# Thalassemia–Hemoglobin C Disease

## A New Syndrome Presumably due to the Combination of the Genes for Thalassemia and Hemoglobin C

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IN PREVIOUS COMMUNICATIONS[1-3] the hematologic effects of the gene for an abnormal hemoglobin, since designated as hemoglobin C, were described in two combinations: A-C, an asymptomatic carrier state in which the hemoglobin consists of a mixture of A (normal adult type) hemoglobin and C; secondly S-C, a variant of sickle cell disease in which sickle hemoglobin S is produced together with hemoglobin C. Another example of the interaction of genetic factors capable of modifying the structure of hemoglobin and red corpuscles is microdrepanocytic disease[4,5] which results from the combination of the sickling gene and the thalassemia gene. The present report deals with still another combination of abnormal genes, hemoglobin C–thalassemia. Evidence will be presented for the coexistence of these two genetic factors in an individual in whose family examples of A-C and of simple thalassemia minor were found.

### Case Report

Larry W., the patient whose anemia directed our attention to the problem, was first seen in the Hematology Clinic of this hospital in September, 1952, at the age of six years, because of a severe anemia of unknown duration. He had been examined in the Out Patient Department in February, 1952, at which time a cardiac murmur was noted. This murmur was thought to be functional. On further study the presence of an anemia was discovered. Routine examination by the technician indicated a hypochromic microcytic anemia, and iron therapy was initiated. There was, however, no indication of either blood loss or dietary deficiency. The therapy was without apparent benefit and after a period of six months the patient was referred to us for further study.

Physical examination revealed a somewhat thin but well developed child with Negroid hair and facies, dark brown skin and moderate pallor of the mucous membranes. The heart was not enlarged but a fairly loud blowing systolic murmur was audible over the precordium. Neither liver nor spleen was palpable, and no other significant abnormalities were found. The hemoglobin at this time was 6.1 Gm. The full hematologic findings are given in table 1. The morphology of the erythrocytes in dried blood films was striking and unlike those we have encountered in any other condition. The most unusual feature was the extreme pleomorphism and the coexistence of hypochromic cells and deeply stained microcytes, some of which had the appearance of microspherocytes. The majority of the red corpuscles were markedly hypochromic and most of these were target cells showing unusually pronounced demarcation of the empty-appearing zones from the hemoglobin-containing zones (fig. 1). While most of the hypochromic cells were of normal or even of increased diameter and regular contour, others were very small, almost resembling fragments. Still others were oval, elliptical, pearshaped or showed tail-like projections. The microspherocytes by contrast were dense, round, fairly uniform. No nucleated red cells were encountered.

The large hypochromic target cells frequently contained coarse basophilic inclusions,

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TABLE 1.—*Hematologic Data of Larry W. and His Family*

| | Hb. Gm.% | RBC mill. | Hemat. vol.% | MCV | MCH | MCC | Target % | Serum Iron γ/cc | Alk. Hb. | Electrophoresis | Blood Group |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Larry (patient)*† | 6.8 (4.1–7.3) | 4.84 | 31 | 63.5 | 13.9 | 22 | 20–60 | 240 | <2% | AC | O N CDe |
| Mr. W. (father) | 14.1 | 4.5 | 43 | 95.5 | 32.4 | 33 | 10–20 | | | AC | O N cDe |
| Mrs. W. (mother)† | 12.0 | 4.6 | 42 | 91 | 26 | 27.5 | | 122 | <2% | A | B N CDe |
| L. M. (twin) | 9.8 | 4.15 | 36 | 87 | 23 | 27 | | 114 | | | B N CDe |
| Brenda | 9.3 | 3.53 | 32 | 91 | 26 | 28.5 | | | | | O N CDe |
| John | 8.0 | 4.5 | | | | | | | | | O N cDe |
| Abbie | 12.3 | 4.2 | 41 | 98 | 29 | 30 | | | | | O N cde |
| Astra | 10.3 | 4.16 | | | | | | | | | O N CDe |
| Freda | 11.3 | 4.94 | | | | | | | | | AB N cDe |
| Lewis | 11.3 | 4.66 | | | | | | | | | B N cDe |

* Reticulocytes per cent, 3.6. Bone marrow hyperplastic; EM ratio 1:1.

† For osmotic fragility, see figures 2 and 5.

