a modification of the method of Bing and Baker, as described by Dacie. The normal range in our laboratory is 1 to 6 mg. hemoglobin per 100 ml. plasma.

**Red cell protoporphyrin** was estimated by a modification of the method of Schwartz and Wickoff. The final purified solution of protoporphyrin was assayed spectrophotometrically on a Unicam Sp500 S.P. and the concentration calculated from the formulae of With. The normal range is 20 to 40 micrograms per 100 ml. packed cells.

**Hemoglobin type** was studied by paper and starch-gel electrophoresis and alkali denaturation. Paper electrophoresis of hemoglobin was carried out using barbitone buffer pH 8.6, ionic strength 0.05, for 16 hours at room temperature. The oxyhemoglobin solution pre-
Fig. 2.—Cases 1 and 4. Osmotic fragility curves. A. Fresh blood (normal range—left hand shaded area). B. Incubated blood (normal range—right hand shaded area).

pared for the alkali denaturation determination was applied as a line with a micropipette (4 µl.). A solution of adult hemoglobin was run concurrently on the same paper strip (Whatman No. 1). Starch-gel electrophoresis was performed by the method of Owen and Got, using gels prepared from hydrolysed starch (Connaught, Medical Laboratories, Toronto). Staining was carried out by the method of Owen, Silberman and Got.

Fetal hemoglobin was sought by the alkali denaturation technic of Singer et al., results being expressed as the one minute denaturation value. Normal range in our laboratory is 0.5 to 2.0 per cent.

Methemoglobin and sulphonemoglobin were sought spectrophotically using the Hartridge reversion spectroscope.

Serum iron was determined by the method of Ramsay, and the unsaturated iron binding capacity by the method of Rath and Finch. Urinary porphobilinogen was sought by the method of Watson and Schwartz.

Glucose-6-phosphate dehydrogenase activity was assayed by the method of Zinkham, Lenhard and Childs as quoted by Hsia; the normal range in our laboratory, using Hsia’s calculations is 140–200 units per 100 ml. of red cells at 25°C.

Red Cell Survival Studies

Radioactive chromium technic. The method used was that of Mollison and Veall, with minor modifications as described by Crawford and de Gruchy. Red cell survival was expressed as (a) the 50 per cent 51Cr survival time and (b) the calculated mean cell life, calculated as described by Hughes Jones and Mollison.

In some cases survival of the patient’s cells in a splenectomized recipient was estimated. All recipients were persons in whom the spleen had been removed at gastrectomy for carcinoma of the stomach; they showed no evidence of metastasis, bleeding, infection or renal insufficiency.

The Ashby differential agglutination method was performed as described by Mollison using powdered anti-M serum supplied by Lederle Laboratories.
NONSPHEROCYTIC CONGENITAL HEMOLYTIC ANEMIA

Family Studies

A detailed family history, including enquiry about consanguinity, was taken in all cases. Patients were questioned about racial history and the occurrence of anemia, jaundice, gallstones, leg ulceration or pigmentation and splenomegaly in grandparents, parents, brothers or sisters and children. A screening blood examination consisting of hemoglobin estimation, reticulocyte count, examination of stained blood film and serum bilirubin estimation was performed in all available relatives and osmotic fragility tests and autohemolysis tests were performed in some relatives.

Cases 1 to 4

These cases belong to the one family and resemble Selwyn and Dacie's Type I.

Case 1

R. G., a married man aged 42 years. At the age of 18 years he suffered from an attack of pneumonia, and on examination was noted to have an enlarged spleen. Blood examination was performed and a diagnosis of acholuric jaundice was made. Until the age of 34 years he was well; he worked hard as a clerk and engaged in fairly strenuous exercise such as swimming. Since then he has been chronically tired and has suffered from attacks of tenderness over the spleen.

On examination he was 67 inches in height and 133 pounds in weight. The sclerae were mildly icteric, the spleen was palpable 3 F.B. below the L.C.M., and the liver was palpable 2 F.B. below the R.C.M. X-ray of the skull was normal. The urine contained no excess porphyrins or hemoglobin.

Special investigations. Blood examination showed a hemoglobin of 12.9 Gm. per cent with 9 per cent reticulocytes, and a total serum bilirubin of 2.08 mg. per cent. In a wet preparation the majority of cells appeared of normal size and shape, although a few cells of slightly increased diameter and a few of decreased diameter were present. Some cells appeared thinner than normal. In the stained film (fig. 1) the majority of cells were of normal shape and normal size, although a few were of slightly increased diameter. Many cells showed slight hypochromia. Approximately 2 per cent of cells appeared as target cells. There was moderate polychromasia and 5 per cent of the cells were stippled; the stippling was predominantly coarse. A few cells which were smaller than normal and which stained a little more deeply than normal were present; the margin of some of these cells was somewhat irregular in outline. Other hematological data are given in table 1. The aspirated bone marrow showed marked normoblastic hyperplasia, with many macronormoblasts.

The osmotic fragility of fresh blood was slightly decreased, and that of blood incubated at 37 C. for 24 hours was markedly decreased compared with normal (fig. 2). The mechanical fragility was normal—8 per cent. The results of autohemolysis were (a) blood alone 5.6 per cent (b) blood with added glucose 1.8 per cent (c) blood with added adenosine 2.4 per cent and (d) blood with added adenosine triphosphate 1.4 per cent.

Red cell survival studies were carried out (a) in the patient himself, (b) in a non-splenectomized recipient and (c) in a splenectomized recipient.

The 50 per cent $^{51}$Cr survival time in the patient was 6 days and the calculated mean cell life 15 days (fig. 3).

In the nonsplenectomized recipient, survival of the same blood was followed simultaneously by $^{51}$Cr and Ashby methods. 250 ml. of blood were taken from the patient (Group ON), tagged with $^{51}$Cr by the usual method and transfused to a Group OM recipient. The 50 per cent $^{51}$Cr survival time was 7 days and the calculated mean cell life 17 days. The mean cell life in the same recipient as measured by the Ashby method was also 17 days (fig. 4).

