

STUDY OF THALASSEMIA MINOR IN THREE GENERATIONS OF AN ITALIAN FAMILY

By ROBERT W. HEINLE, M.D., AND MARGARET RUTH READ, M.D.

A CONSIDERABLE knowledge concerning the heredity and transmission of Cooley's erythroblastic anemia, or thalassemia, has accumulated during the past twenty years but there is still need for more complete data and further genetic studies of families showing the trait.

In 1927, Cooley¹ reported his observations on erythroblastic anemia occurring in Mediterranean peoples. He concluded that the anemia was congenital, but in spite of frequent familial occurrence, he doubted that heredity was a factor since the patients died before puberty and so could not transmit the disease.

It was recognized for the first time in 1937 that a mild, but similar, type of anemia occurred in parents and siblings of individuals with Cooley's anemia. Angelini (quoted by Wintrobe and his associates²) observed that in some instances, the erythrocytes of apparently healthy parents and siblings of patients with Cooley's anemia showed decreased fragility when tested in hypotonic saline. Caminopetros³ independently confirmed this observation in 1938.

Two years later, Wintrobe and co-workers,² Dameshek⁴ and Strauss and co-workers⁵ described a mild form of microcytic hypochromic anemia, resistant to iron therapy, and occurring in Italian families. The anemia was characterized by the frequent occurrence of increased numbers of erythrocytes, with absolute and relative reduction of hemoglobin, bizarre forms of erythrocytes and decreased fragility in hypotonic saline. These authors variously termed the dyscrasia as "target cell anemia," "familial microcytic anemia" and "anemia of adults resembling thalassemia." It was not immediately apparent whether this disease was related genetically to Cooley's Mediterranean anemia, but such a possibility was suggested.

Wintrobe⁶ later confirmed Angelini's and Caminopetros' observation of decreased fragility of erythrocytes in the parents of patients having Cooley's erythroblastic anemia and further pointed out that the blood picture in the parents was identical with the familial microcytic hypochromic anemia which he had previously described.²

In 1942 and 1943, Dameshek⁷ described several anemic states of varying degree of severity ranging from Cooley's erythroblastic anemia to conditions with mild hypochromic anemia, target, oval and stippled cells and increased resistance of the erythrocyte to hypotonic saline. He confirmed the findings of other workers in demonstrating that such blood changes occurred in both siblings and parents of patients with thalassemia. Smith⁸ also demonstrated similar changes in the blood of siblings of patients with Cooley's anemia and discussed the diagnosis of the "trait" or mild form of the disease.

From the Department of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio.

Valentine and Neel,⁹ in reporting studies on parents and siblings of 3 patients with thalassemia and of one person with a similar mild condition, considered the hereditary aspects of the disorder and emphasized the problem of differential diagnosis and the clinical significance of the mild form of the anemia. By statistical analysis of cases collected from the literature, they concluded that the bulk of evidence favored the hypothesis that the mild, microcytic hypochromic anemia was a form of thalassemia which resulted from heterozygosity for a factor which, when homozygous, caused full-blown thalassemia. These authors suggested the term "thalassemia major" for the severe erythroblastic anemias as originally described by Cooley and "thalassemia minor" for the mild microcytic hypochromic anemias characterized by target cells, oval cells and increased resistance of the erythrocytes to hypotonic saline.

Cooley¹⁰ has more recently described a microcytic hypochromic anemia occurring only in males with transmission through the females in a family of Dutch descent. His conclusion that the disease was due to a fundamental constitutional defect of the hematopoietic system unrelated to familial microcytic anemia may be open to some doubt.

In 1945, Neel and Valentine¹¹ reiterated their views regarding "thalassemia major and minor" and the inheritance of the conditions. From a study of a portion of the Italian population of Rochester, N. Y., they concluded that thalassemia major occurred in about 0.042 per cent and thalassemia minor in about 4 per cent.

The present report deals with the occurrence of a microcytic hypochromic anemia in 9 of 13 members of a family of Sicilian descent over a span of three generations. The study was initiated when one of the members of the second generation was found to have a hypochromic anemia which did not respond to iron.

