

THALASSEMIA INTERMEDIA—BIOCHEMICAL AND GENETIC CONSIDERATIONS

Report of a Case

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THALASSEMIA intermedia is a syndrome with clinical and hematologic manifestations ranging in severity between those observed in thalassemia minor and in thalassemia major (Cooley's anemia).¹⁻⁶ The qualifying term intermedia emphasizes the lack of basic knowledge concerning the biochemical and the genetic abnormalities of the various thalassemia syndromes. This paper reports the occurrence of homozygous thalassemia intermedia in an adult with approximately 80 per cent fetal hemoglobin and 3.5 per cent A₂ hemoglobin. The parents and the two children of the patient have thalassemia minor with fetal hemoglobin values in a range of 10 to 17 per cent.

Report of a Case

The propositus, a 37-year-old white man of Croatian descent was first examined at the Cleveland Clinic in September, 1955, because of recurrent superficial suppurative lesions of the legs. In 1940 he underwent an appendectomy; at that time he was told that the spleen was enlarged. In 1947, a mild anemia and enlargement of the spleen were diagnosed as Mediterranean anemia. Jaundice was reported to have been present on several occasions. There were no other specific symptoms. He stated that he never had ulcers of the lower extremities. He had had no symptoms suggestive of biliary colic, and had lost no time from his work as a draftsman.

Physical examination revealed a tall thin man. The mucosae were slightly pale and the sclerae were jaundiced. There were numerous pigmented scars on the anterior aspect of the legs, and an ecthymiform lesion on the right calf. No ulcers were present. Superficial varicose veins and evidence of chronic stasis were present on the lower extremities. The spleen was firm and descended 12 cm. below the costal margin. The edge of the liver was palpable 6 cm. below the costal margin. The results of the examination of the heart, chest, and lungs were normal. The blood pressure was 130/68 mm. of Hg. A roentgen examination showed that the chest, skull, and hands were normal. The cholecystographic study revealed evidence of multiple nonopaque stones.

The patient has been under periodic observation since 1955, and the extent of the hepatosplenomegaly and the severity of jaundice have not changed. The mild hemolytic disease remains well compensated.

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Results of the physical examinations of the parents and the two children of the patient were normal. None of them had splenomegaly, pallor, or jaundice.

Hematologic data. The erythrocyte count of the patient ranged between 4,200,000 and 5,200,000 per cubic millimeter with anisochromia, anisocytosis, and poikilocytosis with oval, tailed, teardrop, and target cells. The hemoglobin has ranged from 10.2 gm. to 12.5 gm. per 100 ml. The reticulocyte count has ranged from 1 to 3.4 per cent. The leukocyte and differential counts have always been within normal limits. The mechanical fragility of the patient's erythrocytes was 4 per cent (normal is 6 per cent). The quantitative osmotic fragility began at a concentration of 0.36 per cent of sodium chloride and was not complete at 0.10 per cent. The test for sickling was negative. The radiochromate-erythrocyte survival study revealed an apparent half-life of 24 days. The range of normal values in our laboratory is from 28 to 32 days. The serum bilirubin was 0.28 mg. per 100 ml., direct, and 3.0 mg. per 100 ml., indirect. The patient's fetal hemoglobin, determined by the alkali denaturation method of Singer, Chernoff, and Singer⁷ ranged between 78 and 83 per cent. The remainder of the hematologic data of the patient and his family is summarized in *Table 1*.

Electrophoretic Studies of Hemoglobin

Studies of the hemoglobin solutions were made by electrophoresis on paper, using barbiturate buffer, pH 8.6, μ 0.05; on agar using citric acid-citrate buffer,⁸ pH 6.2, 0.05 molarity; on starch gel using borate-tris buffer,⁹ pH 8.2; and on starch block using barbiturate buffer,¹⁰ pH 8.6, μ 0.05. A sample of the patient's hemoglobin was chromatographed using amberlite IRC 50 (XE 64)¹¹ and citrate buffer, pH 6.0, sodium-ion concentration 0.15, for elution.

