High Fetal–Hemoglobin C Disease: A New Syndrome

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Hemoglobin C has been reported in combination with several other hemoglobin types, notably A and S, and very rarely, with the gene for thalassemia. To our knowledge, no cases of the interaction of the genes for hemoglobin C and "high fetal" have been reported. This paper deals with such a case.

Case Report and Hemoglobin Analysis

L. N. P., a 64 year old negro female, was admitted to the City of Memphis Hospital on November 19, 1959 because of shortness of breath and weakness of 3 weeks' duration. She had been in apparent good health up until the onset of the present illness.

Physical examination revealed a blood pressure of 190/110 mm. of mercury, slight distention of the neck veins, 2+ pedal and pretibial edema, and stasis dermatitis over both ankles. Her heart was enlarged with the point of maximal impulse in the sixth intercostal space in the left anterior axillary line. No murmurs were present and her lung fields were clear. No scleral icterus was present, and the liver and spleen were not palpable. The remainder of the physical examination was not remarkable.

Chest x-ray revealed moderate cardiac enlargement with the contour of left ventricular preponderance. Electrocardiograms revealed a pattern of left ventricular strain.

Admission laboratory data revealed an hematocrit of 16 volumes per cent, a hemoglobin of 5.8 Gm. per cent, and a red blood cell count of 2,280,000 cu. mm. The white cell count was not elevated. Peripheral blood smears revealed anisocytosis, marked microcytosis, and moderate numbers of target cells. Urine examination revealed a specific gravity of 1.013, 4+ protein, 1+ sugar, and no ketone bodies. Occasional clumps of white cells and granular casts were also present. A guaiac test for occult blood in the feces was negative. The blood urea nitrogen was 51 mg. per cent. Because of the relatively severe anemia, upper and lower gastrointestinal surveys were done to rule out a source of bleeding or a malignancy. These tests were negative. Detailed blood studies were then undertaken.

Paper electrophoresis of the hemoglobin was performed using barbital buffer pH 8.6, 0.06 M. The method used in this Laboratory is the horizontal plate technic with 3½ x 12 inch Whatman #3 MM filter paper strips. Constant voltage of 90 for eight hours using our modification of the power supply of Kebel and Arbogast or 150 volts with the Heathkit power supply was employed.

Quantitation of the amounts of adult (A) and C hemoglobins was done using the Spinco Analytrol on strips run in the Spinco Model R electrophoresis system. The quantity of A hemoglobin present was determined by subtracting from 100 per cent the quantity of alkali resistant hemoglobin and hemoglobin C. Alkali denaturation by the method of Singer et al.2 was employed. Electrophoresis in a starch block using barbital buffer pH 8.6, 0.05 M. was used according to a modification of the method of Masri et al.3

Paper electrophoresis revealed an F-C pattern (fig. 1.). The alkali denaturation test demonstrated 26.7 per cent resistant hemoglobin. Quantitation of the electrophoretic pattern revealed 60 per cent C and 40 per cent for the fast component. This patient is,
Fig. 1.—Paper electrophoretic pattern of the L. P. with controls. Note that the fast component in the patient's hemoglobin is slightly slower than normal adult (A) hemoglobin. This is the usual position of hemoglobin F.

therefore, considered to have 13.3 per cent A hemoglobin. Examination for glutathione reductase revealed a level of 63 per cent (normal range = 50–100 per cent) with a rapid reduction in 1.5 hours (normal = 1–2 hours). Red cells survival studies using Cr51 revealed a decreased survival time, with a half-life of 20 days.

Peripheral blood examination revealed no nucleated red cells but numerous microcytes, spherocytes, and target cells. Bone marrow examination demonstrated only erythroid hyperplasia.

A red cell fragility test revealed decreased fragility with beginning hemolysis at 0.44 per cent NaCl and complete hemolysis at 0.28 per cent NaCl.

Electrophoresis in the starch block failed to demonstrate an $\alpha_2$ component due, perhaps, to our inability to separate the $\alpha_2$ from the hemoglobin C.

She was discharged from the hospital to be followed in the hematology outpatient clinic.

Because of nausea, vomiting and diarrhea of two weeks duration, she was readmitted to the hospital on February 4, 1960. Her blood pressure was 135/65, a considerable decrease over her previous pressures. Orthopnea, paroxysmal nocturnal dyspnea, and slight pedal and pretibial edema were present.