CASE REPORT

Patient L. O., a 37 year old unmarried woman of Sicilian descent, was first seen by us on September 18, 1945, complaining of feeling faint. She had first noted this two years previously and was told by a physician that she had anemia. Therapy with iron, liver extract injections and oral liver-iron preparations were without symptomatic or hematologic benefit. She later experienced fatigue and a sensation of "twitching" in the left leg and hand, although no muscle contractions were ever visible. She had been studied at another hospital on two occasions but was not aware that any diagnosis was made. History of anemia in other members of her family could not be elicited.

On physical examination, the positive findings consisted of slight pallor of the mucous membranes, a metallic first heart sound at the mitral area, bilateral positive Hoffman reflex, and a nystagmus on right lateral gaze with fast component to the right. The liver and spleen were not palpable.

She had been examined by a neurologist who was unable to explain the symptoms and signs and who considered that they might be secondary to the anemia. They have never been adequately explained.

Laboratory data: Erythrocytes 5,570,000 per cu. mm., hemoglobin 10.4 Gm. per 100 cc., leukocytes 5,300; hematocrit 36, mean corpuscular volume 64.9 cu. microns, mean corpuscular hemoglobin 18.7 micromicrograms, mean corpuscular hemoglobin concentration 28.9 per cent. Differential blood count: 58.5 per cent neutrophils, 3.5 per cent eosinophils, 0.5 per cent basophils, 31.0 per cent lymphocytes, 6.5 per cent monocytes. No nucleated red cells were seen. The erythrocytes showed marked anisocytosis and poikilocytosis, and were microcytic hypochromic. Numerous target cells and a few oval shaped cells were present (figs. 1 and 2). The platelets were slightly increased in number. Sedimentation rate was slower than normal, the corrected value being less than zero. This finding was due presumably to delay in rouleau formation resulting from the abnormal shape of the erythrocytes.