In the splenectomized recipient the red cells seemed to have only a slightly shorter than normal survival rate for 11 days, after which they disappeared rapidly from the
Table 1.—Results of Hematological Tests

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>RBC (10^6 per cu. mm.)</td>
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<td>3.7</td>
</tr>
<tr>
<td>Hb. (Gm. per 100 ml.)</td>
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<td>11.4</td>
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<td>Packed red cell volume (%)</td>
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<tr>
<td>MCV (c.R)</td>
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<td>113</td>
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<tr>
<td>MCH (µg.)</td>
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<td>31</td>
</tr>
<tr>
<td>MCHC (%)</td>
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<td>27</td>
</tr>
<tr>
<td>WBC (per cu. mm.)</td>
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<td>17,000</td>
</tr>
<tr>
<td>Reticulocyte count (%)</td>
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circulation. This can be seen clearly in fig. 4 in which the points to the first 11 days fall on a straight line which extrapolated to the time axis gives a mean cell life of 84 days. After 11 days the cells disappeared rapidly, the line drawn through the subsequent points crossing the axis at 37 days. In order to establish that this sudden disappearance of the cells was not due to the toxic effect of the sodium chromate on the red cells, 250 ml. of blood were taken from the patient and transfused to a splenectomized patient. This blood was followed by the Ashby technique alone. As can be seen from fig. 4 the survival curve...
Case 1

WITH SPLEEN

- IN SELF (Cr)

- IN RECIPIENT (Cr Sy)

- IN RECIPIENT (A III)

WITHOUT SPLEEN

- IN RECIPIENT (Cr Sy)

- IN RECIPIENT (A III)

Fig. 4.—Case 1. Red cell life span measured in patient's own circulation and in the circulation of normal recipients, both with and without spleens (see text).

approximates to that obtained for the $^{51}$Cr method. A line drawn through the points obtained from the results during the first 20 days crosses the time axis at 92 days. The line drawn through the points obtained from the results on subsequent days crosses the time axis at 43 days (fig. 4).

Family history. This is positive (fig. 5). R. G. is the second of four children; of the other three one is affected (Case 4) and two are unaffected. Blood examination of both parents was normal. R. G. has two daughters, both of whom are affected (Cases 2 and 3). There is no history of consanguinity. Both parents were born in Australia. Maternal grandparents were British, the paternal grandfather was Welsh and the grandmother Scottish.

Case 2

E. G., a girl aged 5 1/2 years, daughter of Case 1. She was born at full term, following a normal labor, the birth weight being 7 pounds 4 ounces. She thrived from birth and her development has been normal. At the present time her weight and height are within normal limits and her parents regard her as a completely normal child. Clinical examination did not show any abnormalities, her conjunctivae being of normal color and her spleen impalpable. However, her parents state that on several occasions in association with febrile illnesses she has passed dark urine for periods of 24 to 48 hours.

Special investigations. Blood examination showed a hemoglobin of 11.4 Gm. per cent with 14.4 per cent reticulocytes. The appearance of the red cells in the stained blood film was similar to that of the father except that a moderate number of cells showed a slight increase in diameter, only an occasional target cell was seen and only 1 per cent of the cells were stippled. Other hematological data are given in table 1. The osmotic fragility of fresh blood was slightly decreased, and that of blood incubated at 37 C. for 24 hours was markedly decreased compared with normal (fig. 6). Mechanical fragility and autohemolysis were not performed.

Case 3

C. G., a girl aged 3 years, daughter of Case 1. She was born at full term following a normal labor. Her birth weight was 7 pounds 4 ounces. She has developed normally.
since her birth. At the age of two years in association with an upper respiratory tract infection, she passed dark urine for 48 hours, and her spleen became palpable. Since then she has remained well in every respect. Clinical examination at the present time is normal.

**Special investigations.** Blood examination showed a hemoglobin of 12.3 Gm. per cent with 8 per cent reticulocytes. The appearance of the red cells in the blood film was similar to that of the father, except that only an occasional target cell was seen and only 1 per cent of the cells were stippled. The osmotic fragility of fresh blood was slightly decreased, and that of blood incubated at 37 C. for 24 hours was markedly decreased compared with normal (fig. 6). Mechanical fragility and autohemolysis were not performed.

**Case 4**

J. C., a married woman aged 39 years, a younger sister of Case 1. At the age of 18 years she suffered from an attack of cystitis, and on examination an enlarged spleen was found by her medical attendant. Since that time she has suffered from a number of attacks of weakness with jaundice and darkening of the urine. In 1950 her hemoglobin was reported to have dropped below 20 per cent and she was transfused. Her medical attendant has noted that her spleen becomes more enlarged during minor infections such as colds and influenza.

On examination she was 66 inches in height and 140 pounds in weight. There was no conjunctival icterus. The tip of the spleen was palpable on deep inspiration and the liver was palpable 1 F.B. below the R.C.M. There was tenderness to pressure over the gallbladder. Clinical examination was otherwise normal. Cholecystogram revealed the presence of gallstones. The urine contained no excess porphyrins or hemoglobin.

She has one child, a son aged 7 years. He has no symptoms of anemia, has never been jaundiced, and his spleen is not palpable; his blood examination showed no abnormality.

**Special investigations.** Blood examination showed a hemoglobin of 12.3 Gm. per cent with 11 per cent reticulocytes and a total serum bilirubin of 0.48 mg. per cent. The appearance of red cells in the stained film was similar to that of Case 1, except that a moderate number of cells showed a slight increase in diameter, only an occasional target cell was seen, and only 2 per cent of the cells were stippled. Other hematological data are given in table 1.
Cases in the Literature

Holliday has described a family whose disorder in many respects resembles that of our family, and a family described by Haden has some similar features.

Holliday described four cases occurring in an English family. In addition three other members of the family were almost certainly affected and two were probably affected. Two generations were definitely shown to be involved and a third generation was almost certainly involved. The anemia was of mild to moderate degree and caused little interference with general health, being diagnosed in either middle or late adult life. Hemoglobin values ranged from...
Fig. 7.—Case 4. $^{51}$Cr survival curves of red cells followed in a non-splenectomized recipient (with spleen) and in a splenectomized recipient (without spleen).

