THE DIAGNOSIS OF CARRIERS OF COOLEY'S ANAEMIA

BY

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Some 13 years ago Angelini (1937) and Caminopetros (1938a and b) independently postulated the existence of a mild or carrier form of Cooley's anaemia, basing their deductions on the discovery of the frequent occurrence of an increased erythrocytic resistance to saline in certain healthy relatives of clinical cases of this anaemia.

Since then the hereditary and familial nature of the disease has become widely accepted; nevertheless observers still differ in their opinions regarding the best way of detecting the latent or carrier form. Smith (1942) believes that "the occurrence of morphological changes (in the erythrocyte) constitutes a fundamental principle in the detection of the mild trait," and that, although erythrocytic saline resistance was the most reliable single investigation, a normal result would not necessarily invalidate the diagnosis.

Valentine and Neel (1944) agree that the saline resistance may not be so dramatically altered, and believe that the diagnosis rests upon several items, including a low mean cell volume, the presence of stipple cells, resistance to iron therapy, and a relatively normal mean cell haemoglobin concentration.

Dameshek (1946) attaches great importance for the detection of the mild trait to the presence of target or oval cells, and the relationship of haemoglobin content, erythrocyte count, and haematocrit. This relationship is not specifically defined, and he concedes that in many cases "it may not be dramatically abnormal." It would appear, then, that although many carriers present a clearly defined haematological picture, particularly when the erythrocytic resistance to saline is high, there still remains a number of individuals in whom the diagnosis tends to become a matter of personal interpretation of inconstant, and often complex, relationships.

In this paper 13 healthy Maltese, relatives of a patient with severe Cooley's anaemia previously described (Mooney, 1951), have been examined, and the haematological findings analysed in an attempt to define the carrier state more accurately and to discover a reliable method of detecting its presence.

Family History

The paternal and maternal genealogical trees are shown in Fig. 1. The paternal great-grandparents were both of pure Maltese ancestry, and seven children were born of the marriage, four boys and three girls. One of the boys died at the age of 15 after many years of indefinite ill-health. The cause of his death is obscure. The remaining six children were healthy in all respects, and the eldest boy became the grandparent of the family under scrutiny. It will be seen that he married twice. His first wife died in 1919 from influenza, his second in 1946 from a cancer of the colon. Nine children

were born of the first marriage, but only two have survived beyond infancy. These are Mr. B. (father) and J. B. (uncle). The second marriage was to a first cousin, and, although there were 13 children, only three survived beyond the second year of life.

The symptoms manifested by the 17 children who died in infancy were, according to their father and surviving brothers, strikingly similar. All were pale and listless, failed to gain weight, and tended to develop a progressive general weakness which relentlessly deteriorated, until finally death supervened. None survived beyond the age of 2. Whether the deaths were, in fact, all due to Cooley's anaemia is a matter for conjecture.

As the second marriage was to a close relative, it seems reasonable to assume that, in the children of this marriage at any rate, Cooley's anaemia may have played a large part in their deaths. In the first marriage, however, it is more problematical. Certainly it still remains a possibility, as fortuitous consanguinity in marriage is inevitably high in a small island community such as Malta.

Mr. B., the father of the family, married 14 years ago and had seven children. Of these, two died from a severe type of anaemia before this investigation, another is a clinical case of Cooley's anaemia (Mooney, 1951), and the remainder are quite healthy.

J. B. (uncle) is married and has had two children. The first child died from dysentery when 2 weeks old. The second is J. B. (cousin). Of the three surviving children of the grandfather's second marriage, only J. B. (aunt) is married. She has had four children, all of whom are healthy, and the youngest, C. B. (cousin), is one of the individuals who has been scrutinized.

Maternal Origin of Family.—This half of the family is also of pure Maltese descent. The grandfather died at the age of 43 from diabetes, and the grandmother at the age of 66 after an accident. There were eight children of the marriage, four boys and four girls. One girl died from enteric fever in infancy, the second became the mother of the family under scrutiny, the third is married and has had 13 children, all of whom are healthy, and the fourth is unmarried.

