# HEREDITARY ELLIPTOCYTOSIS: A CASE REPORT AND GENETIC STUDY

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ABSTRACT

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By

Margaret Mary Salik

A panel of laboratory measurements has been designed for the study of hereditary anemias. This panel consists essentially of (1) red cell count, (2) hemoglobin concentration, (3) packed cell volume, (4) estimation of reticulocyte count, (5) total bilirubin, (6) characterization of morphologic abnormalities observable on routine smears, (7) examination of all close relatives, (8) determination of as many blood groups and types as is feasible, and (9) examination of chromosomes from lymphocyte cultures for structural, genetic markers.

The potential value of this panel was demonstrated by applying it to the investigation of 41 members of a family affected by hereditary elliptocytosis. Seven males and seven females were found who displayed this abnormality. One young male, the propositus, was manifestly anemic, while the remaining relatives with elliptocytosis all showed evidence of compensated anemia. No linkage to commonly tested blood groups was observed in this family and no visible chromosomal alterations was detected.

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Margaret Mary Salik

A THESIS

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For

My Mom and Dad

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To Reverend Dirk Lieverdink:

"Not in the clamor of the crowded street,
Not in the shouts and plaudits of the throng,
But in ourselves, are triumph and defeat."

- Longfellow

To Reverend Edward Mike:

"You educate to some extent... by what you say, more by what you do, and still more by what you are; but most of all by the things you love."

- Moran

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The author does not intend that the acknowledgments are in decreasing order of importance. If possible, each would be at the top of the page, since each in his own right contributed to this work. Because so many people were generous with their help, I wish to apologize to anyone whose name should appear on this page and does not.

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# INTRODUCTION

Every large hospital laboratory inevitably examines the blood of several thousand subjects each year. There is then a fairly substantial probability that uncommon or even rare genetic characteristics will be encountered one or more times annually. These represent opportunities to profit from the experiments provided by "nature", but we can do so only if we are prepared with a procedure designed to exploit these occasions. The project reported in this thesis is intended primarily to serve as a provisional working model of a panel of procedures for future reference.

Up to the present no standardized set of procedures has been established to follow in the event of a chance encounter with an anemia due to genetic abnormality. An approach is needed that would effectively and efficiently classify the deviation, and thus exploit the opportunity to advance medical science as well as aid the patient and his family. The specific purpose of this project is to establish a reasonable set of procedures to accomplish this end within the usual capabilities of most ordinary hospital laboratories. The standardized approach adopted here may be appropriate now but should be subject to frequent

review in order to incorporate improvements that may become readily available.

The panel of tests proposed here is designed to reveal (1) the intrinsic character of the anemia and (2) the genetic properties of the underlying defeat. Anemias in general, whether hereditary or acquired, can be caused by deficient production, hemorrhage, or premature destruction. Tests in Group 1 (above) are selected to discriminate between these possibilities using ordinary laboratory procedures.

The youngest cells deposited in the circulation from the bone marrow usually retain variable quantities of RNA which impart a slight basophilia to these new cells. These young cells are readily detected and evaluated by reticulocyte counts. It is often assumed for rough clinical estimates that reticulocytes represent one-day-old cells (Lewis, 1970). Theoretically, then, the product of the reticulocyte percentage and total erythrocyte count should provide a quantitative index of the daily activity of the bone marrow. Unfortunately, the inherent statistical error of enumeration procedures (Lewis, 1966) tends to distort both of these measurements, and the resulting estimate of relative bone marrow activity must be interpreted with some caution. Nevertheless, these estimates, however crude, will in most instances reveal significant departures of bone marrow activity from normal levels. For this purpose

the reticulocyte count along with routine erythrocyte and hemoglobin determinations form an important component of the proposed panel.

Anemia secondary to some genetic defect in hemostatic mechanisms resulting in blood loss is not likely to provide much of a diagnostic challenge, unless the hemorrhages are occult. In any event, even relatively small blood losses will in time deplete iron stores and bring on a secondary anemia. Ideally, this should be established by measurements of serum iron and iron-binding capacity, but these tests are not so easily incorporated into a routine screening panel. Many laboratories are quite capable of performing these tests, but unless the blood sample is obtained fairly early in the morning (i.e., before 10 AM), the results are not usable. Where a single patient is involved this creates little serious difficulty, but when it becomes necessary to obtain specimens from siblings and relatives as part of the genetic evaluation, the difficulties are compounded. By including the packed cell volume (PCV) in the panel of tests along with hemoglobin estimates the mean corpuscular hemoglobin concentration (MCHC) can be calculated and used as a fairly reliable but indirect indicator of the adequacy of iron stores.

Although the metabolic rate of mammalian erythrocytes is low, it is not negligible and possesses a degree of importance out of proportion to its magnitude. Normal

erythrocytes endure approximately 120 days in the circulation not only because the metabolic rate of erythrocytes is low but also because the enzymes present are sufficient to last throughout this period. Other cells in the body with metabolic rates as low as or lower than that of erythrocytes may persist for even longer periods than erythrocytes, but these cells are equipped with nuclei and ribosomes, machinery essential for the maintenance or replacement of enzymes consumed even at a low rate. Some hereditary defects seem to shorten the life span of the erythrocyte for mechanical reasons as in sickle cell anemia. By and large, however, a substantial proportion of hereditary anemias are likely to be associated with enzyme defects which shorten the life span of the circulating erythrocyte. If we accept the "one gene-one enxyme" concept of Beadle (1957), it is reasonable to conclude that any new human mutations induced perhaps by accidental irradiation, will be associated with some enzyme loss or deficit, and these may manifest themselves as anemias due to shortened life span.

Several accurate and sophisticated techniques have been devised for measuring the life span of erythrocytes (Ashby, 1919; Weinstein and LeRoy, 1953). Aside from the inherent technical difficulties involved in the application of these methods, they all require multiple samplings of blood over a period of about four to eight weeks. For genetic surveys a more immediate, indirect test becomes

desirable. As discussed by Lewis (1970) the total serum bilirubin concentration can be used to estimate the rate of erythrocyte breakdown as it is related to life span. Total bilirubin measurements can not always be used in a simple automatic way, as these measurements are also a reflection of the capacity of the liver to remove bilirubin. Generally, however, the confusion of liver disease severe enough to produce jaundice with some anemia of hereditary origin is not unlikely. This is especially true in those hospital laboratories where liver function is monitored by more than a single estimate of bilirubin concentration in the serum.

There has been an impressive increase in biochemical knowledge over the past few decades and there has been a corresponding increase in the availability of sensitive, precise chemical measurements. Because of this, there has been a noticeable tendency to overlook the value and importance of morphologic changes in disease. It is, perhaps, appropriate then to point out that the unique character of the erythrocyte's structure is maintained by a highly complicated and inter-related series of biochemical characteristics and changes. Target cells, sickle cells, and spherocytes readily come to mind as examples of subtle genetic changes first detected by morphologic criteria and still only partially explained by biochemical changes. The

value of a careful scrutiny of the erythrocytes as they appear on ordinary smears is self-evident.