The blood urea nitrogen rose from 90 mg. per cent on admission to 106 mg. per cent on the day before death. The CO$_2$ combining power on admission was 5 milliequivalents per liter. Other laboratory findings were a hematocrit of 10 volumes per cent, hemoglobin of 3.25 Gm. per cent and red blood cell count of 1,080,000/cu. mm. The reticulocytes were 4 per cent and the white cell count was 6,900/cu. mm. with a normal differential. Her stool guaiac test was negative. Her urine had a specific gravity of 1.007 with negative sugar and 3+ protein. An arm to tongue circulation time using Decholin was 14 seconds and her venous pressure was 138 mm. of water. In spite of intensive therapy she became severely oliguric and expired four days after admission.

**Autopsy Findings**

The patient was well developed and nourished and had no icterus or dependent edema. Dense fibrous adhesions were encountered between the lung and the thorax. The heart was hypertrophied, weighing 500 Gm.. The cardiac valves were normal. The lungs were
severely congested and edematous weighing 580 and 430 grams, right and left respectively. Patchy bronchopneumonic consolidation was present throughout both lungs.

The liver weighed 1710 Gm. and was passively congested. No gross hemosiderosis was present. The spleen weighed 430 Gm. The kidneys were bilaterally contracted, weighing 90 and 80 Gm., right and left respectively.

The remainder of the organs were normal.

Microscopic examination revealed very severe arteriolonephrosclerosis, intercapillary glomerulosclerosis, focal myocardial fibrosis, and focal acute bronchopneumonia.

Prussian blue stains done on the liver, kidneys, spleen, and bone marrow revealed intense hemosiderosis of the spleen and slight hemosiderosis of the liver. The kidneys and bone marrow were not remarkable in their iron content. Periodic acid-Schiff (PAS) stained sections of the spleen revealed no PAS positive “foam cells”.

Death was considered to be due to terminal bronchopneumonia in a patient with uremia secondary to severe arteriolonephrosclerosis and diabetic glomerulosclerosis.

**FAMILY STUDY**

The propositus (L.N.P.) was married but had no living children. Her one pregnancy resulted in a living female infant who died at about one week of age. Only one sibling, A.L.N., is living. She was found to have an A-F hemoglobin pattern of electrophoresis with 19.7 per cent alkali resistant hemoglobin. Both parents are dead and no known relatives of the father can be found. We know of a number of maternal relatives, but have been unable to contact and examine all of them. Two maternal uncles, M.H. and L.H., were found to have A hemoglobin and less than one per cent alkali resistant hemoglobin and no elevation of A₂ on starch electrophoresis. A maternal first cousin, B.H., was found to have 2.2 per cent alkali resistant hemoglobin, moderate anemia, and microcytes in his peripheral blood smear. Unfortunately, no A₂ levels were done on this patient. His mother, C.H., was found to have the “high fetal” gene, but no hemoglobin C, and no elevation of the A₂ component.

Figure 2 demonstrated the electrophoretic patterns of the propositus (L.N.P.), her sister (A.L.N.), and two uncles (N.H. and L.H.), two aunts (P.H. and C.H.) and her first cousin (B.H.), the son of C.H.

Figure 3 summarizes the family study.

**DISCUSSION**

The incidence of hemoglobin C in all of its combinations (i.e., A-C, S-C, C-C, etc.) is 2.3 per cent in the Memphis Negro, based on a study of 2,800 unselected adult Negro blood samples examined by one of us over the past three years.⁴ Not quite so accurate a figure for the incidence of the “high fetal” gene can be given, but an estimate of slightly less than 0.2 per cent, based on the finding of an elevation in the fetal hemoglobin to above 15 per cent in 2500 unselected adult Negroes in a study underway in this laboratory and collaborated by findings in the Department of Hematology, is probably reasonable. The expected frequency of such a case of high fetal-hemoglobin C disease in the Negro population of Memphis can thus be calculated to be approximately 0.005 per cent. The incidence of this disease in Caucasians cannot be calculated, but due to the extreme rarity of hemoglobin C in this group (0 per cent in 1250 unselected samples in our laboratory) and to our having found no cases of the high F gene, it must be much less than 0.001 per cent.
Fig. 2.—Electrophoretic comparison of several members of the propositus' family. A. N. is the sister, M. H. and L. H., uncles, P. H. and C. H., aunts, and B. H., a first cousin. All are relatives on the maternal side.