Fig. 8.—Case 4. Calculated red cell life span in a non-splenectomized recipient (with spleen) and splenectomized recipient (without spleen).
7.8 to 15.2 Gm. per cent, and reticulocyte counts from 4.5 to 24 per cent. The anemia was normocytic or moderately macrocytic. The stained blood film of the most extensively studied patient was described as follows: “the red cells appear uniformly large and well coloured. Poikilocytes were rare; anisocytosis was slight; fair numbers of stippled cells were present, but polychromatophilic cells were not observed. Moderate numbers of target cells were seen.” The bone marrow showed macronormoblastic hyperplasia and 23 per cent of the orthochromatic normoblasts had a stippled cytoplasm. Stippled cells ranged from 0.4 to 9 per cent in this subject, and were described as “occasional,” “not noted,” and “frequent” in the other three subjects. The red cell osmotic fragility was slightly diminished and the mechanical fragility was a little increased compared with a single normal control.

Haden described “hereditary hemolytic jaundice without spherocytosis” affecting five members of three generations in a family of Hungarian origin (Family B). The anemia was macrocytic and of moderate severity, and caused little disturbance of general health. There was mild reticulocytosis (highest value 7 per cent). The outstanding feature was the presence of stippled cells, in numbers up to 4.7 per cent. There was no spherocytosis and the osmotic fragility of fresh blood was normal. Splenectomy was not performed on any of the patients.

**SUMMARY OF THE CHARACTERISTICS OF CASES 1–4 AND SIMILAR CASES IN THE LITERATURE**

**Hematologic Features**

*Red cell morphology.* The red cells are of normal shape, of normal or slightly increased diameter and are moderately hypochromic. A proportion of cells appear as target cells (from an occasional cell to 2 per cent in our cases and in “moderate numbers” in Holliday’s case). Basophil stippling is present (from 1 to 5 per cent in our cases; in one of Holliday’s cases from 0.4 to 9 per cent, and in the other three cases described as “occasional,” “not noted,” and “frequent”; in Haden’s cases up to 4.7 per cent stippled cells were present). It will be seen that there is some variation within families in the number of target and stippled cells. It should be noted that the basophil stippling has been reported in other cases of atypical congenital hemolytic disease, which appear to differ from our Type I, e.g., the case reported by Feinberg and Watson.

In all our cases there was a small proportion of cells which were smaller than normal and which stained somewhat more deeply than normal; the margin of some of these cells was irregular, giving the appearance of irregularly contracted cells. The presence of these cells was not mentioned by Holliday or Haden. Reticulocyte counts are moderately increased, in our cases ranging from 8 to 14.4 per cent, Holliday’s cases from 4.5 to 24 per cent and Haden’s from 0.7 to 7 per cent.

*Osmotic fragility.* The osmotic fragility of fresh blood is either slightly decreased (our Cases 1, 2 and 3 and Holliday’s case) or normal (our Case 4 and Haden’s case). The osmotic fragility of incubated blood has been
determined only in our cases; it showed a much greater resistance to hemolysis than did normal incubated blood, the hemolysis in the lower saline dilutions being actually less than that of the fresh blood.

Mechanical fragility. This was normal in our two cases (1 and 4) in which it was performed. In Holliday's case it was a little increased compared with a single normal control.

Autohemolysis was slightly increased in our two cases (1 and 4) in which it was measured; it was diminished although not to the same extent as in normal blood by the addition of glucose, adenosine and ATP (there was no significant difference in the degree of correction of autohemolysis by these substances).

Clinical Features

Family history. The disorder is familial. Two generations of our family were affected; in Holliday's family two generations were definitely and a third generation was almost certainly involved; in Haden's family three generations were involved.

No definite conclusions about inheritance can be drawn from this limited data, but it appears probable that the disorder is inherited as a Mendelian dominant. There is no evidence of the disease in the parents of our Cases 1 and 4; however, it is possible that one of the parents carried the gene for the disorder, but that the gene penetrance was low and thus this parent appeared unaffected.

Age of clinical onset and severity. In general the disorder is not severe, a mild degree of anemia with moderate reticulocytosis being usual. Symptoms of anemia are mild or absent, and cause little interference with general health; thus the disorder may not be diagnosed until adult life when the spleen is palpated in a routine medical examination or when symptoms of cholelithiasis develop, or when blood examination is carried out because another member of the family is found to be affected. Our Cases 1 and 4 were not diagnosed until early adult life; at the age of 18 splenomegaly was found in both incidentally on general medical examination for some other disorder. Cases 2 and 3 who are still children are regarded by their parents as normal children and the disorder was discovered in both only as a result of routine examination because their father was affected. Holliday's cases were all diagnosed in adult life, at the ages of 48, 68, 45 and 37 years. Haden's presenting case was diagnosed at the age of 25 years, her only symptom being moderate exhaustion; the mother, two sisters, one brother, and one daughter of this patient had no complaints referable to the disorder and were diagnosed only when blood examination was carried out after diagnosis was made in the presenting patient.

Splenectomy. Splenectomy has not been performed in any of the cases described. However, red cell survival studies in Cases 1 and 4 of our series, suggest that splenectomy may cause a moderate partial remission.

Mechanism of hemolysis. This is uncertain. The plasma hemoglobin was normal in Case 4 and only slightly increased in Case 1 suggesting that hemolysis did not occur intravascularly to any great extent. Comparison of the
survival of red cells from Cases 1 and 4 in splenectomized and nonsplenectomized recipients shows that the absence of the spleen results in a considerable improvement in red cell survival, particularly over the first 10 to 20 days (fig. 4, 8). This suggests that the spleen plays at least some part in the destruction of the abnormal cells.

**CASES 5 TO 7**

These cases belong to different families and resemble Selwyn and Dacie's Type II.