The remaining tests form a group of attributes which are potentially useful in analyzing the genetic inter-relationships. Eventually sets of attributes affected by one or more closely linked genes will serve as phenotypic labels for each man's 23 chromosomal pairs. However, until this goal is achieved, the individual blood group systems generally called "public antigens" provide a more or less satisfactory substitute. Theoretically, measurements of activity levels of various enzyme systems would be equally useful, but as a practical matter, it is generally easier to categorize red blood cells; the equipment is relatively simple and readily available in all laboratories, and if panels of appropriate sera are available along with Coomb's serum, the subject's system pattern can be quickly determined. Such a pattern is likely to manifest considerable individuality, but rather than being unique, may serve to reveal certain common genetic properties.

Examination of relatives is, of course, basic to any genetic study. Questions of dominance or of autosomal or sex linkage can be discovered in no other way. Since the entire panel, with the possible exception of lymphocyte cultures, should be applied to all of the relatives, the desirability of simple or easy tests is clear.

Perhaps simpler or easier methods will become available some day for the culture of cells and the examination of the structure of their chromosomes. The methods now available are not particularly difficult, but they are time consuming and expensive. It is not easy to defend the inclusion of this examination as part of a routine panel, because the probability of obtaining useful data may not be proportional to the time and expense. The same discouraging statistical prospect faces every prospector, but because there are many prospectors, the small proportion that "strike it rich" comprises an impressive number. If it is at all possible to obtain a culture and photographs of a spread of chromosomes at least from the propositus, the opportunity to do so should not be neglected.

An opportunity to demonstrate the potential utility of the panel of studies recommended here arose when a patient at Butterworth Hospital (Grand Rapids, Michigan) was discovered to have elliptical erythrocytes in his peripheral smear. This opportunity was unusually fortunate, since virtually all of the relatives lived within a radius of twenty miles and were relatively readily available.

## REVIEW OF LITERATURE

Hereditary elliptocytosis is a congenital abnormality of erythrocytes characterized by elliptical or ovoid cells and sometimes hemolytic anemia. The presence of elliptical erythrocytes was first observed by Dresbach, who wrote, "In this short note the writer desires to place on record a peculiar anomaly in human red blood corpuscles. This interesting variation came to notice in the histologic laboratory of Ohio State University in October, 1902. The class at that time was studying the human corpuscle. . . . The student in whose blood these corpuscles were found was a healthy mulatto about twenty-two years of age."

Variations exist in estimates of the incidence of elliptocytosis. Dacie (1960) said that this anomaly is encountered in virtually all ethnic groups with a relatively low frequency of four per million population. Bannerman and Renwick (1962) report an apparent incidence of one in 4,000, but this figure is not to be taken as an estimate of the true incidence of the condition since it refers to a selected hospital population. It should be noted that no estimate of incidence in a general population is available; all reported estimates in the literature are based on hospital populations. (USA: one in

2,000--Bannerman and Renwick, 1962). Wintrobe (1964) reported estimates of the incidence of hereditary elliptocytosis in the general population at about 0.04%.

Cheney (1932) reported that considerable interest has centered about the microscopic examination of the bone marrow in order to determine whether the elliptic cell forms could be identified before they entered the blood stream. The bone marrow is normal in individuals whose blood contains elliptocytes. Since the underlying cause of the erythrocyte abnormality is still unknown, diagnosis depends entirely on morphological findings. No abnormalities have been discovered in the hemoglobin of the patients (Dacie, 1960). Elliptocytosis has been reported in sporadic association with other genetically determined disorders such as sickle cell trait, hemoglobin-C trait, heterozygous B-thalasemia, hereditary telangiectasia, and glucose-6-PD deficiency (Baehaner, 1968).

The elliptocyte is a dumbell-shaped structure in one plane and an ellipse in another plane. Rebuck's (1968) morphologic studies indicated a bipolar massing of hemoglobin. He said that this bipolar massing might be expected to weaken the intermolecular binding forces, as well as to render the polar surfaces more resistant to crenation.

Dacie (1960) and Wintrobe (1964) said that although the condition is usually harmless, it has been found to be

associated with evidence of increased blood destruction in about 12% of those carrying the trait. Elliptocytosis manifests itself in three degrees: (1) the latent form, with no signs of hemolysis observed, but elliptical cells on the peripheral blood smear; (2) compensated form, in which hemolysis is compensated entirely by an increased erythropoiesis; and (3) the non-compensated form, which is characterized by hemolytic anemia.

Geerdink, Helleman and Verloop (1966) pointed out that elliptocytosis does not influence the hemoglobin concentration, the packed cell volume, or the erythrocyte count per unit volume of blood. Their patients showed a compensated hemolysis, regardless of whether they belonged to families in which a genetic linkage between elliptocytosis and Rhesus factor exists. The reticulocyte count may be normal in elliptocytosis. In non-anemic patients with fully compensated hemolysis it is normal. In patients with overt hemolysis the reticulocyte counts may reach 20% or even higher (Cheney, 1932; Marshall, et al., 1954; Motulsky, et al., 1954; Dacie, 1960; Geerdink, Helleman and Verloop, 1966).

Hereditary elliptocytosis is the heterozygous manifestation of a rare allele. The gene is considered to be a conditional, autosomal dominant (Bannerman and Renwick, 1962), because we do not know the homozygous effect of this gene. Autosomal refers to inheritance on chromosomes other

than the sex chromosomes, X and Y. Hallmarks of dominant, autosomal inheritance are: (1) children do not show the trait unless at least one parent does; (2) the trait appears with equal frequency in males and females; (3) if the trait is rare, most, if not all, persons who have it will be heterozygotes; and (4) when heterozygotes marry unaffected mates, half the children can be expected to show the trait (Moody, 1969). Genes for hereditary elliptocytosis are suitable for linkage investigations because they are detectable in the heterozygote. Morton (1956) pointed out that elliptocytosis may be classified genetically into at least two types, depending on the presence or absence of close linkage with the Rhesus locus. Clinical and linkage data suggest that hemolysis occurs less often or less severely in Rhesus-linked elliptocytosis than in those not so linked (Bannerman and Renwick). Lawler (1954) demonstrated linkage between the locus for Rhesus factor and elliptocytosis. Her work demonstrated that the gene for elliptocytosis was traveling on the same chromosome as $R_2$ (DcE), and there is no example of crossing over. The gene for elliptocytosis has been found linked with most of the other alleles at the Rhesus locus. The other alleles at this locus are: r (dce), $R_1$ (DCe) and $R_o$ (Dce). A comparative study of the degree of hemolysis in the various families disclosed that the patients in one "non-linked" family showed a more markedly pathological

hemolysis than those in one "linked" family. This suggested that both genetically and biochemically at least two types of elliptocytosis exist (Geerdink, Helleman and Verloop, 1966).