The four brothers are all alive. The eldest, A. A. (uncle), is married and has had four children. Two of these were twins who died in infancy from dysentery, and the other two were both girls, who are now also married. The second brother, G. A., is married, but has no children. The remaining two sons were both serving in the Navy and were abroad during this investigation.

Investigations

Routine examination of blood included a full count, a differential white cell count, calculating various erythrocytic indices, estimating serum bilirubin, and the titre of Brucella antibodies, and the Kahn reaction for syphilis. In all but four members of the family a special study was made of the reticulocytosis and stippling of the red cells.

Red Cell Count.—An accurate red cell count is notoriously difficult to achieve, and various investigators have estimated the coefficient of variation to be as high as 7–8% (Magath, Berkson, and Hurn, 1936; Berg, 1945). In order to minimize this error and consequently obtain more accurate estimations of the mean cell thickness, diameter,

FIG. 1.—Genealogical tree of the affected family.

TABLE I
RESULTS OF HAEMATOLOGICAL INVESTIGATIONS

Name	Age	R.B.C.s per c.mm.	Hb. % (100% = 14.5 g.)	Colour Index	Mean Corpuscular Haemoglobin Concentration (%)	Mean Cell Volume (μ)	Mean Cell Dia- meter (μ)	Mean Cell Thick- ness (μ)	Stipple Cells (per c.mm.)	Reticu- lo- cytes (per c.mm.)	Target Cells	Standard Devi- ation in Size of R.B.C.s (s)	Titre of <i>Brucella melitensis</i> Antibody
Grandfather	66	4,970,000	87	0.88	32.3	78.5	7.71	1.69	19,500	—	Present	0.675	Nil
Father	33	5,520,000	87	0.79	28.7	79.6	7.52	1.79	6,000	32,000	"	0.502	"
J.B. (brother)	12	5,040,000	80	0.79	29.7	77.3	7.39	1.80	18,000	60,000	Absent	0.545	"
C.B. (brother)	10	4,930,000	87	0.88	30.8	81.1	7.17	2.00	9,500	50,000	Present	0.570	1/50
M.B. (sister)	4	4,660,000	87	0.94	29.3	90.0	7.54	2.03	3,000	24,000	Absent	0.462	Nil
T.B. (brother)	1	4,970,000	84	0.86	—	—	—	—	5,000	—	"	—	Nil
J.B. (uncle)	38	5,170,000	91	0.88	33.0	77.3	7.24	1.92	14,000	33,000	Present	0.560	Nil
J.B. (cousin)	12	4,560,000	87	0.95	33.6	82.2	7.49	1.90	5,000	—	Absent	0.587	1/25
R.B. (aunt)	16	5,000,000	82	0.82	33.0	73.0	7.56	1.63	2,000	—	Present	0.744	"
C.B. (cousin)	4	4,870,000	80	0.82	31.2	70.8	7.28	1.70	7,000	—	"	0.735	Nil
A.A. (uncle)	52	5,000,000	96	0.96	32.8	85.0	7.60	1.87	6,000	32,000	Absent	0.547	1/25
G.A. (uncle)	36	5,300,000	104	0.98	31.5	87.0	7.40	2.08	<250	25,000	"	0.440	Nil
Mother	36	4,390,000	81	0.94	30.9	89.6	7.28	2.03	3,000	36,000	"	0.478	1/25

and volume, two separate counts were done by a technician and myself, and instead of the usual 80 small squares 160 were counted in each case. The mean of the counts was taken as correct, and by this means the coefficient of variation was reduced to approximately $\pm 3\%$.

Stipple Cell Count.—The method employed was that recommended by Whitby and Britton (1946), using a simple methylene blue stain, and is considered to be superior to the ordinary Leishman film in the search for stipple cells.

The remaining investigations were performed by the commonly accepted techniques. Fully oxygenated blood was used in the estimation of the mean cell volume.