**Case 5**

D. F., a single man aged 20 years. He was born two weeks prematurely, and weighed 5½ pounds at birth. He was jaundiced at birth and was still jaundiced when he left hospital at the age of three weeks. During the first 22 months of life he was transfused three times. At the age of 22 months his abdomen commenced to swell and splenectomy was performed. Between the ages of 7 and 14 years, he was given five transfusions, each of one pint, because of attacks of weakness. These attacks were associated with darkening of the urine and were not precipitated by infections or any other known factor. He was of small stature until the age of 13 years when he started to grow rapidly. At school he played football and cricket and was a keen swimmer. He now works as a bank clerk.

On examination he was a well developed man, 68 inches in height and 142 pounds in weight. The sclerae were moderately icteric and liver was palpable 3 F.B. below the R.C.M. Clinical examination was otherwise normal. The urine contained no excess porphyrins or hemoglobin.

**Special Investigations.** Blood examination showed a hemoglobin of 10.4 Gm. per cent with 56 per cent reticulocytes and a total serum bilirubin of 5.5 mg. per cent. Other hematological data are given in table 1.

In a wet preparation red cell rouleaux formation appeared normal. Most of the cells appeared of greater than normal diameter, and a few appeared thinner than normal with a tendency to fold. A few small rounded microcytes and a few small crenated cells were seen together with a small number of irregularly contracted cells. In the stained film the majority of red cells were round, of moderately increased or normal diameter with relatively little variation in size, and were normally hemoglobinized (fig. 9). There was marked polychromasia, but only a very occasional stippled cell. A small number of target cells were present and a few cells contained Howell-Jolly bodies. A few irregularly crenated and contracted cells, most but not all of which were smaller than normal, were present. Over 50 per cent of the cells contained Pappenheimer bodies, and siderotic inclusions were numerous in the film stained with potassium ferrocyanide. There was no spherocytosis.

The osmotic fragility of fresh blood was normal but that of blood incubated at 37 C. for 24 hours showed a greater than normal increase (fig. 10). The mechanical fragility was at the upper limit of normal–8 per cent. The results of autohemolysis were (a) blood alone 26 per cent, (b) blood with added glucose 28 per cent, (c) blood with added adenosine 27 per cent, and (d) blood with added adenosine triphosphate 5.6 per cent.

The results of biochemical studies are given in table 2. The red cell survival studies were not performed.

**Family history.** This is negative. D. F. is the youngest of three children. Blood examinations of the mother, father, brother and sister were normal. The grandparents, both maternal and paternal, are dead but had not suffered from anemia or jaundice. Three maternal uncles, four paternal uncles and five paternal aunts gave no relevant history. Both parents and grandparents were born in Australia. Paternal great grandparents were Irish and maternal Scottish. There is no history of consanguinity.

**Case 6**

J. M., a married man aged 42 years. He has been told that shortly after birth he was jaundiced and had an enlarged spleen. As an infant and child he frequently attended hospital for the treatment of jaundice and anemia, and at the age of 13 years his spleen
Fig. 9.—*Case 5.* Photomicrograph of blood film. (× 820).

Fig. 10.—*Cases 5 and 6.* Osmotic fragility curves. A. Fresh blood (normal range—left hand shaded area). B. Incubated blood (normal range—right hand shaded area).
was removed. He was transfused immediately before splenectomy but prior to this had not required transfusion. Between the age of 17 and 24 years he did moderately heavy farm work and was able to work eight hours a day without fatigue. Since the age of 24 years he has suffered from asthma and bronchitis and has noticed decreasing exercise tolerance with dyspnea and fatigue on exertion, but he has been able to work as a cleaner and leading fireman. Between the ages of 30 and 40 years he received four blood transfusions totalling 10 pints, each transfusion being given at the time of a surgical procedure, e.g., operations on his nose or antrums and teeth extractions. In 1953 laparotomy was performed and unsuccessful search for an accessory spleen was made.

On examination he was of short stature, 62 inches in height and 112 pounds in weight. The sclerae were mildly icteric and the liver was palpable one F.B. below the R.C.M. Examination of the chest showed evidence of chronic bronchitis and emphysema. Clinical examination was otherwise normal. Plain x-ray of the gallbladder area did not reveal any radio-opaque gallstones. X-rays of the skull and hands were normal.

Special investigations. Blood examination showed a hemoglobin of 10.4 Gm. per cent with 42 per cent reticulocytes and a total serum bilirubin of 1.54 mg. per cent. In a wet preparation the cells showed normal rouleaux formation, and appeared of normal thickness. A few small irregularly contracted cells were present. In the stained film the majority of red cells were round, of moderately increased or normal diameter, with relatively little variation in size. Most cells appeared normally hemoglobinized but a proportion were slightly hypochromic. There was fairly marked polychromasia and a very occasional stippled cell. A small number of target cells were present and a few cells contained Howell-Jolly bodies. Pappenheimer bodies were present in over 70 per cent of cells, and siderotic inclusions were numerous in the film stained with potassium ferrocyanide. A few irregularly crenated and contracted cells, most of which were smaller than normal, were present. There was no spherocytosis. Other hematological data are given in table 1.

The aspirated bone marrow showed marked normoblastic hyperplasia.

The osmotic fragility of fresh blood was normal, but that of blood incubated at 37 C. for 24 hours showed a greater than normal increase (fig. 10). The mechanical fragility was normal-6 per cent. The results of the autohemolysis test were (a) blood alone 8 per cent, (b) blood with added glucose 7 per cent, (c) blood with added adenosine 2 per cent, and (d) blood with added adenosine triphosphate 2 per cent.

The results of the biochemical studies are given in table 2.

The 50 per cent ^125I survival time was 3 days, and the calculated mean red cell life was 7 days.

Family history. This is negative. His mother died of carcinoma of the bowel at the age of 65 years; she had not suffered from anemia or jaundice. His father is alive and well. J. M. is the third of five children, the eldest and the younger two being alive and well. The second child, a girl, died at the age of eight months, the cause of death being uncertain. J. M. has two children, a boy aged 14 years and a girl of 12 years. Blood examination of the father, two sisters, one brother, and both children was normal. The father is of Scottish descent and the mother of English descent. There is no history of consanguinity.