The incomplete family study in this case seems to indicate that the "high fetal" gene was derived from the maternal side. No members from the father's side could be found for study, but the hemoglobin C gene was possibly derived from him. The part the American Indian ancestry played in this family is not known, but the studies of Rucknagel, Scott et al., and Pollitzer et al. indicate...
### Table 1.—Hemoglobin C-thalassemia disease

| Age | 68 | 29 | 6 | 10 | 7 | 1½ | 3 | 64 |
| Race | Negro | Negro | Negro | White | White | Negro | Negro | Negro |
| Sex | F | F | M | M | M | M | F | F |
| Hemoglobin, Gm. % | 12.7 | 11.5 | 6.1 | 9.11 (7.7) | 9.11 (7.9) | 10.4 | 6.7 | 6.3 |
| Hematocrit, % | 36 | 34 | 31 | — | 34 | 29 | 25 | 18 |
| MCV./cu.mm. | 59 | 60 | 63.5 | 67.3 | 63 | 72.5 | 50 | 67 |
| MCH, % | 21 | 20 | 13.9 | 21.5 | 20.2 | 26.0 | 13 | 23.7 |
| MCHC, % | 35 | 34 | 22 | 31.9 | 32.1 | 35.8 | 27 | 35 |
| Reticulocytes, % | 3.8 | 2.1 | 3.6 | 2.3 | 2.3 | 1.1 | 4.0 | 8.3 |
| Osmotic fragility | decreased | decreased | decreased | decreased | decreased | decreased | decreased | decreased |
| Serum bilirubin | mg./100 ml. | 0.9 | 0.6 | 0.4 | 0.4 | 1.8 | 0.8 | — | 0.4 |
| Target cells, % | 43 | 47 | 20-60 | 30 | 30 | 53.0 | 25 | 18 |
| Fetal hemoglobin, % | 0 | 2.7 | 2 | 1.4 | 0.4 | 10.9 | 2 | 26.7 |
| C-hemoglobin, % | 77.4 | 74.1 | 29 | 93 | 9.0 | 81.3 | 85 | 60.0 |
| Serum iron./100 ml. | — | — | 240 | 81 | 50 | 120 | — | 75 |
| LIBC, mg. % | — | — | 200 | 420 | — | 151 | — | — |
| Spleen, palpable | none | none | none | 3 cm. | 3 cm. | none | none | none |

| Symptoms | none | none | anemia | none | none | none | none | anemia |
| Ceruloplasmin | — | — | — | — | — | — | — | — |
| Micro spherocytes % | — | — | — | — | — | — | — | 35 |
| Erythrocyte sedimentation rate, mm./hr. | — | — | — | — | — | — | — | 52 |
| Wintrobe | — | — | — | — | — | — | — | 0 |
| Nucleated erythrocytes | + | + | — | — | — | — | — | — |

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would seem to indicate the abnormal genes were not derived from this source.

The finding of very high fetal hemoglobin levels in two members of the patient’s family is of particular interest. Some elevation in the fetal hemoglobin level is not uncommon in patients with the thalassemia syndrome, but levels of 15-50 per cent in the heterozygous state are very uncommon. Aksoy has recently reported four cases of “thalassemia minor” in which the fetal hemoglobin levels were 28 to 59 per cent. That this elevation in fetal hemoglobin was hereditary is beyond question (see family study) and in this respect appears to differ from the family reported by Lorber, in which elevated fetal hemoglobin levels were seen in two Caucasian siblings whose parents had normal fetal hemoglobin levels and no evidence of thalassemia or an abnormal hemoglobin. Singer et al. and Went and MacIver have discussed the co-existence of hemoglobin S with a “high fetal” gene. Went and MacIver called this the “F gene” and state that it “appears to control the fetal hemoglobin level regardless of which other hemoglobin types are present. The precise relationship between this gene and the thalassemia gene is uncertain.” This “F gene” was found unassociated with the usual findings in “classical thalassemia trait.” It is the “F gene” of Went and MacIver that we believe to be present in the propositus and other members of her family. Recently, Eng et al. have reported a case of hemoglobin E-thalassemia syndrome with very high fetal hemoglobin levels. However, no genetic studies were done.

The possibility that the propositus represents the interaction of the genes for hemoglobin C and thalassemia must be considered. The presence of anisocytosis and poikilocytosis, microcytes, and decreased osmotic fragility is in favor of thalassemia minor. The presence of hemoglobin C is proven by paper electrophoresis. The absence of an elevated A2 fraction in the sister (A.L.N.) and an aunt (C.H.) with the “high F” gene strongly mitigates against such a diagnosis. However, in a recent paper the absence of an elevation in A2 in patients with clinical thalassemia minor and who were children of patients with thalassemia major was discussed. The authors suggested that “it is possible that alpha and beta thalassemias may differ by allowing or not allowing the proportion of A2 to rise in compensation.”