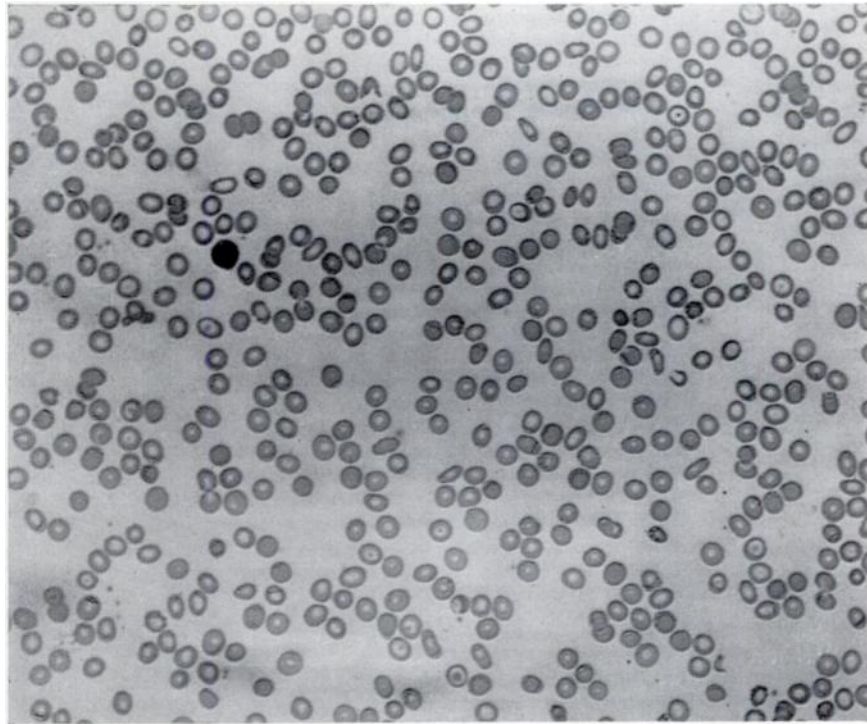


FIG. 1. Patient L. O., second generation. Peripheral blood. $\times 385$

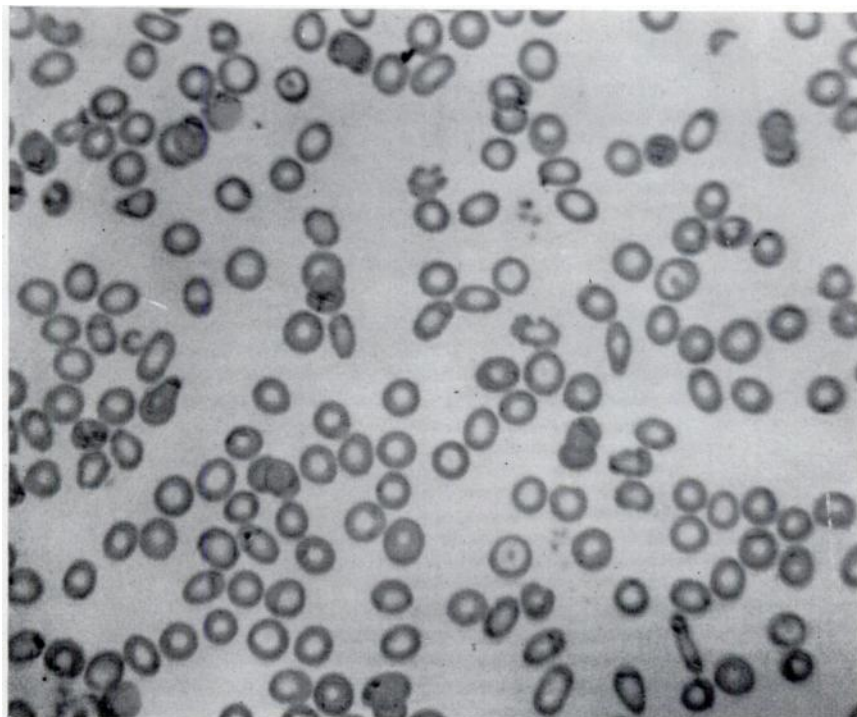
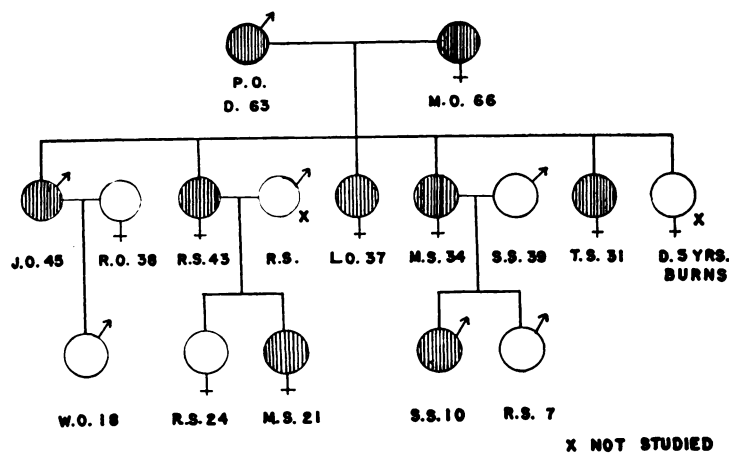


FIG. 2. Patient L. O., second generation. Peripheral blood showing target cells, oval cells, poikilocytes and hypochromia. $\times 640$



FAMILIAL MICROCYTIC ANEMIA

FIG. 3. Occurrence of thalassemia minor in three generations of a family of Italian origin. Numbers after initials indicate age of patients in years