Case 7

P. J., a married man aged 36 years. He was well until the age of 15 years when his conjunctivae were noticed to be yellow. At the age of about 23 years he began to notice fatigue, pallor and mild dyspnea on exertion. In July 1948 at the age of 25 years he was admitted to hospital with acute abdominal pain; his gallbladder, which contained gallstones, and his appendix were removed, and at operation his spleen was noticed to be enlarged. In November 1946 his spleen was removed; following this he felt less tired, and his conjunctivae became less yellow. In 1954 ulceration developed above the ankle on the inner and outer aspects of both legs and over the middle of the left shin; this ulceration has persisted. In August 1955 a barium meal performed because of epigastric
Table 2.—Results of Biochemical Studies

<table>
<thead>
<tr>
<th></th>
<th>Case Number</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
<th>Type 5</th>
<th>Type 6</th>
<th>Type 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum bilirubin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) direct (mg.% )</td>
<td>0.48</td>
<td></td>
<td></td>
<td>0.24</td>
<td>0.3</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) indirect (mg.% )</td>
<td>0.72</td>
<td></td>
<td>0.24</td>
<td>4.9</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) total (mg.% )</td>
<td>1.20</td>
<td>0.48</td>
<td>5.2</td>
<td>1.22</td>
<td>4.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hemoglobin type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) paper electrophoresis</td>
<td>Hb A only</td>
<td>Hb A only</td>
<td>Hb A only</td>
<td>Hb A only</td>
<td>Hb A only</td>
<td>Hb A only</td>
<td>Hb A only</td>
<td>Hb A only</td>
</tr>
<tr>
<td>(b) starch-gel electrophoresis</td>
<td>Hb–A2 within normal range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) one minute alkali denaturation value (normal 0.2–2.0%)</td>
<td>3.1%</td>
<td></td>
<td></td>
<td>3.5%</td>
<td>3.0%</td>
<td>2.4%</td>
<td>1.2%</td>
<td></td>
</tr>
<tr>
<td><strong>Iron studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) serum iron (µg./100 ml.)</td>
<td>200</td>
<td></td>
<td>162</td>
<td>356</td>
<td>310</td>
<td>305</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) total iron binding capacity (µg./100 ml.)</td>
<td>355</td>
<td></td>
<td></td>
<td>356</td>
<td>310</td>
<td>305</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) % saturation</td>
<td>57</td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma hemoglobin</strong></td>
<td>normal 1–6 mg./100 ml.)</td>
<td>8.2</td>
<td></td>
<td>5.0</td>
<td>9.25</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Red cell protoporphyrin</strong></td>
<td>normal 20–40 µg./100 ml packed cells</td>
<td>10</td>
<td></td>
<td></td>
<td>39</td>
<td>63</td>
<td>22</td>
<td>25.4</td>
</tr>
<tr>
<td><strong>Sulphemoglobin and methemoglobin</strong></td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Urinary porphobilinogen</strong></td>
<td>Absent</td>
<td></td>
<td></td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Glucose-6-phosphate dehydrogenase activity</strong> (Normal 140–200 U/100 ml. red cells at 25 C.)</td>
<td>310</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>415</td>
<td></td>
</tr>
</tbody>
</table>
pain revealed a duodenal ulcer. He occasionally passes dark urine but he has no attacks suggestive of a major hemolytic crisis. At present his main symptom is tiredness, but nevertheless he is able to swim and play tennis.

On examination he was fairly well built, 71 inches in height, and 156 pounds in weight. The sclerae were mildly icteric. The liver was palpable 1 F.B. below the R.C.M. and there was ulceration of the legs as described above.

*Special investigations.* Blood examination showed a hemoglobin of 11.2 Gm. per cent with a reticulocyte count of 30 per cent, and a total serum bilirubin of 2.6 mg. per cent. In the stained film the majority of cells were round, of normal or moderately increased diameter with relatively little variation in size, and were normally hemoglobinized. There was fairly marked polychromasia but no strippling. A small number of target cells were present and a few cells contained Howell-Jolly bodies. Cells with irregularly crenated and contracted margins, some showing well defined pointed spicules were present; they were smaller than the average cell and often stained somewhat more deeply than normal. These cells were present in only smaller numbers in films made from freshly drawn blood from the ear, but were more numerous (approximately 10 per cent) in films made from blood which had stood in syringe for several minutes. A very few cell fragments were seen. Pappenheimer bodies were present in over 50 per cent of the cells and siderotic inclusions were numerous in the film stained with potassium ferrocyanide. An occasional macronormoblast was present.

The osmotic fragility of fresh blood was normal, but that of blood incubated at 37 C. for 24 hours was decreased compared with normal (fig. 12). The mechanical fragility was at the upper range of normal—8 per cent. The results of autohemolysis were (a) blood alone 11 per cent, (b) blood with added glucose 9 per cent, (c) blood with added adenosine 11 per cent, and (d) blood with added ATP 2.5 per cent.

The results of biochemical studies are given in table 2.

Radioactive chromium survival studies were kindly performed by Dr. P. Lamond and Dr. P. George. The 50 per cent $^{51}$Cr survival time of the patient's cells in his own
circulation was 5 days, and the calculated mean red cell life 17 days. The 50 per cent 51Cr survival time of normal compatible cells transfused into the patient was 25 days and the calculated mean red cell life 100 days.

Family history. P. J. is the second of five children, all males. Blood examination of the father, mother and four brothers revealed no abnormality, except a slightly raised reticulocyte count (3.4 per cent) in the youngest brother. P.J. has two children, boys aged 5 and 3 years, neither of whom had any clinical or hematological evidence of hemolytic anemia, although the younger boy had an iron deficiency type of anemia with a hemoglobin of 9.7 Gm. per cent. Both parents are of English descent. There is no history of consanguinity.