Studies of osmotic fragility have yielded varied results. In asymptomatic elliptocytosis the results are normal; in the presence of hemolysis the osmotic fragility may or may not be increased. Few studies of the effect of incubation at 37°C for 24 hours have been made. It is suggested that, in patients whose osmotic fragility was increased before incubation, the effect of incubation will be similar to that in hereditary spherocytosis with hemolysis occurring in 0.85% sodium chloride. It is clear that, unlike the results noted in hereditary spherocytosis, the osmotic fragility of elliptocytosis may not be increased above the normal despite the presence of overt hemolysis (Marshall, et al., 1954; Dacie, 1960).

Treatment consists of blood transfusions in cases of aplastic crises. In hemolytic anemia, splenectomy is the most effective treatment, with improvement in anemia, reduction in jaundice and less evidence of regeneration. The blood picture after splenectomy shows an increase in the number of microelliptocytes and bizarre-shaped cells. It is likely that this picture is due to increased life-span of the erythrocytes. The tendency is for time-expired elliptocytes to undergo fragmentation, and the fragments

to circulate for a longer period as a result of the removal of the filtering action of the spleen (Motulsky, et al., 1954; Dacie, 1960; Figure 4).

## Summary of Literature Review

1. No hemoglobin abnormalities have been demonstrated in hereditary elliptocytosis.
2. The basic defect for this erythrocyte abnormality remains unknown.
3. Hereditary elliptocytosis manifests itself in three forms:
   a. latent form
   b. compensated form
   c. hemolytic anemia
4. No treatment is required for hereditary non-hemolytic elliptocytosis. Splenectomy is advised for the hemolytic form.
5. Genetically, hereditary elliptocytosis is either Rhesus-linked, or non-linked, the latter being the more hemolytic.

## METHODS AND MATERIALS

The family studied consisted of 41 members of German-Dutch descent. They ranged in age from 6 to 85 years. They reside in the Western Michigan area of Grand Rapids, Martin and Kalamazoo. This family was very interested in the project. They were very helpful and co-operative in providing information and, most important, their blood samples.

Blood was drawn using B-D Vacutainers, 3218, 15-ml. capacity and 4739 (3204Q), 7-ml. capacity, using the disposable 20-ga. x 1-1/2 inch needle (100) in the reusable plastic adapters.

### Bilirubin Level

The total bilirubin values were determined on a sequential, multi-phasic Auto Analyzer[1] by the Jandrassik and Grof method.

### Hemoglobin Concentration

The hemoglobin, in Gms. per 100 ml., was measured using cyanmethemoglobin reagent. Whole blood (0.02 ml.)

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[1]SMA 12/60, Technicon Instruments Corporation, Tarrytown, New York 10591.

was measured, using a disposable pipet,[2] into 5 ml. of Drabkin's solution[3] dispensed from an Oxford Pipettor.[4] When the dilution was made, it was allowed to stand 10 minutes before reading in a Hemophotometer.[5]

## Packed Cell Volume (Hematocrit) Determinations

The packed cell volume (PCV) determinations were made using heparinized tubes,[6] 75-mm. length, inner diameter 1.1 - 1.2 mm., filling them two-thirds full with whole blood. They were then flame-sealed, cooled, and spun for 5 minutes in a Hematocrit-Electrifuge.[7] They were read on an International Micro-Capillary Reader.[8]

## Chromosome Cultures

Chromosome studies were performed using the TC Chromosome Culture Kit.[9] See Appendix B for procedure.

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[2] S/P diSPO Micro Pipet (20 microliters ± 1/2%. P4518-20). Scientific Products, Allen Park, Michigan.

[3] Hycel Cyanmethemoglobin, Hycel, Inc.

[4] SP Oxford Pipettor, Oxford Laboratories, P 5058-1.

[5] Fisher Hemophotometer, Flo-Thru Model, Fisher Scientific Co.

[6] Clay-Adams, No. 1037.

[7] Chicago Surgical and Electrical Co., Model No. 30.

[8] International Equipment Co., Model CR.

[9] Difco Laboratories.

Reticulocyte Count

The reticulocyte count was made using the procedure of E. Whitehouse, M. T. (ASCP), Wayne County General Hospital. Equal parts of whole blood and staining fluid were mixed in a capillary tube, let stand for ten minutes, and mixed well. Thin smears were then made and air-dried. Red blood cells stain a light blue; the reticulum stains a deep blue. Reticulum cells were counted under oil immersion without counterstaining or fixation. Two counts of 1,000 cells each were made using two different slides. The average percent was recorded. See Appendix A for staining fluid.

Blood Typing

One drop of Anti-A and Anti-B, Blood Grouping Serum (Human)[10] was placed in separate wells on a cold blood typing plate to determine ABO classification. A drop of 40 - 50 % cell suspension was added after mixing by rotation for two minutes; reactions were read and recorded.

The above procedure was repeated for the Rh typing, with the exception that a warm typing plate was used for the two-minute timing period. Typing sera included:

Anti-$Rh_o$ (Anti-D) Serum.[11]

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[10]For slide test and modified tube test. Ortho Diagnostics, Raritan, New Jersey, 08869.

[11]Ibid.

Anti-rh' (Anti-C) Serum.[12]

Anti-rh" (Anti-E) Serum.[13]

Anti-hr' (Anti-c) Human.[14]

Anti-hr" (Anti-e) Human.[15]

Spectrogen II[16] was used to enhance the agglutination for Anti-e in I-1, 3, and 5, and II-5. An Indirect Coombs technique was used.

Anti-K (Kell) Typing

Anti-K Serum. (Anti-Kell) Human for Indirect Coombs Test.[17] Cells were washed three times in saline before use. Two drops of the antiserum were placed in a small test tube, 1 drop of a 4-6% cell suspension was added and mixed. Positive control (Tencell Panel, No. 8). Incubated at 37°C for 15 minutes. Washed 3 times with saline. Two drops of Anti-Human (Coombs) serum[18] was added to the button of washed cells, mixed, then centrifuged. The cells were resuspended by gentle shaking. Read macroscopically.

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[12] Ibid.

[13] Ibid.

[14] Spectra Biologicals, Oxnard, California, 93030.

[15] Ibid.

[16] Ibid.

[17] Ibid.

[18] Ibid.

## RESULTS

Of the people tested, 14 had elliptical cells in their peripheral blood smears and increased reticulocyte counts. Of the 14 affected, seven were males and seven were females.

A comparison of hemolysis in the elliptocytotic patients and the normal controls is shown in the results in Table 1.

Table 1.--Comparison of hemolysis in elliptocytic patients and normal controls.

| | | Gms./100 ml. Hemoglobin (± 1 S.D.) | mgs.% Bilirubin (± 1 S.D.) | % Reticulocytes (± 1 S.D.) |
|---|---|---|---|---|
| Normal | Male | 14.8 ± 0.4 | 0.4 ± 0.1 | 1.2 ± 0.4 |
| El + | Male | 14.1 ± 2.0 | 0.8 ± 0.1 | 3.5 ± 0.5 |
| Normal | Female | 13.6 ± 0.9 | 0.3 ± 0.1 | 1.0 ± 0.4 |
| El + | Female | 13.2 ± 0.9 | 0.8 ± 0.1 | 2.4 ± 1.1 |

The lymphocyte cultures showed no evidence of a marker chromosome. A marker gene is defined as a gene utilized in investigations to indicate the presence of a certain chromosome with its genetic contents (Moody, 1969).