Table 1.—*Hematologic data of patient and family*

Family relation; age, years	Erythrocytes, million/cu. mm.	Hemoglobin, gm./100 ml.	Mean corpuscular volume, cu. μ	Reticulocytes, percentage of total
Patient				
37	4.2-5.2	10.2-12.5	80-82	1-3.4
Father				
60	5.3	12.2	80	3.2
Mother				
56	4.8	10.8	80	4.5
Daughter				
10	5.0	12.0	78	1.6
Son				
9	4.3	9.5	78	2.2
Wife	4.7	12.4	87	Not determined

Results obtained by paper electrophoresis indicated that approximately 90 per cent of the patient's hemoglobin had a mobility similar to that of fetal hemoglobin. To establish its identity more conclusively the material was subjected to chromatographic analysis. The characteristics of the major fraction of the patient's hemoglobin corresponded perfectly with those of samples of known fetal hemoglobin. The hemoglobins of the patient and his family, when studied by electrophoresis on agar, showed a fraction with mobility faster than that of normal adult hemoglobin (A_1) and identical with that of known fetal hemoglobin (*Fig. 1*). Both parents and both children of the patient had high concentrations of fetal hemoglobin (*Table 2*).

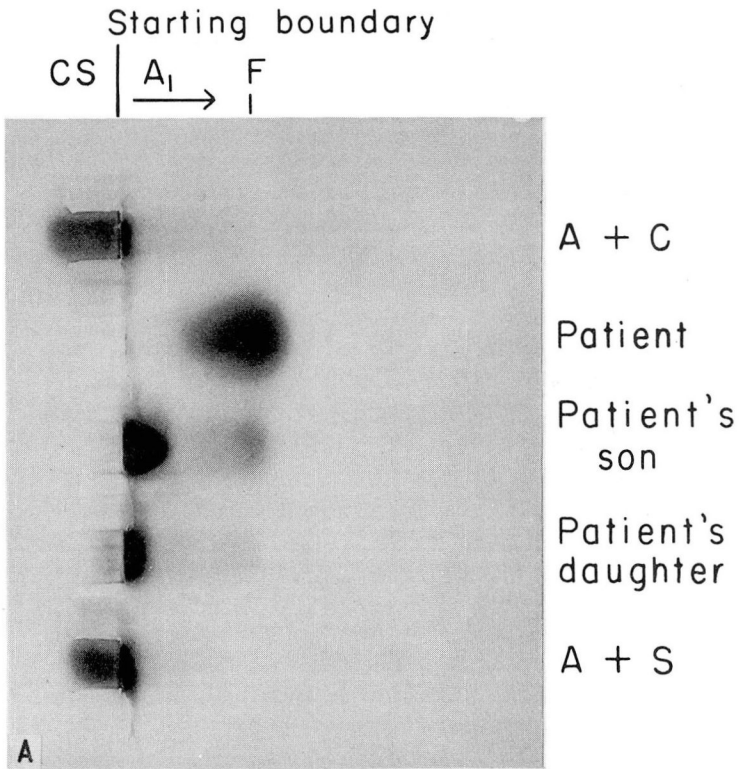
Discussion

Though much information has been acquired in the past thirty years in regard to thalassemia, there are still many puzzling biochemical and genetic aspects. On the basis of the familial distribution of thalassemia it has been suggested^{1,12-14} that the mild form of the disease, known as thalassemia minor, occurs when there is heterozygosity for a gene that in the homozygous state produces the more severe thalassemia major. The genetics of thalassemia would follow the pattern depicted by Valentine and Neel¹⁴ (*Fig. 2*). However, the mechanism is much more complex. On the basis of Valentine and Neel's simple hypothesis, it is difficult to explain the great variation in the clinical, hematologic, and biochemical manifestations of thalassemia. The spectrum of the thalassemia syndromes ranges from those characterized by minimal erythrocytic abnormalities to tha-

Table 1.—*Continued*

Erythrocyte abnormalities	Fetal hemoglobin,* percentage of total	Diagnosis
Anisochromia, anisocytosis, poikilocytosis; oval, tailed, teardrop, and target cells	78-83	Thalassemia intermedia
Anisocytosis, poikilocytosis; oval, tailed, and target cells	10	Thalassemia minor
Anisochromia, anisocytosis, poikilocytosis; oval, tailed, and target cells	14	Thalassemia minor
Anisocytosis, poikilocytosis; oval and target cells	17	Thalassemia minor
Anisochromia, anisocytosis, poikilocytosis; oval and target cells	13	Thalassemia minor
None	1.2	Normal