Comments on Cases 5, 6 and 7

Case 5 and 6 are similar in the following respects: red cell morphology (apart from slight hypochromia in Case 5), osmotic fragility, mechanical fragility, age of onset, effect of splenectomy, and family history. The autohemolysis picture is similar in that autohemolysis is increased, is not reduced by glucose but is reduced by ATP, but differs in that in Case 6 it is reduced by adenosine whereas in Case 5 it is not. Thus in general the two cases closely resemble one another and can be grouped together.

Case 7 resembles Case 5 and 6 in red cell morphology, mechanical fragility, autohemolysis (as in Case 5), and family history. However, it differs in the following respects: (a) the osmotic fragility of blood incubated at 37 C. for 24 hours was decreased, and (b) clinical manifestations of the disease were not obvious until the age of 15 years. Splenectomy did not cure the disorder but resulted in some symptomatic improvement. Nevertheless, despite these differences it seems reasonable at the present time to group Case 7 with Cases 5 and 6 which it resembles in most respects, particularly in autohemolysis.
NONSPHEROCYTIC CONGENITAL HEMOLYTIC ANEMIA

SUMMARY OF THE CHARACTERISTICS OF CASES 5–7 AND SIMILAR CASES IN THE LITERATURE

There are seven other cases in the literature which are similar in most respects to our cases: those of Baty,29 Dacie et al.30 (Cases 1 and 4), Nelson,30 Saint and Hunt,31 von Hememann32 and Horsfall.33 The most important features of these cases are summarized in table 4. By courtesy of Dr. Horsfall, we have been able to make further observations on his patient.

From a consideration of our three cases and the seven in the literature, i.e., 10 cases in all, the characteristics of this type of nonspherocytic congenital hemolytic disease may be summarized as follows:

Hematologic Features

Red cell morphology. In all except Nelson’s case only post-splenectomy descriptions of red cell morphology are available. The majority of cells showed a fairly uniform round macrocytosis, of moderate to marked degree, MCV values ranging up to 128 cμ; these macrocytes show only a mild variation in size and little or no poikilocytosis. The cells were either normally hemoglobinized or very slightly hypochromic. Pappenheimer bodies and siderocytes were numerous in all cases (sometimes being present in over 50 percent of cells), except in Nelson’s case, in which the only film described is presplenectomy. A small number of cells with irregularly crenated or contracted margins, a few of which were smaller than normal and stained slightly more deeply than normal, were noted in six cases: our three cases, Dacie’s two cases, Horsfall’s case. Stippling was not a feature, being either absent, or present in only a very occasional cell.

The degree to which splenectomy has contributed to the changes is uncertain, but it is probable that the numerous Pappenheimer bodies and siderocytes, the small number of target cells and Howell-Jolly bodies are the result of splenectomy; it is probable also that the presence of cells with an irregularly crenated and contracted margin is largely due to or is accentuated by splenectomy.

Osmotic fragility. The osmotic fragility of fresh blood was normal (6 cases) or slightly decreased (4 cases); in the latter cases it is possible that the decrease is due to splenectomy. The osmotic fragility of incubated blood was measured in 8 cases; it showed a greater than normal increase in 7 cases, and was decreased in 1 (our Case 7).

Mechanical fragility was measured in 7 cases; it was normal in 6 cases and slightly increased as compared with a single normal control in Nelson’s case.

The rate of autohemolysis of whole blood alone was increased in all 7 cases in which the test was performed and it was not reduced by the addition of glucose. However, in all 3 of our cases and in Horsfall’s case which we tested, autohemolysis was markedly reduced by the addition of ATP; adenosine caused a reduction in autohemolysis in Case 6, but not in Cases 5 or 7 or in Horsfall’s case (table 3).
Table 3.—Results of Red Cell Autohemolysis Studies

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Type I</th>
<th>Type II</th>
<th>(Horsfall's Case)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood alone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal range</td>
<td>1.0–3.5%</td>
<td>26%</td>
<td>22%</td>
</tr>
<tr>
<td>(mean 2.4%)</td>
<td></td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Blood with added glucose</td>
<td>1.8%</td>
<td>28%</td>
<td>21%</td>
</tr>
<tr>
<td>Normal range</td>
<td>0–0.7%</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>(mean 0.34%)</td>
<td></td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Blood with added adenosine</td>
<td>2.8%</td>
<td>27%</td>
<td>29%</td>
</tr>
<tr>
<td>Normal range</td>
<td>0–0.8%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>(mean 0.35%)</td>
<td></td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Blood with added adenosine triphosphate</td>
<td>1.4%</td>
<td>5.6%</td>
<td>25%</td>
</tr>
<tr>
<td>Normal range</td>
<td>0–0.8%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>(mean 0.35%)</td>
<td></td>
<td>2.5%</td>
<td>(12%)</td>
</tr>
</tbody>
</table>

*This value is bracketed, as, by error, a lesser amount of ATP was added.

Clinical Features

Family history. There was no family history in 6 cases, a probably positive family history in 3 cases, and a possible history in 1 case. Investigation of the families of our 3 cases failed to reveal the disease in any other members; in Baty's, Nelson's and Saint and Hunt's cases there was no family history but family studies were not performed. In 3 cases (Dacie et al. Case 1, von Hennemann and Horsfall's cases) there was evidence of involvement of other sibs, but no evidence of the disease in either of the parents. In Dacie's Case 3, the mother's blood contained 6 per cent reticulocytes and she possibly had a mild form of the disease.

It is not possible to decide definitely, from the limited data available, whether or not the disorder is genetically determined. However, the fact that in three cases there was evidence of involvement of other sibs suggests that it probably is genetically determined. It appears most likely that the gene is a recessive, as none of the cases described showed any definite evidence of the disease in either their parents or their children.

Age of clinical onset and severity. Nine of the 10 cases presented in infancy, and one (our Case 7) at the age of 15 years. In general the defect is severe, as suggested by the early clinical onset, the short mean red cell life span (7 and 17 days in Cases 6 and 7 respectively) the markedly raised reticulocyte counts, and the markedly increased autohemolysis.