Differential Saline Resistance Test (Quantitative Fragility Test).—As Creed (1938) has pointed out, fragility varies considerably with the degree of oxygen saturation, so fully oxygenated blood was used in this estimation. The technique was based upon that of Creed (1938), except that a range of concentrations of 0.48% to 0.16% saline was employed in preference to the more usual 0.72% to 0.28%. It was also found that a more easily readable graph was obtained by plotting the percentage of unhaemolysed cells as abscissae against saline concentration.

Price-Jones Curve.—As no elaborate apparatus was available for this estimation, the simplified apparatus described by Humble and Belyavin (1948) was used. The films were stained by 1% eosin for one minute after preliminary fixation in methyl alcohol.

The remainder of the investigations were performed by the usual techniques. Fully oxygenated blood was used in the estimation of the mean cell volume.

Results

The results are most conveniently expressed in the tables and graphs. Table I is a simple record of the various haematological indices obtained. Table II reveals the number of abnormal readings found in each individual.

The results of the differential or quantitative fragility tests are depicted in Figs. 2, 3, and 4. The Kahn test results and serum bilirubin were normal in each case.

Discussion

From Table II it is clear that G. A. is the only member of the family examined in whom all investigations are normal. The remaining individuals reveal at least three abnormal indices, and an increase in both erythrocytic resistance to saline and the stipple cell count is common to all.

Fragility of Erythrocytes.—Many observers have recorded examples of normal erythrocyte fragility in both mild and severe forms of the disease (Cooley, 1928 ;

FIGS. 2, 3, and 4.—Results of quantitative saline resistance tests in members of the family.

TABLE II
NUMBER OF ABNORMAL READINGS IN CERTAIN MEMBERS OF THE FAMILY

	Mean Cell Dia- meter	Mean Cell Volume	Mean Cell Thick- ness	Target Cells	Stipple Cell Count	Quantitative Erythro- cyte Saline Resis- tance	Standard Deviation in Size of R.B.C.s
Grandfather	+	N	—	+	+	+	+
Father	+	N	—	+	+	+	+
J.B. (brother)	+	—	—	N	+	+	+
C.B. (brother)	N	N	N	+	+	+	+
M.B. (sister)	+	N	N	N	+	+	N
J.B. (uncle)	N	—	N	+	+	+	+
J.B. (cousin)	+	N	N	N	+	+	+
R.B. (aunt)	+	—	—	+	+	+	+
C.B. (cousin)	N	—	—	+	+	+	+
Mother	N	N	N	N	+	+	N
A.A. (uncle)	+	N	N	N	+	+	+
G.A. (uncle)	N	N	N	N	N	N	N

N = result was within normal limits; + = result above normal limits; — = result below normal limits.

Baty, Blackfan, and Diamond, 1932 ; Smith, 1942 ; Valentine and Neel, 1944 ; Dameshek, 1946). In each of these, however, the result was invariably expressed merely as a record of the beginning and end-points of haemolysis. In this series the fragility tests of both Mrs. B. and M. B. (sister), if expressed in these terms, would certainly have been reported within normal limits. Nevertheless, by a more critical quantitative method, a significant increase in the resistance becomes apparent in both (Figs. 3 and 4).

Stipple Cells.—The occurrence of stipple cells in severe anaemia and leukaemia is well recognized, but their presence in healthy individuals is almost entirely confined to asymptomatic cases of mild plumbism. The constant appearance of stipple cells here is, therefore, of considerable significance. Furthermore, as this cell has also been reported in severe cases of Cooley's anaemia by many observers (Cooley, 1928 and 1929 ; Haden, 1934 ; Dameshek, 1940 ; Neel and Valentine, 1945), there would appear to be grounds for the belief that its presence indicates the existence of some factor common to both major and minor Cooley syndromes. The nature of the stipple cell itself has given rise to some speculation. Although most observers agree that it is immature, and closely related to the reticulocyte, Smith (1942) believed it to be a degenerate erythrocyte. As the stipple cell occurs in symptomless carriers with a relatively normal count, he believed it unlikely that there would be evidence of regeneration in the peripheral blood of such cases. Nevertheless, the weight of evidence is against this view. Whitby and Britton (1933) established a close relationship between the sum of polychromatic and stipple cells and the reticulocytes. Rosegger (1936), too, has shown that stippling, polychromasia, and reticulum have similar iso-electric points. The data from this family support a relationship between the stipple cell and reticulocyte in this condition.