*Measured according to the alkali denaturation method of Singer, Chernoff, and Singer.⁷



AGAR ELECTROPHORESIS

Fig. 1 A. Electrophoretic patterns of hemoglobin of the patient and his family; agar electrophoresis.

lassemia major characterized by a severe hemolytic anemia, splenomegaly, bone changes, and a greatly shortened life span of the patient. Thalassemia intermedia, between these two extremes, may present a complicated genetic pattern that is heterozygous, doubly heterozygous, or homozygous (*Fig. 3*). Double heterozygosity represents a combination of genes for thalassemia and for abnormal hemoglobins, for example, sickle-thalassemia. The complexity of the problem is further illustrated by the occasional lack of correlation between the clinical severity of the disease and the hemoglobin abnormalities. In thalassemia, the percentage of fetal hemoglobin usually is higher in the homozygous states. Our patient, in spite of having approximately 80 per cent of fetal hemoglobin, had only a mild hemolytic anemia. Other observations have been recorded¹⁵ in which adult members of a number of families, each with a great percentage of fetal hemoglobin, have had only mild erythrocytic abnormalities.

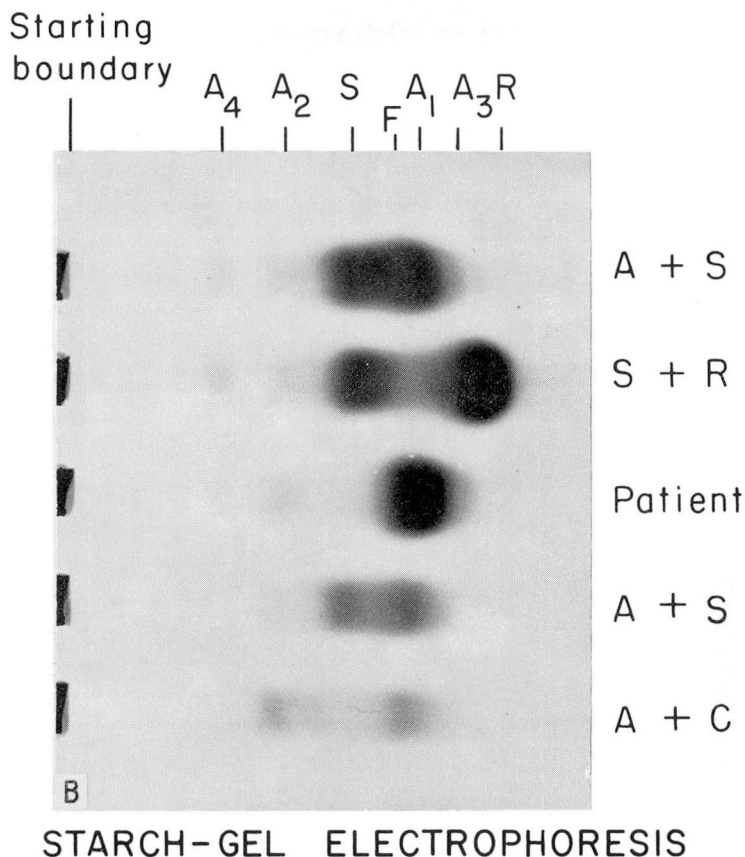
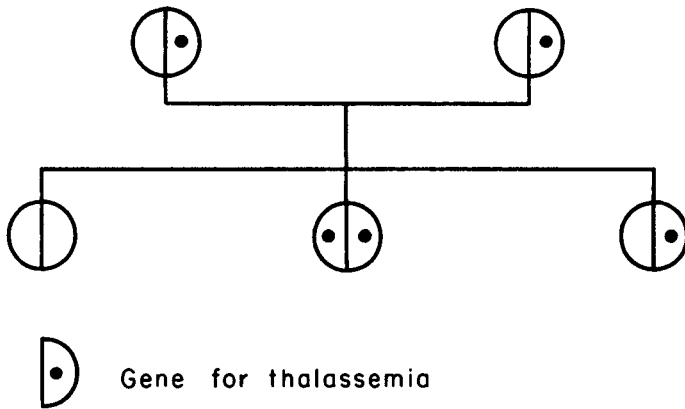


Fig. 1 B. Electrophoretic patterns of hemoglobin of the patient and his family; starch-gel electrophoresis.