Splenectomy appeared to be without beneficial effect in 7 cases, but probably caused slight improvement in 3 cases. Sections of the spleen were not available for study in our cases. The only fairly definite Type II case in which the splenic histology is reported is that of Nelson; the spleen showed no congestion of the pulp with blood. Other reported cases in general show considerable difference in splenic histology from hereditary spherocytosis; there is as a rule much less congestion of the pulp, more reticulum cell hyperplasia and more extramedullary erythropoiesis.
Mechanism of hemolysis. It appears that the premature destruction of the red cells in this type of congenital hemolytic anemia is related to a defect in their glycolytic mechanism. Human red cells obtain their energy chiefly, if not entirely, from glycolysis; this energy is essential for the maintenance of the structural integrity of the red cell. A defect of glycolysis in these cases is suggested by the following observations: (1) the increased red cell autohemolysis was not corrected by glucose but was corrected by ATP, (2) Selwyn and Dacie found that the red cells in two patients with this disorder were unable to utilize glucose at the normal rate, the observed utilization being on 25 and 30 per cent of the calculated amount, and (3) the red cells in our cases had a low ATP content. Thus it seems most likely that the destruction of the red cells in this disorder is related to a defect in glycolysis which results in an impairment of energy production and thus in an impairment of structural integrity; this in turn results in premature destruction of the red cells by the normal mechanisms of destruction. The plasma hemoglobin was normal in Case 5 and was only slightly increased in Case 6; this suggests that hemolysis does not occur intravascularly to any great extent.

The mechanism by which ATP reduces hemolysis in the red cell autohemolysis test is not certain; most probably it does so by acting as an energy source, but the possibility exists that it acts in some unknown way other than as an energy source. It is generally stated that ATP does not readily penetrate the erythrocyte membrane. Thus if the added ATP does act as an energy source reconstituting the ATP supply of the cell, it is necessary to explain how it enters the cell. Recent work by Kashket and Denstedt suggests a possible mechanism; they found that the addition of ADP to a suspension of washed erythrocytes resulted in an increase in the rate of glucose utilization by the cells. It appears that ADP is converted by adenylate kinase, which they have shown to be present in the erythrocyte membrane, to equimolar quantities of ATP and AMP as set out below:

\[ 2 \text{ADP} = \text{ATP} + \text{AMP} \]

The ATP so formed can pass from the membrane into the interior of the cell or into the external medium.

It is possible therefore, that when ATP is added to blood in the autohemolysis test, it is converted to ADP by the action of a phosphatase in the plasma; the ADP so formed may then be converted in the erythrocyte membrane to ATP and AMP: some of the ATP enters the cell and reconstitutes, at least in part, the ATP supply of the cell and reduces autohemolysis.

Classification of the Nonspherocytic Congenital Hemolytic Anemias

At the present time it is not possible to put forward a complete classification of these anemias, either on the basis of clinical and hematological features or on the basis of pathogenesis.

Clinical classification. From the clinical and hematological point of view the classification of Selwyn and Dacie into Type I and Type II on the basis of the autohemolysis test is probably the most satisfactory. Type I is the more
### Table 4.—Summary of Cases in the Literature Resembling Type II

<table>
<thead>
<tr>
<th>Author</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Red cell morphology</th>
<th>Genotypic fragility (a) fresh blood (b) blood incubated at 37°C for 24 hours</th>
<th>Mechanical fragility</th>
<th>Auto-hemolysis</th>
<th>Family history &amp; studies</th>
<th>Age of clinical onset</th>
<th>Effect of splenectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratliff</td>
<td>3</td>
<td>M</td>
<td>Red cell morphology not described.</td>
<td>(a) Normal</td>
<td>--</td>
<td>--</td>
<td>No family history but blood studies not given.</td>
<td>Infancy</td>
<td>Splenectomy aged 3 years. Probable improvement but marked active hemolysis persisted.</td>
</tr>
<tr>
<td>Nelson</td>
<td>7</td>
<td>F</td>
<td>Pre-splenectomy. Macrocytosis, anisocytosis and polychromasia. No evidence of spherocytosis, fragmentation or bizarre shaped cells. MCV 94-125 cu. (pre-splenectomy). 100-114 cu. (post-splenectomy). MCHC 30%. Reticulocytes 17%. The post-splenectomy blood film is not described.</td>
<td>(a) Normal</td>
<td>--</td>
<td>--</td>
<td>Family not investigated but no family history.</td>
<td>Infancy</td>
<td>Hemolysis persisted following splenectomy but anemia moderately improved. Splenic histology. No splenic pulp congestion.</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Sex</td>
<td>Age</td>
<td>Post-splenectomy</td>
<td>Family Study</td>
<td>Other Observations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>--------------</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saint &amp; Hunt¹</td>
<td>F</td>
<td>34</td>
<td>Post-splenectomy: “The red cells were of uniformly macrocytic appearance. About 10% of red cells contained basophil-staining Pappenheimer bodies.” MCV 121 ca. MCHC 31%. Reticulocytes 28%.</td>
<td>Increased</td>
<td>Family studies not performed.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>von Henne mann²</td>
<td>F</td>
<td>20</td>
<td>Post-splenectomy: “The most convincing (abnormality) was the considerable macrocytosis, which dominates the picture.” Also anisocytosis, poikilocytosis,* polychromasia, normoblasts, Howell-Jolly bodies and occasional target forms. Reticulocytes 25–97%. Bone marrow normoblasts.</td>
<td>“Lowered” Increased</td>
<td>Both parents healthy but not studied. One sister anemic from birth, received a number of transfusions, died aged 8 months. One brother unaffected.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horsfall³</td>
<td>M</td>
<td>6</td>
<td>Post-splenectomy. The majority of cells show a moderate fairly uniform round macrocytosis. There is quite marked polychromasia but no stippling. A small proportion of cells are irregularly crenated and contracted; many of these are smaller than normal. No spherocytosis or fragmentation. A small number of target cells and a few Howell-Jolly bodies seen. Pappenheimer bodies in over 50% of cells.</td>
<td>(See Table 3.)</td>
<td>Elder brother yellow at birth and died aged 3 years of anemia. Younger sister severely anemic with high reticulocytes one month after birth. Death after splenectomy at one year. Family studies performed by present authors. No evidence of hemolytic disease in either parent or in one sister tested.</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*The degree of poikilocytosis is not described.
common. Perusal of the literature suggests that Type I probably represents several or even more types of nonspherocytic congenital hemolytic anemia which have in common a similar autohemolysis test, but which differ in clinical severity, red cell morphology and osmotic fragility; our Cases 1–4 simply represent one subgroup of Type I. On the other hand the clinical and hematological features of Type II seem to be more constant. It is hoped that future cases reported will be described in complete clinical and hematological detail, and in particular that studies on the incubation osmotic fragility and on autohemolysis will be carried out to enable comparison with other cases in the literature, so that in due course an accurate classification can be made.