TABLE I

Patient	Sex	Generation	Age	Hgb.		W.B.C. × 10 ³	P.C.V.	M.C.V.	M.C.H.	M.C.H.C.	Bizarre cells	Target cells	Retic.	Spleen below costal margin	Ict. Ind.	Fragility Test % saline	
				R.B.C. × 10 ⁶	Gm.											Begin	Comp.
P. O.	M	1	63	4.02	11.0	5,550											
				3.90	10.4	7,110											
M. O.	F	1	66	5.50	12.0	5,900	40	72.7	21.9	30.0	+	+	0	8	0.46	0.30	
L. O.	F	2	37	5.57	10.4	5,300	36	64.9	18.7	28.9	+++	+++	0	0	0.38	0.20†	
R. S.	F	2	43	4.84	10.4	5,150	34.5	71.5	21.6	30.2	++	+	0.5	8	0.34	0.20	
J. O.	M	2	45	6.33	12.7	8,500	45	71.0	20.1	28.2	++	++	2	2	0.42	0.20†	
T. S.	F	2	31	6.53	11.9	10,900	41	62.7	18.2	29.1	+	+	1	8	0.38	0.20‡	
M. S.	F	2	34	5.33	11.0	10,200	38	71.4	20.8	29.2	+++	+++	0.1	0	5	0.36	0.20†
M. S.	F	3	21	6.99	12.2	6,700	38	54.4	17.4	32.1	+++	+	0	0.5	0	0.42	0.22†
R. S.	F	3	24	4.90	13.4	7,950	42.5	86.7	27.2	31.5	0	0	0.5	0	0.44	0.30‡	
R. S.	M	3	7	5.03	13.1	10,200	41	81.5	26.0	31.2	0	0	0.3	0	6	0.44	0.32‡
S. S.	M	3	10	6.42	11.5	7,300	40	62.3	17.8	28.7	+	+++	0.2	0	4	0.44	0.22†
W. O.	M	3	18	5.37	15.9	7,500	47.5	88.6	29.7	33.5	0	0	0	0	0	0.46	0.24‡
R. O.	F	S-2*	38	4.68	13.4	6,500	44	94.0	28.7	30.4	0	0	0	0	0	0	‡
S. S.	M	S-2*	39	5.36	15.6	6,500	46	86.8	29.1	31.7	0	0	0	0	0	0.44	0.32‡

* S-2 = spouse of member of second generation.

† Platelets estimated from smear, increased in number.

‡ Platelets estimated from smear, normal in number.

She was seen again one month later with the same complaints and laboratory findings. There had been no response to iron therapy. Saline fragility test showed beginning hemolysis at 0.38 per cent and

complete hemolysis at 0.20 per cent. X-rays of the skull, humerus and hands showed a slight degree of demineralization without specific changes.

It was felt that much of the patient's symptomatology was on a nonorganic basis, especially since she was inclined to place the blame for her symptoms on her work (machine operator), and since it seemed likely that the anemia had antedated the onset of her symptoms.

A tentative diagnosis of familial microcytic anemia was made and it was arranged to study other members of her family.

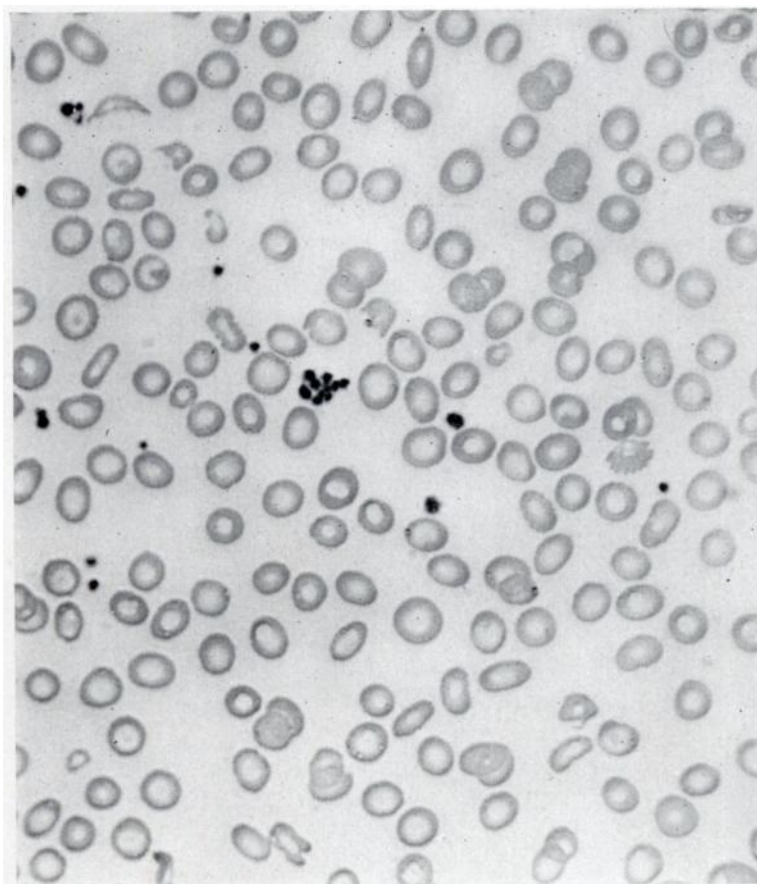


FIG. 4. Patient S. S., third generation. Peripheral blood showing hypochromia, target cells, oval cells and poikilocytes. $\times 640$