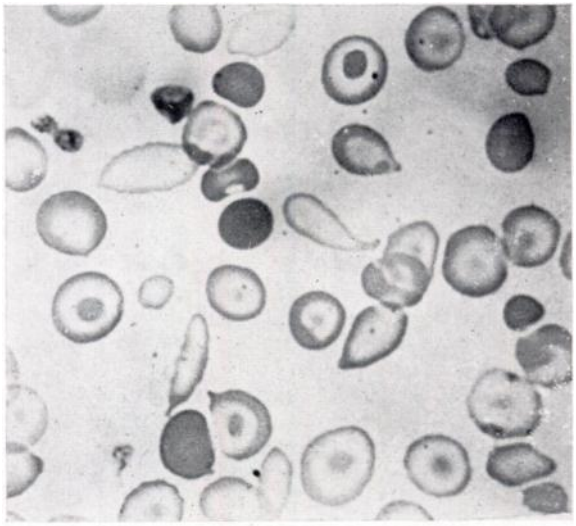

FIG. 1.—Film of Larry W.'s blood showing marked target cell formation, hypochromasia, ovalocytosis, poikilocytosis and also small, well stained cells resembling spherocytes.

usually clustered in the dense portions of the cell. Less than 1 per cent of all cells were siderocytes. The granulocytes and platelets of the blood were not remarkable.

There was no sickling of the erythrocytes in sodium metabisulfite preparations. The osmotic fragility of the patient's erythrocytes is graphically shown in figure 2. Hemolysis began at 0.66 per cent saline and was not complete at 0.12 per cent. When incubated at 37 C. for 18 hours the resistance of the erythrocytes to hypotonic saline was even greater.

The serum iron was 240 γ per cent with the iron-binding capacity of the serum 200 γ per cent. The total serum bilirubin was 0.4 mg. per cent. The fecal urobilinogen excretion was 3 mg. per day.

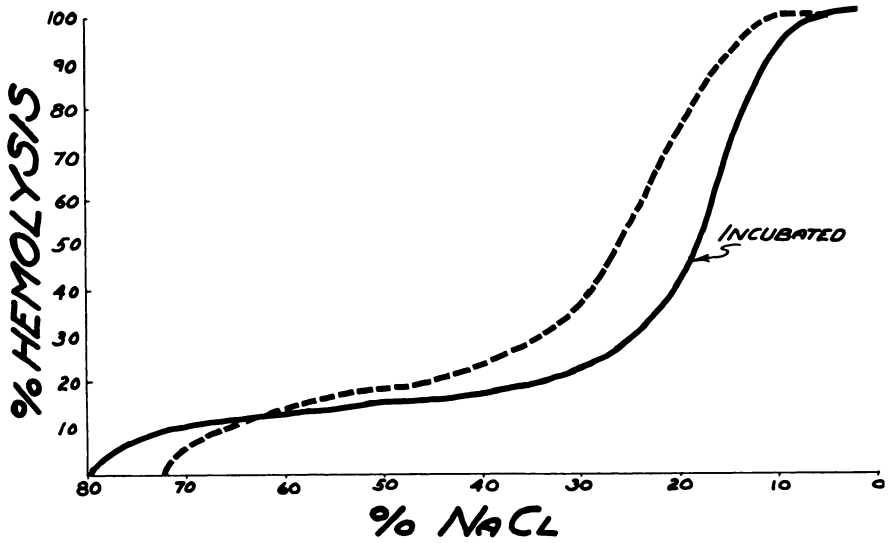


FIG. 2.—Osmotic fragility of Larry W.'s erythrocytes, showing the presence of unusually fragile as well as unusually resistant cells.

The bone marrow was cellular and showed moderate erythroid hyperplasia. The predominant erythroid precursor was the polychromatic normoblast, which did not appear remarkable with respect to hemoglobin content or size. Only a few cytoplasmic inclusions similar to those seen in the peripheral blood were found in normoblasts. Siderotic granules on the other hand were demonstrated with the prussian blue reaction in 80 per cent of the normoblasts.