It is usually seen as a satellite or an extra appendage or constriction on a chromosome.

The genes for Rhesus and elliptocytosis ("linked" form) are three crossover units apart which is indicative of a very close genetic linkage. The recombination value when linkage is present is estimated to be 3.3 ± 2.3% with a 90% fiducial limit of 8.6% (Morton, 1956). Crossing-over is defined as an exchange of genetic material between homologous chromosomes occurring during meiosis. Meiosis is the process by which diploid precursor cells give rise to haploid gametes (ova and sperm). The frequency of crossovers is dependent on the distance between the two gene loci under study, i.e., if genes lie close together the frequency of crossover is low, if the genes lie far apart the crossover frequency is high. Our studies in this area show that this family has the "non-linked" form of hereditary elliptocytosis. We have no knowledge of how the gene for elliptocytosis was transferred to generation I. We assume an initial crossover and that the original progenitors were heterozygous for the Rhesus system. Resulting from the initial crossover we have I-3, El+ with $R_2$, and I-5, El+ with r.

Considering II-5 thru 14, we have a minimum of one crossover, El+ with r; II-5, 9, and 13 are the non-crossovers; II-5 is El+ with $R_2$, while II-9 and 13 are El- with r.

In family I-5 and 6, II-15 thru 20, and III-13 thru 25, we assume the El+ gene to be linked with r. The family II-15 and 16, and III-13 thru 17, though most severely affected with this anomaly, gives little information due to the homozygous rr of II-15.

Family II-17 and 18, and III-18 thru 22 shows an incidence of three crossovers and three non-crossovers. The former are III-18, 19 and 20. The latter are II-18, III-21 and 22.

Three crossovers and one non-crossover are found in family II-19 and 20 and III-23 thru 25. In the first instance we have III-23, 24 and 25. II-20 belongs to the latter category.

We have an incidence of seven crossovers and seven non-crossovers. This represents approximately a 1:1 ratio which is not indicative of a close genetic linkage.

Table 2.--Results of hematologic procedures.

| Ident. | Age | Wt. | Blood Types ABO | Kell | Rhesus | Weiner | Hgb. gm/100 ml | PCV % | Retic. % | Bilirubin mg/100 ml | Ellipto. % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GENERATION I | | | | | | | | | | | |
| 1 | 83 | 150 | B | - | D-ccEe | $R_2r$ | 12.6 | 38 | 1.2 | 0.2 | 0 |
| 2 | 85 | 130 | A | - | D-Ccee | $R_1r$ | 15.0 | 46 | 1.9 | 0.7 | 0 |
| 3 | 81 | 115 | B | + | D-ccEe | $R_2r$ | 13.2 | 38 | 3.9 | 3.9 | 100 |
| 4 | | | A | - | D-Ccee | $R_1r$ | | | | | |
| 5 | 70 | 130 | AB | + | D-ccEe | $R_2r$ | 11.2 | 34 | 6.7 | 1.7 | 100 |
| 6 | 67 | 135 | A | - | D-Ccee | $R_1r$ | 12.8 | 39 | 1.0 | 0.2 | 0 |
| 7 | | | ? | | | | | | | | |
| 8 | | | ? | | | | | | | | |
| GENERATION II | | | | | | | | | | | |
| 1 | 58 | 145 | O | - | ddccee | rr | 14.8 | 44 | 0.7 | 0.2 | 0 |
| 2 | 62 | 165 | O | - | D-CCee | $R_1R_1$ | 16.0 | 46 | 0.7 | 0.4 | 0 |

Table 2.--Continued.

| Ident. | Age | Wt. | Blood Types ABO | Kell | Rhesus | Weiner | Hgb. gm/100 ml | PCV % | Retic. % | Bilirubin mg/100 ml | Ellipto. % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | | | ? | | | | | | | | |
| 4 | | | ? | | | | | | | | |
| 5 | 53 | 172 | AB | + | D-ccEe | $R_2r$ | 15.6 | 45 | 2.5 | 0.3 | 22 |
| 6 | 53 | 125 | A | - | D-CCee | $R_1R_1$ | 14.9 | 43 | 1.3 | 0.7 | 0 |
| 7 | | | ? | | | | | | | | |
| 8 | | | ? | | | | | | | | |
| 9 | 51 | 175 | B | + | D-Ccee | $R_1r$ | 15.8 | 44 | 0.9 | 0.1 | 0 |
| 10 | 51 | 118 | B | - | D-ccEe | $R_2r$ | 13.0 | 40 | 0.6 | 0.2 | 0 |
| 11 | 50 | 175 | B | + | ddccee | rr | 14.9 | 45 | 2.2 | 0.4 | 25 |
| 12 | 42 | 135 | A | - | D-Ccee | $R_1r$ | 12.7 | 39 | 0.8 | 0.4 | 0 |
| 13 | 47 | 158 | AB | + | ddccee | rr | 15.2 | 44 | 2.0 | 0.3 | 0 |

Table 2.--Continued.

| Ident. | Age | Wt. | Blood Types ABO | Kell | Rhesus | Weiner | Hgb gm/100 ml | PCV % | Retic. % | Bilirubin mg/100 ml | Ellipto. % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 46 | 150 | O | - | D-Ccee | $R_1r$ | 13.8 | 41 | 0.9 | 0.2 | 0 |
| 15 | 44 | 160 | A | - | ddccee | rr | 14.8 | 44 | 3.8 | 0.4 | 24 |
| 16 | 40 | 138 | A | - | D-Ccee | $R_1r$ | 12.7 | 40 | 0.8 | 0.3 | 0 |
| 17 | 43 | 175 | A | - | D-CCee | $R_1R_1$ | 15.5 | 46 | 1.5 | 0.5 | 0 |
| 18 | 42 | 110 | A | + | D-Ccee | $R_1r$ | 13.9 | 42 | 1.9 | 0.6 | 90 |
| 19 | | | A | - | ddccee | rr | | | | | |
| 20 | 41 | 110 | B | + | D-Ccee | $R_1r$ | 12.5 | 39 | 2.4 | 0.4 | 75 |
| GENERATION III | | | | | | | | | | | |
| 1 | 31 | 125 | O | - | D-Ccee | $R_1r$ | 14.5 | 43.5 | 0.8 | 0.3 | 0 |
| 2 | | | ? | | | | | | | | |
| 3 | | | ? | | | | | | | | |
| 4 | 14 | 120 | B | + | ddccee | rr | 14.8 | 42.5 | 0.8 | 0.2 | 0 |

Table 2.--Continued.