Table 1 and figure 3 show the results of findings in the other living members of the family, including the parents of the patient, her brothers and sisters, and the third generation of her nieces and nephews, in addition to two of three spouses of members of the second generation. The patient's father (P. O.) had died, at age 63 years, two years before we saw the patient, L. O., but he had been admitted to another hospital in 1942 where blood studies had been performed and where a diagnosis of duodenal ulcer and nonfunctioning gall bladder had been made. His laboratory findings were made available to us and are recorded in table 1.

A sister of the patient, L. O., died at age 5 years, of burns. She was never investigated for the presence of anemia and was thought to have been entirely well. All the other members of the family thought they were in good health.

It will be noted (table 1, figure 3) that the parents of the patient, all 5 living members of the second generation and 2 of 5 members of the third generation apparently had identical blood findings. The number of target cells and bizarre forms varied somewhat but the essential features were identical. A photomicrograph of the blood of S. S., a 10 year old nephew of the patient (L. O.) is shown in figure 4 and a photomicrograph of the blood of M. S., aged 21, a niece of the patient is shown in figure 5.

The 9 affected members of this family were individuals with apparent good health, capable of doing a full day's work without unusual fatigue. There were no signs or symptoms which would have led to a diagnosis of a blood dyscrasia except the presence of palpable spleens in 5 of the 9 affected individuals.

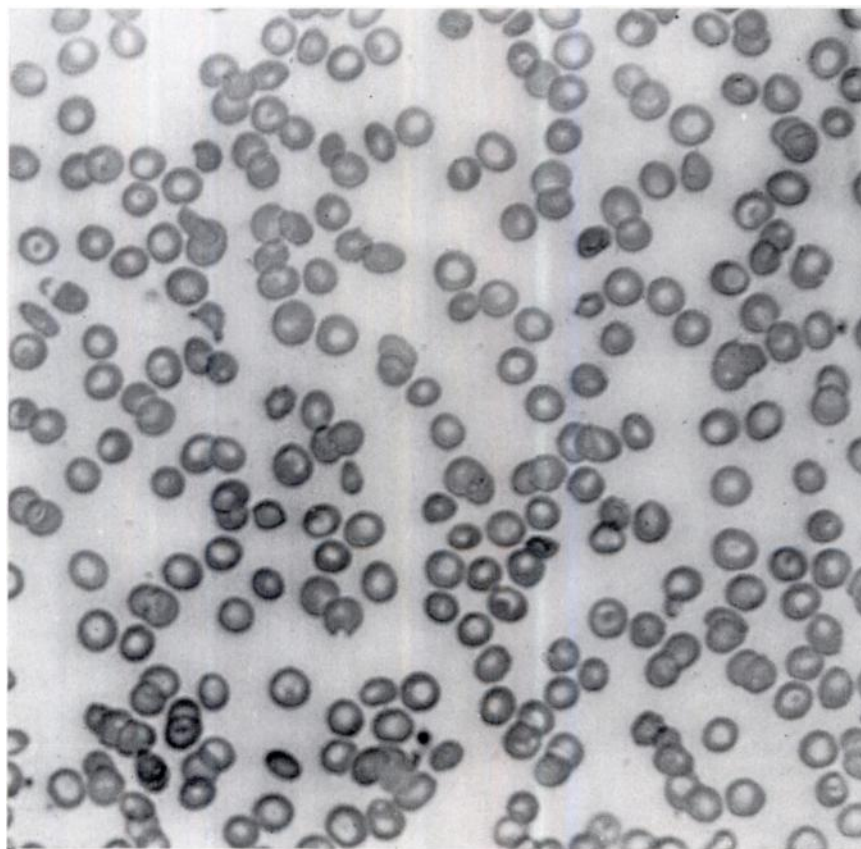


FIG. 5. M. S., third generation. Peripheral blood showing target cells, oval cells and poikilocytes. $\times 640$

Thus, such cases are usually discovered coincidental to examination for other reasons or during a special study of a group of patients. The individuals are of Italian or Sicilian descent. The spleen is palpably enlarged in some cases but the enlargement is not great as compared with others of the blood dyscrasias, and the finding is not constant. Palpable enlargement of the liver did not occur in this series.