## FAMILY STUDIES

The family of Larry W. was unusual in that the mother had had eight children by five different fathers. Figure 3 shows the "pedigree." The patient and

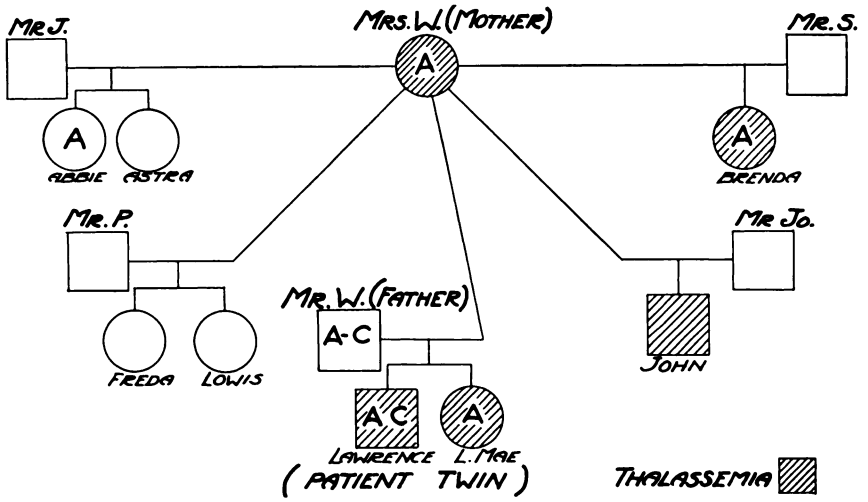


FIG. 3. Pedigree of the family of Larry W. The cross hatching signifies the thalassemia trait. Where electrophoretic analysis of the hemoglobin has been made, the letters indicate the type of combination found.

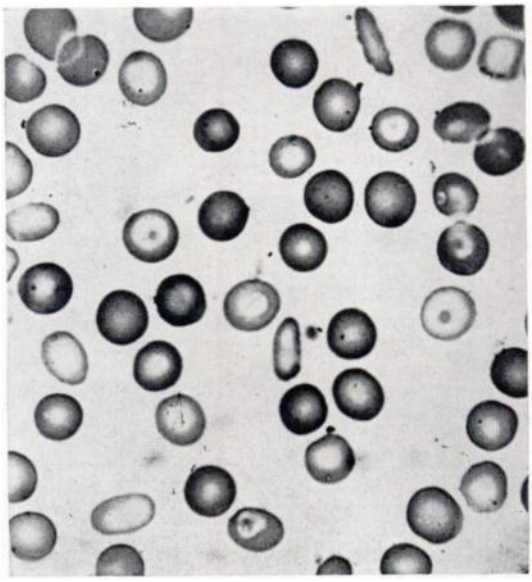

FIG. 4.—Blood film of the patient's mother showing the presence of hypochromic cells and ovalocytes.

one younger sister were by the same father; each two of the four older children and each of the younger siblings had a different father. The patient's father was a carrier of the "C" gene. The mother and three of her children had thalassemia minor.

Mrs. W., the mother, was a thirty-one year old American Negress who was in good health. Her family was not available for study. There was no known admixture with white Mediterranean stock. There were no significant findings

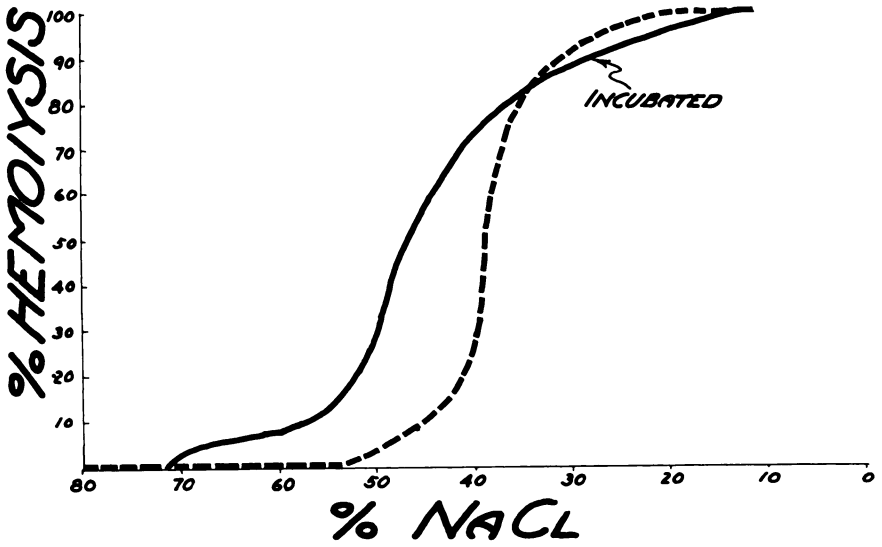


FIG. 5.—Osmotic fragility of Mrs. W.'s erythrocytes.