| Ident. | Age | Wt. | Blood Types | | | | Hgb. gm/100 ml | PCV % | Retic. % | Bilirubin mg/100 ml | Ellipto. % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ABO | Kell | Rhesus | Weiner | | | | | |
| 5 | 21 | 175 | A | + | ddccee | rr | 15.7 | 45 | 1.4 | 0.4 | 0 |
| 6 | 19 | 115 | A | - | ddccee | rr | 12.7 | 38 | 1.9 | 0.3 | 0 |
| 7 | 16 | 110 | A | - | ddccee | rr | 13.7 | 40 | 1.1 | 0.5 | 0 |
| 8 | | | ? | | | | | | | | |
| 9 | | | ? | | | | | | | | |
| 10 | 15 | 128 | A | + | ddccee | rr | 13.4 | 41 | 0.8 | 0.3 | 0 |
| 11 | 13 | 90 | A | + | D-Ccee | $R_1r$ | 12.5 | 36.5 | 1.2 | 0.2 | 0 |
| 12 | 8 | 80 | A | + | D-Ccee | $R_1r$ | 13.6 | 40 | 0.8 | 0.4 | 0 |
| 13 | 16 | 110 | A | - | D-Ccee | $R_1r$ | 14.2 | 42 | 3.8 | 0.7 | 100 |
| 14 | 15 | 144 | A | - | D-Ccee | $R_1r$ | 15.6 | 45 | 4.6 | 1.3 | 100 |
| 15 | 10 | 78 | A | - | D-Ccee | $R_1r$ | 13.3 | 41 | 0.1 | 0.3 | 0 |

Table 2.--Continued.

| Ident. | Age | Wt. | Blood Types ABO | Kell | Rhesus | Weiner | Hgb. gm/100 ml | PCV % | Retic. % | Bilirubin mg/100 ml | Ellipto. % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *16 | before splenectomy | | | | | | 7.2 | 23.5 | 11.4 | | |
| 16 | 9 | 77 | A | - | D-Ccee | $R_1r$ | 11.0 | 34 | 3.2 | 0.8 | 26 |
| 17 | 6 | 60 | A | - | ddccee | rr | 11.9 | 36 | 2.0 | 0.5 | 94 |
| 18 | 20 | 100 | A | - | D-CCee | $R_1R_1$ | 14.1 | 42 | 2.0 | 0.3 | 30 |
| 19 | 19 | 140 | A | - | D-CCee | $R_1R_1$ | 15.6 | 46 | 2.0 | 0.8 | 63 |
| 20 | 16 | 165 | A | - | D-Ccee | $R_1r$ | 16.5 | 48 | 1.4 | 0.7 | 0 |
| 21 | 15 | 150 | A | + | D-CCee | $R_1R_1$ | 15.5 | 46 | 1.4 | 0.7 | 0 |
| 22 | 12 | 75 | A | + | D-CCee | $R_1R_1$ | 13.6 | 42 | 1.2 | 0.6 | 0 |
| 23 | 19 | 119 | AB | - | D-Ccee | $R_1r$ | 13.0 | 37 | 0.8 | 0.3 | 40 |
| 24 | 16 | 140 | AB | + | ddccee | rr | 14.2 | 42 | 1.5 | 0.6 | 0 |
| 25 | 13 | 110 | O | + | ddccee | rr | 13.6 | 39 | 1.3 | 0.5 | 0 |
| 26 | Adopted | | | | | | | | | | |

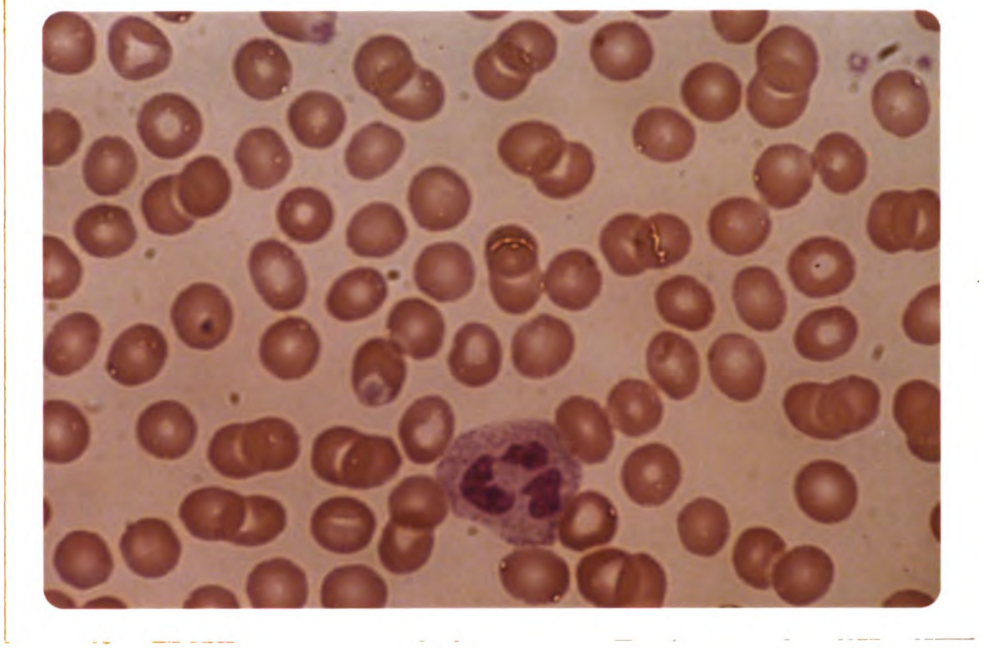

Figure 1.--Normal erythrocytes (1000X).

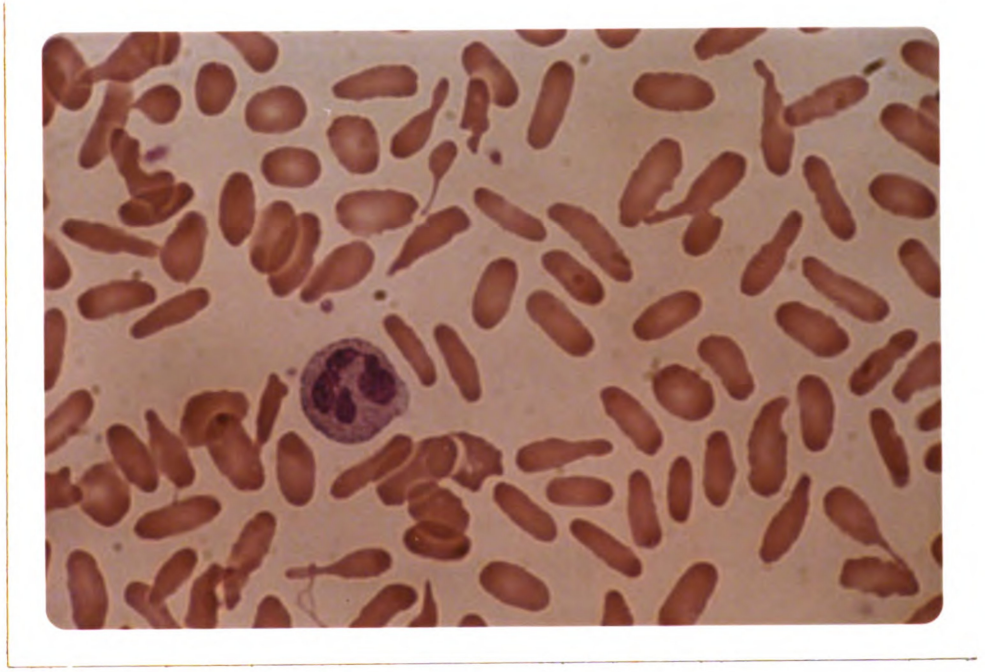

Figure 2.--Elliptocytes (1000X).