The erythrocyte count is frequently elevated above normal and in this series was as high as 7,000,000 per cu. mm. The hemoglobin content and hematocrit values are low, of course, so that the mean corpuscular volume and mean corpuscular hemoglobin values are considerably less than normal. The mean corpuscular hemoglobin concentration is not reduced as much, a further indication of the microcytosis. Examination of the blood films confirm these data. Hypochromia is obvious in addition to which there are variable numbers of target cells, and cells of bizarre shape. Some of the smaller erythrocytes appear to be well-filled with hemoglobin.

The saline fragility test was abnormal in all 9 of the affected individuals in this series. In some, he-

molysis began at about normal concentrations, 0.42 to 0.46 per cent saline, indicating that a part of the population of erythrocytes had normal osmotic properties and were, presumably, of normal shape. In some, hemolysis began at much lower levels, indicating that all of the erythrocytes were flatter than normal. Complete hemolysis occurred at lower than normal concentrations in all cases, ranging from 0.20 to 0.24 per cent with the exception of the mother who had complete hemolysis at 0.30 per cent, only slightly below normal.

In this series there was no increase in reticulocytes, stippled erythrocytes or nucleated red cells. The leukocyte and differential blood counts were normal in all of the affected individuals. In some cases, the number of platelets appeared to be slightly increased as estimated from the blood films. Platelet counts were not made. X-rays of bones showed no specific lesions in the one case (L. O.) on whom they were made. The anemia is completely resistant to iron therapy.

DISCUSSION

This asymptomatic, microcytic hypochromic anemia is not in itself of great importance except as the condition may fail to be diagnosed or diagnosed incorrectly. Of significance, however, is its relation to thalassemia major, a more severe disease, usually fatal during childhood. In this series, two Italians with the mild form of the disease, thalassemia minor, produced 6 children, 5 of whom are known to have had an identical mild form of the condition. It would appear at first hand, therefore, that the trait, dominant in both the parents, was simply inherited by the children. That such is not the case, however, has been demonstrated by the work of others and is further confirmed by the occurrence of the condition in the third generation of this series, in which only 2 of 5 were affected.

From the literature, and from this series, it seems probable that the severe form of the anemia, thalassemia major, results from homozygosity of an inherited factor while the milder form, thalassemia minor, results from heterozygosity of the same factor. According to this idea, both parents were heterozygous, since they had the mild form of the disease. They would be expected to have children who were homozygous (thalassemia major), heterozygous (thalassemia minor) and completely free of the trait in a ratio of 1:2:1. That all 5 of the second generation studied had thalassemia minor, signifying heterozygosity, was therefore apparently due to chance occurrence in a small sample. The statistical probability of this occurrence being due to chance is 1 in 14 (P value about 0.07, borderline significance).

The third generation in this series, however, fits the idea very well. In this case, if one parent were heterozygous and the other free of the trait, half the offspring would be expected to be heterozygous (thalassemia minor) while half would be expected to be unaffected. The occurrence of thalassemia minor in 2 of 5 members of the third generation agrees with this concept.

The mechanism of production of the abnormal erythrocytes is not understood. Valentine and Neel¹² were able to produce target cells experimentally, both in vitro and in vivo, by increasing the tonicity of the solution in which the cells were suspended. Whether this represents the natural mechanism of production of target cells has not been established.

SUMMARY

1. Three generations of a family of Italian descent were studied. Nine of 13 members were found to have thalassemia minor.

2. Genetic studies indicate that this mild, microcytic hypochromic anemia characterized by the presence of target and elliptical cells and other bizarre forms and by increased resistance to hypotonic saline, results from heterozygosity of an inherited factor, which, when homozygous, produces thalassemia major or Cooley's Mediterranean anemia.

3. If individuals heterozygous for this factor (thalassemia minor) marry other heterozygous individuals, one quarter of the offspring can be expected to be homozygous (thalassemia major), one half heterozygous (thalassemia minor) and one quarter free of the trait.

4. The presence of thalassemia minor apparently did not interfere with the general health of affected members of this family and did not appear to shorten life expectancy. The importance of the condition lies in its relation to thalassemia major.

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