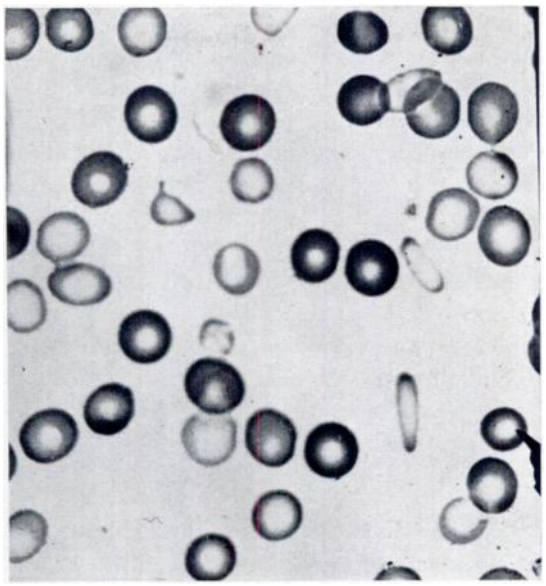

FIG. 6.—Blood picture of patient's twin sister, Lulu Mae. Note similarity to the blood picture of the mother (fig. 4).

on physical examination. The erythrocyte constants were indicative of slight microcytic hypochromasia. In stained films, Mrs. W's erythrocytes gave the impression of consisting of a "double population." The majority of the cells were round and well filled with hemoglobin. There were scattered target cells, hypochromic microcytes, occasional red cell fragments and oval and elliptical cells (fig. 4). The serum iron was well within normal limits. The resistance to hypotonic saline was increased (fig. 5).

The father's blood was remarkable only in that it contained between 10 and 20 per cent target cells.

The half siblings, Abbie and Astra and Freda and Lewis, were hematologically essentially normal. The patient's full sister, actually his twin, had a mild hypochromic microcytic anemia. Her blood picture was almost identical with that of her mother, and like it, gave the impression of a double population of red corpuscles (fig. 6). The two younger half siblings, John and Brenda, were moderately anemic and likewise had the blood picture of thalassemia minor.

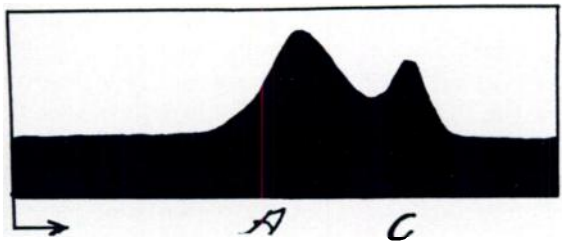


FIG. 7. Electrophoretic pattern of patient's hemoglobin. Cacodylate buffer at pH 6.5.

## Special Studies

The bloods of the oldest half sibling, the patient, his twin sister and the youngest half sister, as well as both parents, were tested for abnormal hemoglobin components by paper electrophoresis and in the standard Tiselius apparatus. As shown in table 1, the mother's hemoglobin gave a normal pattern of A hemoglobin, as did Abbie's, L. Mae's (twin) and the youngest half sibling's Brenda. The patient's hemoglobin consisted of a mixture of A and C, in the proportions of 71 per cent and 29 per cent (fig. 7). The father's hemoglobin, likewise, contained the components A and C, as shown by standard Tiselius technic and paper electrophoresis. Owing to artifacts in the pattern, the quantitative calculation of the components had to be omitted in his case.

Determinations of alkali-resistant hemoglobins (hemoglobin F) were made in the case of the patient and his mother. In both instances less than 2 per cent alkali-resistant hemoglobin was found.

## Discussion

From the observations available thus far[1–3, 6, 7] it appears that the uncomplicated hemoglobin C trait (A-C) produces an asymptomatic carrier state such as was found in the father of our patient, and manifest only in the frequency of target cells in the blood. It is clear, therefore, that the C gene alone cannot be responsible for the striking blood picture observed in the patient, even though his hemoglobin proved to be the combination A-C. The evidence indicates that in addition to the hemoglobin C trait this child harbors the thalassemia gene and that the interaction of these traits—which singly are relatively benign—has produced a new and severe type of anemia. The experience with microdrepanocytic disease [4, 5, 8] and with hemoglobin C–sickle cell disease[1–3] has shown that the presence of an abnormal hemoglobin can materially modify and enhance the effect of concomitant abnormal traits.