In 1955, Kunkel and Wallenius¹⁰ applied the technic of starch-block electrophoresis (pH 8.6) to the separation of hemoglobin. They found that normal adult hemoglobin separated into three components: main (A_1), slow (A_2), and fast (A_3). Masri, Josephson, and Singer¹⁶ reported the presence of another component, A_4 , with mobility slower than A_2 . The maximal normal concentration of A_2 is 3.5 per cent. It is usually high in thalassemia, yet Kunkel and Wallenius¹⁰ reported several patients with homozygous thalassemia and normal amounts of A_2 . The affected members of our patient's family had increased amounts of A_2 hemoglobin with the exception of the son of the proband. The values of A_2 show no correlation with the clinical severity of thalassemia, nor is there any apparent correlation of the amount of A_2 with that of fetal hemoglobin.¹⁷

Fig. 2. Genetic mechanism of thalassemia (after Valentine and Neel¹⁴).Table 2.—*Electrophoretic studies of hemoglobin of patient and family*

Family relation	Type of hemoglobin, percentage of total					Supporting medium used
	F	A ₁	A ₂	A ₃	A ₄	
Patient	89.6* 90	— 10	3.5 —	3.8 —	2.1 —	Starch gel and starch block Agar
Father	89.6* 15	— 85	3.5 —	4.8 —	2.1 —	Starch gel Agar
Mother	87.9* 15	— 85	4.6 —	5.5 —	2.0 —	Starch gel Agar
Daughter	85* 10	— 90	4.5 —	4.5 —	6.0 —	Starch gel and starch block Agar
Son	92.2* 15	— 85	2.3 —	4.0 —	1.5 —	Starch gel and starch block Agar

*Resolution of F and A₁ not clear; therefore percentages include both.

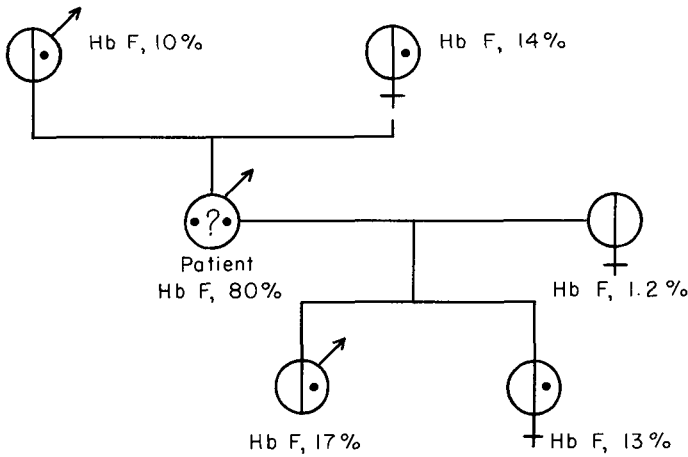


Fig. 3. Pedigree of the family of the patient reported, with individual percentages of fetal hemoglobin.

The preceding considerations seem to lend support to the concepts of Chernoff^{15,18,19} in regard to the genetics of thalassemia. He suggests that the thalassemia syndromes are caused by a series of multiple, interrelated genetic defects, not necessarily closely linked, which in various combinations give rise to a graduated series of hematologic aberrations from a mild asymptomatic state to thalassemia major. He speculates that at least one of the genes involved in the thalassemia syndromes is either identical with or closely related to the gene for fetal hemoglobin. Some patients may well be homozygous for the hemoglobin-F gene, and heterozygous for genes that control other manifestations of thalassemia. This might be the genetic explanation of the high percentage of fetal hemoglobin in our patient in spite of his position in the intermediate area of the thalassemia spectrum.

Summary

A case report is presented of a patient having thalassemia intermedia with a high percentage of fetal hemoglobin (78 to 83 per cent) and a mild, compensated hemolytic anemia. It is suggested that the patient may be homozygous for the hemoglobin-F gene, but perhaps heterozygous for genes responsible for other manifestations of thalassemia.

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