Mean Cell Volume, Diameter, and Standard Deviation in Size.—It will be seen that the average M.C.V. is inclined to be on the low side of normal, while the

average M.C.D. tends to be high. These figures, although of no absolute diagnostic value, give a certain amount of information about the usual morphology of the erythrocyte in the carriers of this anaemia. Similarly, although the standard deviation in the size of the erythrocyte is of little specificity from a diagnostic point of view, nevertheless it will be seen that in the majority of cases (75%) it is raised.

Target Cells.—Target cells have long been thought to be of profound significance in Cooley's anaemia, largely due to the work of Dameshek (1943), who regarded them as congenitally defective cells, and believed their presence to be essential in both mild and severe forms of this anaemia. Target cells, however, were first described in a case of sickle-cell anaemia (Haden and Evans, 1937), and Barrett (1938) in his monograph records them in a variety of conditions, including obstructive jaundice with or without anaemia, in idiopathic steatorrhoea, after splenectomy (whatever the cause of the operation), and in a variety of anaemias whenever the colour index falls below 0.8. They have been recognized more recently (Valentine and Neel, 1944) in cases of Laennec's cirrhosis and cardiac decompensation, and I have found them occasionally in cases of untreated pernicious anaemia. In the present investigation, target cells were present in less than half of the cases, and although they are undoubtedly common in Cooley's anaemia, particularly in the severe form, they can hardly be regarded as either pathognomonic or as a *sine qua non* in mildly affected individuals.

Mean Cell Thickness.—Although every member of the family, except G. A., shows a low colour index, the mean corpuscular haemoglobin concentration is normal. This relationship, combined with the large cell diameter and low volume mentioned above, would appear to indicate a low mean cell thickness. It is surprising, therefore, to discover that this index falls below the normally accepted limits of 1.8μ to 2.3μ in only five individuals (Table I). The explanation may be in the limits themselves: thus a "normal" cell with a thickness of 1.8μ must also possess a volume of $78 \text{ c}\mu$ and a diameter of 7.4μ , a most unusual combination in a healthy individual. What appears to be of greater significance is the discovery that the mean erythrocyte thickness in all, except G. A., lies below the average of 2.15μ . This coincides with the views of Bradford and Dye (1936), who, from a study of five severe cases of Cooley's anaemia, concluded that leptocytosis was probably the fundamental defect in that condition.

From these findings it is clear that the most consistently recurring abnormalities in the blood picture of the individuals examined are the increased stipple cell count and quantitative saline resistance. Indeed, in only one individual (G. A.) are these normal, and in this single case all other indices are also normal. Both these indices have been found to be raised in all severe cases, whenever they have been estimated. It would appear, therefore, that as these findings are rare in other types of anaemia, their presence in many of the relatives of sufferers from Cooley's anaemia is in itself strong *a priori* evidence for assuming the existence of the carrier state in those persons.

None of the other indices reveals such constant abnormalities, and yet, on the assumption that all but one of the individuals examined are, in fact, carriers, it is possible to deduce a considerable amount of information about the usual morphology of the erythrocyte in this condition. Thus, the cell thickness would appear

to be always below average, the diameter to be usually increased, and the volume low. Target cells may or may not be present, and there is usually a mild accompanying degree of anisocytosis. In all cases there is a subclinical anaemia, in which the colour index is below unity, but the mean corpuscular haemoglobin concentration remains relatively normal. These indices, however, are subject to wide fluctuations in any one case, and at the best represent the average picture.

Summary

The haematological findings in 13 healthy relatives of a case of Cooley's anaemia have been analysed in an attempt to discover a simple means of detecting the presence of the mild trait.

In only one individual were normal readings found in all haematological investigations.

An increased stipple cell count and raised quantitative erythrocytic resistance to saline were found in all the remaining individuals, and it is submitted that by means of these two tests carriers of the trait may be detected.

The haematological picture usually found in the mild trait is outlined.

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