Herman and Conley, in their study of three generations of a Negro family with high fetal hemoglobin levels in the adults, concluded that “the genetic condition resulting in persistence of fetal hemoglobin is allelomorphic with the genes responsible for hemoglobins A, S and C, at a locus now usually designated Hb ASC.” They further postulated that the primary effect of this disorder is a failure of production of hemoglobin A, with formation of fetal hemoglobin occurring as an indirect result, and concluded that “the genetic abnormality causing persistence of fetal hemoglobin is clearly different from the thalassemia gene.”

In the three cases of S-F disease studied by these authors, no hemoglobin A was found. In the propositus reported here, there was about 10 per cent of the
hemoglobin pigment that was neither hemoglobin C nor alkali resistant (F) according to our quantitative studies. As multiple determinations were done, both by us and an independent laboratory, this observation must be considered valid. We assume that this 10 per cent of the total pigment represents hemoglobin A. In both natural and artificial mixtures of hemoglobins A and F, we have observed a decreased rate of migration of the forward edge of the A component, which is in good agreement with that seen in this case. If, then, hemoglobin A is indeed present in this case, the gene for "high fetal" must not completely suppress its formation in all cases.

Table 1 gives the findings in the seven fully reported cases of C-thalassemia. Note that the fetal hemoglobin levels in these cases are all much lower than that seen in the present case. The remainder of the hematologic values are in good agreement, as shown in the last column.

Exactly where this case fits in, if at all, with the thalassemia syndromes is not clear. It seems most probable that it is unrelated.

The size of the normal spleen is subject to considerable variation due, in part, to a decrease in size with age and to racial differences. We have found that the "normal" spleen in the adult Negro averages 101 ± 10 Gm. in weight with a range of 60 to 130 Gm. This average weight and weight range is significantly lower than in Caucasians, who average 128 ± 10 Gm. and whose range is from 80 to 180 Gm. These weights are based on the examination of 250 "normal" spleens from adult patients with electrophoretically proven A hemoglobin.

During the past four years we have autopsied 29 patients with electrophoretically proved hemoglobin C. Twenty-six of these had hemoglobin C-trait (A-C hgb.), two had sickle cell-hemoglobin C disease, and one had high fetal-hemoglobin C disease. The spleens were quite uniformly enlarged, averaging about one and one-half times the normal size in the A-C patients, four times the normal size in the S-C patients, and over four times the normal size in the single high fetal-hemoglobin C case. This splenic enlargement in all of the various C-syndromes has not been previously reported.

In addition, one case from an unrelated adult with the high fetal gene has been examined at autopsy. The spleen in this case and in the propositus (L. N.P.) did not contain foam cells with their P.A.S. positive material reported by Gupta et al.23 This observation lends further support to the concept that the high fetal gene is not related to classical thalassemia.

**Summary**

A case of interaction of the genes for hemoglobin C and "high fetal" has been reported, with an incomplete family study. The "high F" gene seems to behave as a dominant one.

A discussion of the relationship, if any, with this disease to "classical" C-thalassemia was presented. The evidence is inconclusive, but this case does not fit the picture of C-thalassemia as previously reported and is considered to be a separate condition.
HIGH FETAL-HEMOGLOBIN C DISEASE

SUMMARIO IN INTERLINGUA

Es reportate un caso del interaction del genes pro hemoglobina C e pro hemoglobina fetal alti-proportional. Un non complete studio familial es includite. Le gen “alti-fetal” pare comportar se como dominante.

Es discutite le relation - si un tal existe - inter iste morbo e le “classic” morbo de hemoglobina C plus thalassemia. Le evidentia non es conclusive, sed le caso es clarmente incongrue con le tableau de previemente reportate combinationes de hemoglobina C con thalassemia e debe esser reguardate como un condition separate.

REFERENCES


No correlation was noted between the position of the oxygen dissociation curve of eight human fetal bloods and the percentage of fetal hemoglobin as determined by the alkali denaturation technic. The importance of performing these determinations at constant pH is stressed.—A. I. C.


A modification of the starch gel method of Smithies using a discontinuous buffer system consisting of tri-EDTA-borate buffer for the starch gel and veronal buffer for the electrode chambers is described. More sharply defined separations of hemoglobins are claimed using this combination. Particularly good separations were achieved with the Hgb A₂ fraction of adult hemoglobins. The author describes a survey of normal individuals and patients with thalassemia for their Hgb A₂ content. The normal range of Hgb A₂ was found to be 2.0 to 5.0 per cent with an average of 3.7 per cent. The latter figure is higher than those reported with the starch block technic. Patients with thalassemia minor were found to have an average of 8.0 per cent Hgb A₂ with a range of 0.8 to 15.2 per cent. A. I. C.
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