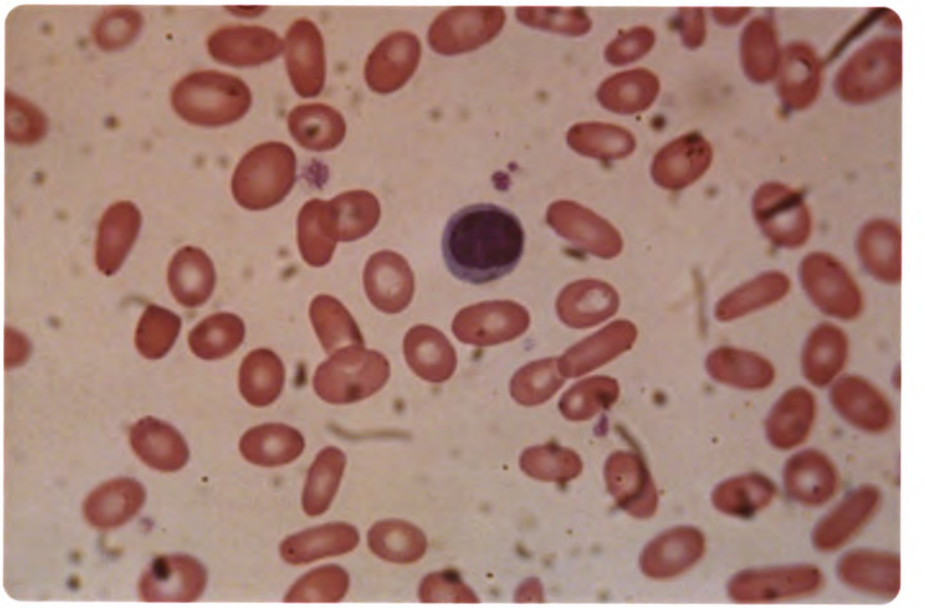

Figure 3.--Elliptocytes, III-16, 1000X
before splenectomy.

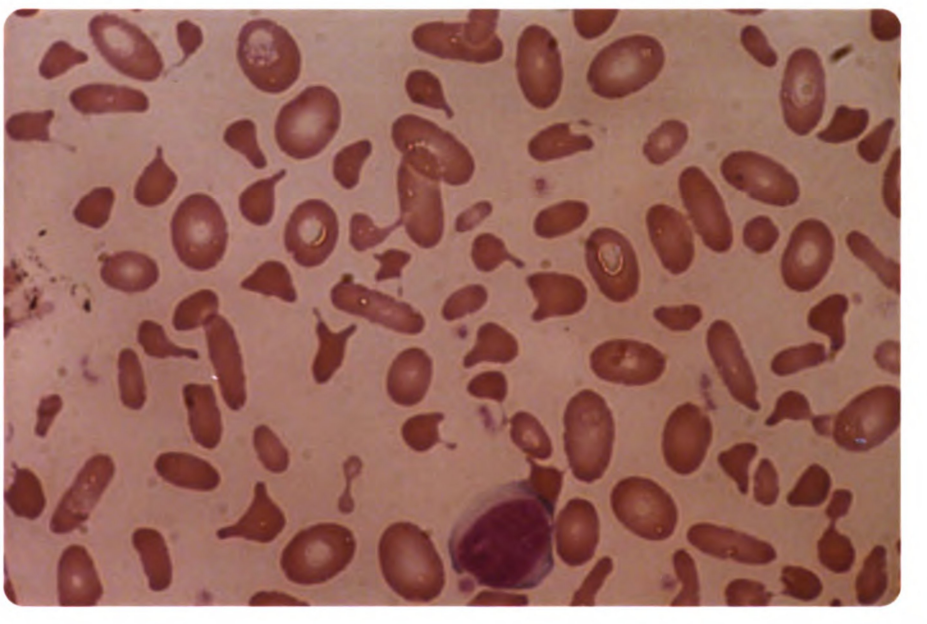

Figure 4.--Elliptocytes, III-16, 1000X
after spenectomy.

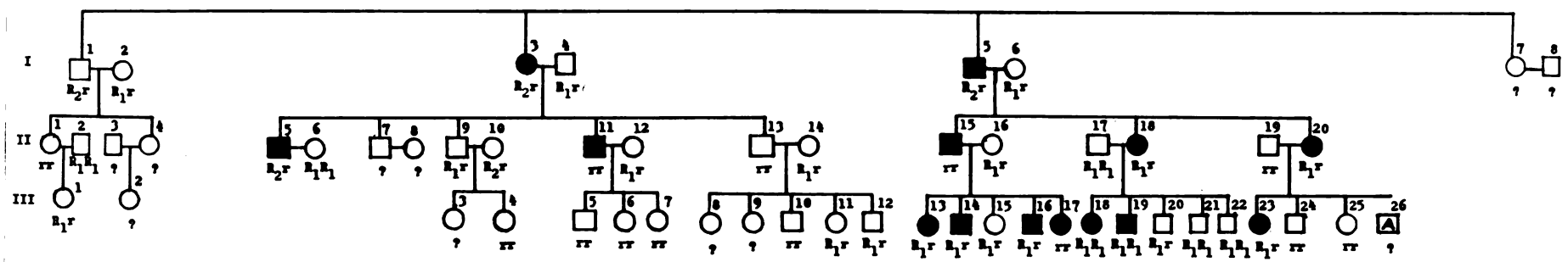

Figure 5.--Pedigree tree.