The proof that the patient has hemoglobin C rests securely on the electrophoretic analysis of his hemoglobin. The evidence for the presence of the thalassemia gene is less direct, since at the present time the thalassemic syndromes are recognized only on essentially morphologic grounds. Within these limits, however, the findings are quite conclusive. The mother, though not anemic, has a clear cut abnormality of the blood, manifested by the presence of ovalocytes, microcytes and hypochromic cells. The interpretation of these findings as an expression of thalassemia minor is greatly strengthened by the finding of identical morphologic changes in the blood of three of Mrs. W.'s children, namely Lulu Mae (the patient's twin sister) and the half siblings, John and Brenda. Moreover, Mrs. W.'s serum iron level is normal and the presence of iron deficiency is thus excluded. It is evident from Mrs. W.'s own blood picture and from the fact that her four older children are hematologically normal that she is heterozygous for the thalassemia gene. The patient, whose father could have contributed only the gene for C, could inherit the thalassemia gene only in single dose. That he actually did so is made overwhelmingly likely by the hypochromasia and microcytosis shown by the majority of his erythrocytes. Iron deficiency was ruled out by the high serum iron levels, the failure to respond to

therapy and by the presence of abundant iron granules in the normoblasts of the bone marrow.[9]

The possibility might be considered that thalassemia minor, i.e., the heterozygous state with respect to the thalassemia gene[10–12] might account for the anemia of Larry W., and that the presence of the C trait is merely coincidental and does not contribute to the development of the blood picture. This interpretation is highly unlikely in view of the following facts:

1. Although the heterozygous state for thalassemia may be variably expressed and in some instances may produce anemia of significant degree,[12, 13] the severity of the anemia in the patient could not be accounted for by the thalassemia gene alone. So severe an anemia in so young an individual could be explained only either by a homozygous state (which can be excluded here) or by the interaction with another factor which is furnished in this case by the proved C trait. It will be necessary to examine future cases of severe anemia in heterozygous individuals with the thalassemia syndrome for the presence of C or some comparable factor.

2. In the three other members of the family whose blood showed the usual thalassemia stigmata, but no C trait, the condition was clinically and hematologically quite benign.

3. The qualitative and quantitative tendency toward extreme target cell formation is not in our experience characteristic of the thalassemia minor syndrome but is associated frequently with the presence of hemoglobin C, though it is of course not specific.

The conclusion appears justified, therefore, that the anemia described here is the result of the interaction of the gene for hemoglobin C with that for thalassemia, the patient being heterozygous for each, and that another example of enhancement of the effects of one pathologic gene by another has been uncovered. No conclusions can be drawn from this particular instance with regard to the question whether the gene for hemoglobin C and that for thalassemia are allelomorphs. Silvestroni and Bianco[5] have recently presented evidence that the gene for the abnormal hemoglobin S segregates independently from the thalassemia gene and that the two are not allelic. If, as has been suggested, A, S and C are allelomorphs, C is not likely to be allelic to the thalassemia gene.

The exact mechanism of the anemia in the syndrome described is not clear. Unfortunately, erythrocyte survival studies were not feasible. It is highly probable, in spite of the low urobilinogen excretion, that an element of hemolysis is present, but the most striking feature is that shared with thalassemia major, the hypochromic microcytosis. The slight degree of reticulocytosis is a puzzling feature of the case, especially in view of the fairly marked erythroid hyperplasia of the bone marrow. A second feature which is noteworthy in a thalassemia syndrome of such severity is the lack of splenic enlargement. Finally, the coexistence of normochromic or hyperchromic microcytes with decreased resistance and hypochromic target cells is noteworthy. The blood picture seems to us to be distinctive, and we have been unable to find comparable descriptions in the available literature.

The salient clinical features of this case are those of a severe anemia, thus far

without crises and without hepatosplenomegaly or skeletal changes. Further experience will be needed before the full range of clinical manifestations can be delineated.


## Summary

An unusual type of severe chronic hypochromic microcytic anemia in a Negro is described and attributed to the interaction of the hemoglobin C gene with the thalassemia gene. The patient and his father were shown to be carriers of the C trait, the mother and several siblings of the thalassemia gene.

## Summario in Interlingua

Un typo inusual de sever chronic anemia microcytic hypochromic in un negro es describite e attribuite al interaction del gen pro hemoglobina C con le gen pro thalassemia. Le patiente e su patre esseva demonstrabilemente portatores del tracto C durante que le matre e plures del altere infantes esseva portatores del gen pro thalassemia.


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