Pathogenesis. The available evidence suggests that the basic defect in the nonspherocytic congenital hemolytic anemias is a defect of red cell metabolism or structure, or both. It appears that at least several types of biochemical abnormality exist. An enzyme defect similar to that of “primaquine sensitive hemolytic anemia” is present in some cases. However, in the two cases of our series studied, Cases 1 and 6, this defect did not appear to be present, as the glucose-6-phosphate dehydrogenase activity was not reduced (table 2); indeed it was increased, presumably due to the fact that reticulocytes have an increased concentration of this enzyme. Robinson, Loder and de Gruchy have shown that the cells from Cases 1 and 6 show definite abnormalities in the distribution of phosphorylated intermediates of the glycolytic cycle both in fresh cells and in incubated cells; furthermore the abnormal patterns differ significantly in the two cases.

It is obvious that further biochemical studies are necessary before a more accurate classification of these disorders can be made. In particular, studies of red cell glycolysis and of the composition of the cell surface and stroma are required to determine the number of different types of biochemical defects in these anemias, and whether a particular biochemical defect results in a particular pattern of hematological change in red cell morphology, osmotic fragility and autohemolysis.

Summary

1. The clinical and hematological features of seven cases of nonspherocytic congenital hemolytic anemia occurring in four different families are presented, together with family studies. Four cases resemble Selwyn and Dacie’s Type I and three cases their Type II. Cases in the literature similar to our cases are reviewed.

2. The Type I cases showed the following features: normocytic or slightly macrocytic anemia with mild hypochromia and some stippling; a decreased or normal osmotic fragility of fresh blood and a decreased fragility of incubated blood; a slightly increased autohemolysis corrected by glucose and by ATP. The family history was positive and clinical onset was usually in adult life. Survival studies suggest that splenectomy may result in partial remission.

3. The Type II cases showed the following features: a marked uniform macrocytosis with numerous Pappenheimer bodies (postsplenectomy); a normal osmotic fragility of fresh blood and an increased fragility of incubated blood; an increased autohemolysis uncorrected by glucose but corrected by
NONSPhEROcytic CONGENITAL HEMOLYTIC ANEMIA

ATP. The family history was often negative and clinical onset was usually in infancy. Splenectomy in general appeared to produce no benefit.

4. The classification and pathogenesis of these anemias is discussed. It is pointed out that Type I is not a homogeneous group, and that our cases simply represent one subgroup of this type. Type II appears to be a more homogeneous group. Preliminary biochemical studies suggest the difference in clinical types may be determined by differences in biochemical defects.

SUMMARIO IN INTERLINGUA

1. Es presentate le characteristicas clinic e hematologic de septe casos de congenite anemia hemolytic nonspherocytic occurrente in quatro familias. Studios familial es includite. Quatro del casos es simile al typo I de Selwyn e Dacie; le altere tres es simile al typo II de ille autores. Le casos de character simile al nostres que es trovate in 1 litteratura es revistate.

2. Le casos del typo I exhibiva le sequente characteristicas: Anemia normocyctic o levemente macrocytic, con debile hypochromia e un certe maculation; reducite o normal fragilitate osmotic de sanguine fresc e reducite fragilitate de sanguine incubate; un leve augmento del autohemolyse que es corrigibile per glucosa o adenosinotriphosphato. Le historia familial esseva positive. Le declaration clinic occurreva usualmente a etates adulte. Studios de supervi-ventia suggere que splenectomia va possibilemente resultar in un remission partial.

3. Le casos del typo II exhibiva le sequente characteristicas: Marcate e uniforme macrocytosis con numerose corpores de Pappenheimer (post le splenectomia); un normal fragilitate osmotic de sanguine fresc e un augmento del fragilitate in sanguine incubate; un augmentate autohemolyse, on corrigibile per glucosa sed corrigite per adenosinotriphosphato. Le historia familial esseva frequentemente negative. Le declaration clinic occurreva usualmente al infantia. A generalmente parlar, splenectomia non pareva resultar in ulle beneficios.

4. Le classification e le pathogenese de iste anemias es discutite. Es signalate que typo I non es un gruppo homogenee e que nostre casos representsimply un subgruppo de iste typo. Type II es apparentemente un gruppo plus homogenee. Studios biochimic de character preliminari pare indicar que le differentias inter iste typos clinic es possibilemente determinate per differentias in le defectos biochimic.

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A review has been made of the case records of eight children suffering from acquired hemolytic anaemia who were admitted to the Royal Alexandra Hospital for Children, Sydney, during the period 1954 to 1957. Four of the eight children were of Mediterranean racial extraction in the Australian community.

Recovery from the illness was complete in each of the eight cases; in seven, the duration of the hemolytic episode was less than four weeks. It is suggested that acquired hemolytic anaemia in childhood is usually an acute disease of short duration. In one case, excessive hemolysis persisted for more than two years; for 16 weeks of this period, reticulocytopenia was present. In one case the hemolytic anaemia followed closely upon ingestion of fresh broad beans. Inquiry has established the fact that the common broad bean grown in Australia is Vicia fava. The glutathione content of the erythrocytes of this child, estimated more than one year after the hemolytic episode, was found to be significantly reduced.—G. C. de G.
Nonspherocytic Congenital Hemolytic Anemia

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