Legend

- Normal
- Elliptocytosis
- A - Adopted
- ? - Not available at the time of the study.
- * - Propositus

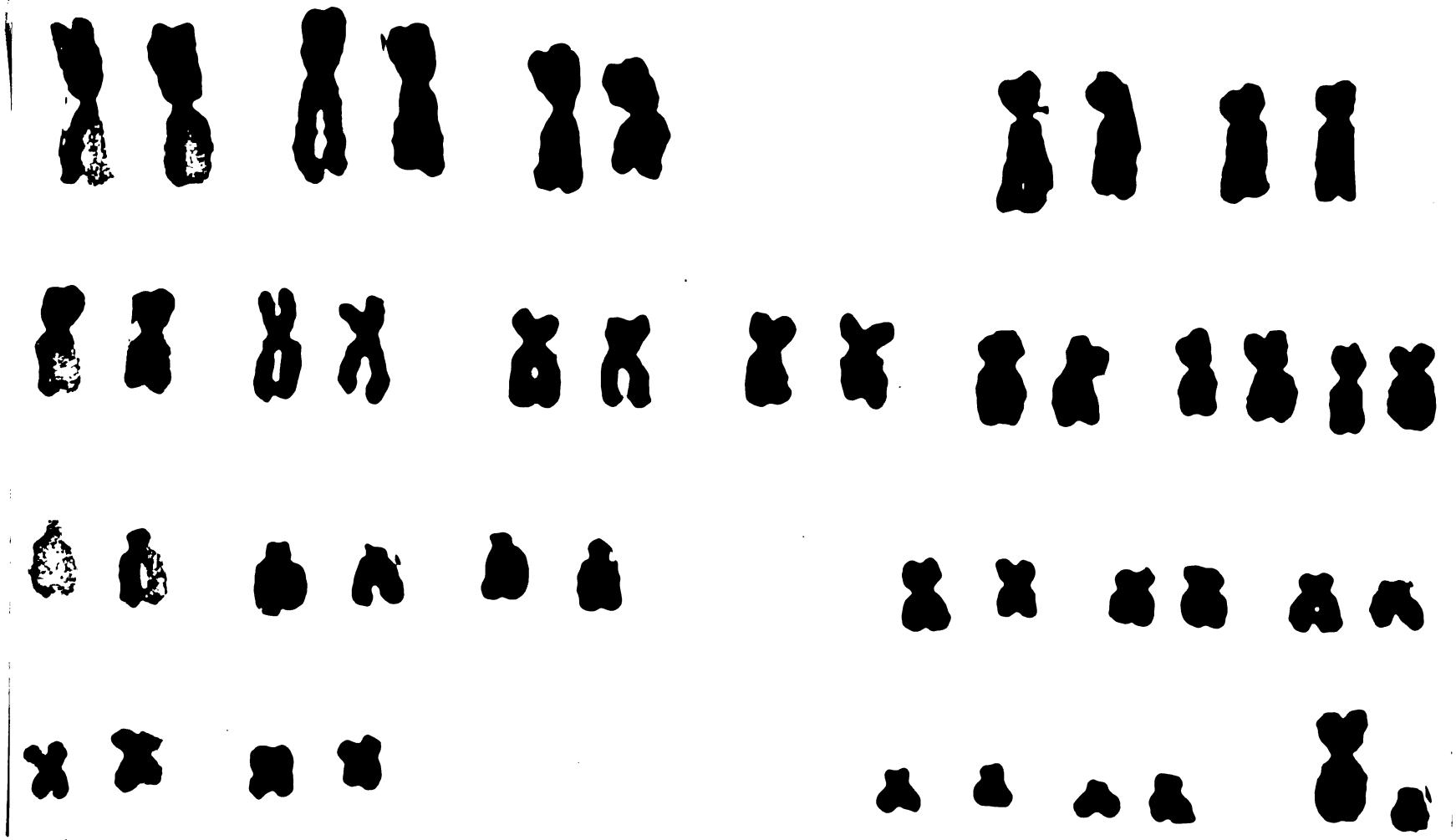



Figure 6.--Karyotype of propositus. Karyotype of III-16, the propositus assembled from metaphase figure derived from peripheral blood lymphocytes. A normal chromosome constitution is present (46 XY).

## DISCUSSION

The gene determining the presence or absence of elliptocytosis in the family studied here is located on the same chromosome carrying the alleles determining the presence of the Rh factor. The Rh and El genes are probably on one of the longer chromosomes and the frequency of crossing-over suggests that they are at opposite ends. Since no clear difference in severity of anemia is noted here between those cases of elliptocytosis that are Rh+ and Rh-, the Rhesus factor does not seem to aggravate or ameliorate the effects of the El gene.

From the reticulocyte percentages and bilirubin levels observed here it is clear that there must be increased marrow activity and corresponding increase in rate of erythrocyte destruction. However, since there is no significant difference in hemoglobin concentrations between the El+ and El- groups, we may conclude that there is a compensated "anemia" in most of these subjects. This implies, then, that there is a shortened erythrocyte lifespan associated with elliptocytosis. This raises the question as to whether the premature destruction of these erythrocytes is a mechanical consequence of the altered shape or a consequence of some unidentified enzyme

deficiency. On theoretical grounds the possibility that elliptical cells would be subject to more mechanical stress and trauma than a round cell seems unlikely. In some non-mammalian species (birds, reptiles) the erythrocytes are normally oval or elliptical. Furthermore, the slightly elongated configuration of these cells would seem to be more streamlined and adapted to minimizing the effects of hydrodynamic forces on the cell.

Since it has already been established that the elliptical shape is produced after the cell develops in the marrow and enters the bloodstream (Cheney, 1932), and since there are cases showing more severe anemia due to a gene on separate chromosomes (i.e., unassociated with the Rhesus factor), it is reasonable to conclude that elliptical form and the shortened life-span results from some inadequacy in one of the energy-producing biochemical sequences. In the cases associated with Rh factor the determinant gene results in a deficiency at one point in the process, while in those instances not associated with Rh the determinant gene is located on some other chromosome but produces a more severe enzyme deficiency at some other point in the same biochemical sequence. Unfortunately, these questions were not raised until after the data had been accumulated and examined; in retrospect it is now obvious that it would have been desirable to make some effort to determine whether the enzyme defect involved the

Embden-Meyerhof anaerobic glycolytic sequence or the pentose shunt. If the pentose shunt were involved, a simple Heinz body test would have yielded results similar to that observed in deficiencies of glucose-6-phosphate (G-6-PD) deficiencies. If the Embden-Meyerhof sequence were involved, this might be more difficult to establish, but differences in osmotic fragility before and after incubation with and without glucose in the medium might have provided a starting point.

We may conclude from the experience in applying the proposed panel of tests that even though this was designed to yield a maximum of information, the inclusion of simple tests to narrow down the location of a genetically determined enzyme deficiency would be desirable.

## SUMMARY AND CONCLUSIONS

A study of the distribution of hereditary elliptocytosis among 41 members of a family yielded results conforming to established genetic patterns for this anomaly. Using readily available laboratory procedures supplemented with a chromosome analysis, our results indicate that the life-span of the elliptocytes is shortened and that in most instances anemia is not manifested because of compensatory increase in erythropoiesis by the bone marrow.

# REFERENCES CITED

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Ashby, W. The determination of the life span of the transfused blood corpuscles in man. J. Exper. Med., 29:267, 1919.

Baehner, R. L. Hereditary Elliptocytosis and Primary Renal Tubular Acidosis in a Single Family. Amer. J. Dis. Ch., 115:414, 1968.

Bannerman, R. M., and Renwick, J. H. The Hereditary Elliptocytosis: Clinical and Linkage Data. Ann. Hum. Gen., 26:23, 1962.

Beadle, G. W. The genetic basis of biological specificity. J. Aller, 58:392, 1957.

Bishop, C., and Surgenor, D. M. The Red Blood Cell. Academic Press, 1964.

Bless, S. R. Hereditary Elliptocytosis: Selected Studies in Genetics and Anemia. Ann. Int. Med., 68:1164, 1968.

Chalmers, J. N. M., and Lawler, S. D. Data on linkage in man: elliptocytosis and blood groups I. Families 1 and 2. Ann. Eugen., Cambr., 17:267, 1953.

Cheney, G. Elliptic Human Erythrocytes. JAMA, 98:878, 1932.

Dacie, J. V. The Hemolytic Anaemias, Part I. Grune and Stratton, Inc., 1960.

Dresbach, M. Elliptic Human Red Cells. Science, 19:469, 1904.

Fujii, T., Moloney, W. C., and Morton, N. E. Data on linkage of ovalocytosis and blood groups. Amer. J. Hum. Gen., 7:72, 1955.

Geerdink, R. A. Hereditary Elliptocytosis and Hyperhaemolysis. Act. Med. SC., 179:715, 1966.

Goodall, H. B., Hendry, D. W. W., Lawler, S. D., and Stephen, S. A. Data on linkage in man: elliptocytosis and blood groups II. Family 3. Ann. Eugen., Cambr., 17:272, 1953.

Goodall, H. B., Hendry, D. W. W., Lawler, S. D., and Stephen, S. A. Data on linkage in man: elliptocytosis and blood groups III. Family 4. Ann. Eugen., Cambr., 18:328, 1954.

Lawler, S. D., and Sandler, M. Data on linkage in man: elliptocytosis and blood groups IV. Families 5, 6 and 7. Ann. Eugen., Cambr., 18:328, 1954.

Lewis, A. E. Biostatistics. Reinhold Publishing Corporation, New York, 1966.

Lewis, A. E. Principles of Hematology. Appleton-Century-Crofts, New York, 1970.

Marshall, R. A., Bird, R. M., Bailey, H. K., and Beckner, E. Genetic Linkage Between Ovalocytosis and the Rh Blood Type. J. Clin. Invest., 33:790, 1954.

Moody, P. A. Genetics of Man. W. W. Norton and Company, Inc., New York, 1969.

Morton, N. E. The Detection and Estimation of Linkage Between the Genes for Elliptocytosis and the Rh Blood Type. Am. J. Human. Genet., 8:80, 1956.

Motulsky, A. G., Singer, K., Crosby, W. H., and Smith, V. The Life Span of the Elliptocyte. Blood, 9:57, 1954.

Nijenhuis, L. E., and Geerdink, R. A. Deviating Segregations in a Family with Elliptocytosis. Ann. Hum. Gen., London, 33:413, 1970.

Race, R. R., and Sanger, R. Blood Groups in Man. F. A. Davis Company, Philadelphia, 1962.

Rebuck, J. W. An Unsuspected Ultrastructural Fault in Human Elliptocytes. AM. J. Clin. Path., 49:19, 1968.

Weenberg, E. The Structure of the Spleen and Hemolysis. Ann. Rev. Med., 20:41, 1969.

Weinstein, I. M., and LeRoy, G. V. Radioactive sodium chromate for the study of survival of red blood cells. J. Lab. & Clin. Med., 43:368, 1953.

Wintrobe, M. M. Clinical Hematology. 6 th Edition, Lea and Febiger, Philadelphia, 1967.

# APPENDICES

## APPENDIX A

Reticulocyte Stain

| | |
|---|---|
| New methylene blue (Color Index 927) | 0.1 gm. |
| Potassium oxalate | 1.6 gm. |
| Distilled water | 100.0 ml. |

Filter before using

## APPENDIX B

Procedure for Chromosome Culture

TC Chromosome Culture Kit
Code 5842
Difco Laboratories
Detroit, Michigan

For chromosomal analysis of leukocytes from peripheral blood.

1. Draw 10 cc of blood from a patient who has fasted for at least three hours. Transfer to a Blood Separation Vial. Mix by inversion. Let stand at room temperature, or 37°C, for 1-3 hours until at least 4 ml. of Plasma-Leukocyte suspension has separated.
2. Reconstitute each required bottle of chromosome medium with a vial of warm (37°C) chromosome reconstituting fluid.
3. Inoculate each bottle of rehydrated chromosome medium with 1.5-2.5 ml. of plasma-leukocyte suspension from the blood collection vial.
4. Incubate the inoculated bottle in a vertical position for three days at 37°C. Important to maintain proper pH.
5. Add one vial of chromosome arresting solution to each bottle and tilt once or twice to insure mixing.

6. Incubate an additional 3-6 hours at 37°C. Resuspend cells.
7. Transfer entire culture to a 12 ml. conial centrifuge tube. Centrifuge for 12 minutes at 800 rpm.
8. Pour off supernatant.
9. Add 5-6 ml. of warm (37°C) Hanks Solution. Resuspend cells.
10. Centrifuge at 800 rpm. for 5 minutes.
11. Aspirate all but 0.5 ml. of supernatant.
12. Resuspend the packed cells in the supernatant.
13. Add 1.5 ml. of warm (37°C) distilled water slowly while shaking.
14. Incubate the suspension at 37°C for 10 minutes.
15. Centrifuge at 600 rpm. for 5 minutes.
16. Aspirate the supernatant.
17. Add, without disturbing the cell button, 3-4 ml. of freshly prepared fixative consisting of 1 part Glacial Acetic Acid and 3 parts of methanol.
18. Let cells soak in fixative for 30 minutes.
19. Resuspend cells.
20. Centrifuge at 600 rpm. for 5 minutes. Discard supernatant.
21. Resuspend in 3-4 ml. of fresh fixative. Let stand for 5 minutes. Centrifuge at 600 rpm. for 5 minutes.
22. Aspirate the supernatant.

23. Add 0.25-0.5 ml. of fresh fixative to the button of cells to get a hazy suspension.
24. Use acid clean microscope slides, chilled in a beaker of distilled water in the refrigerator.
25. Shake excess water from slides and add 2-3 drops of cell suspension. Tip the slide to spread suspension.
26. Slides are then treated with Giemsa stain.
27. Examine the slides under the microscope.

# VITA

The author was born on January 31, 1938, in Grand Rapids, Michigan. She graduated from Catholic Central High School in June of 1955 and attended Grand Rapids Junior College and Aquinas College. After receiving a Bachelor of Science Degree in Medical Technology from Aquinas College in May 1960, she was accepted for Medical Technology internship at Butterworth Hospital, Grand Rapids. She completed her internship, June 30, 1959, and successfully passed the National Registry Examination in July, 1959.

In January 1965, the author assumed the duties of Hematology Section Chief, which required teaching medical technology students basic techniques and principles as well as developing and trying out new procedures.

In September 1969, she was granted a one-year educational leave to pursue the Master's Program in Clinical Laboratory Science at Michigan State University. Upon completion of her program she will resume her duties at Butterworth Hospital Laboratory.