

A Family With Red Cell Pyrimidine 5'-Nucleotidase Deficiency

By Isaac Ben-Bassat, Frida Brok-Simoni, George Kende, Fanny Holtzmann, and Bracha Ramot

Congenital hemolytic anemia associated with pyrimidine 5'-nucleotidase deficiency is reported in two siblings. Both have had moderate chronic hemolytic anemia, splenomegaly, and jaundice since early infancy. The peripheral blood smear is characterized by striking red cell basophilic stippling. As this feature has been found in all previously reported cases, it should be the clue to the diagnosis.

SIX PATIENTS, belonging to five kindreds, with chronic hereditary hemolytic anemia, marked red cell basophilic stippling, and a deficiency of pyrimidine 5'-nucleotidase (P5N) are described by Valentine et al.¹ These cases were previously diagnosed as belonging to the "high red cell ATP syndromes" due to erroneous identification of the large amounts of pyrimidine nucleotides in these cells as adenine nucleotides.^{2,3} In this report we provide data on an additional kindred with this red cell enzymatic deficiency.

MATERIALS AND METHODS

Hematologic data were obtained by standard techniques. All the glycolytic enzymatic activities, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione peroxidase, glutathione reductase, adenylate kinase, and reduced glutathione were assayed as previously described.⁴ ATP was determined using the Boehringer Mannheim Corp. kit. P5N activity assay and spectral analysis of deproteinized red cell extracts were performed according to Valentine et al.¹ P5N activity was measured using uridine 5'-monophosphate as the substrate and expressed in IU, the activity required to dephosphorylate 1 μ mole of substrate/g Hb/hr. Red cells were separated into age groups, using the phthalate ester density gradient method as described previously.⁵ Globin chain ratios were determined according to the method of Clegg et al.⁶ with slight modifications.⁷

CASE REPORT

S.E. was first seen in 1969 at the age of 7 mo for evaluation of a hemolytic anemia diagnosed at the age of 3 mo. She was the second child of Ashkenazi Jewish parents originating in Eastern Europe and without known consanguinity. The girl was born after a normal pregnancy and delivery weighing 3300 g and had no history of neonatal jaundice. A complete hematologic workup at that time did not reveal any known red cell abnormality. Subsequently, she continued to have mild chronic hemolysis with jaundice. Her hemoglobin level ranged between 8 and 10 g/dl, but she did not require blood transfusions. At age 6, the girl was in the 15th percentile; her intelligence and psychomotor development were normal and except for jaundice and palpable spleen and liver edges, the physical examination was normal.

E.E. was the younger brother of the propositus, born in 1972. He was diagnosed as having congenital hemolytic anemia at the age of 7 mo. His hemoglobin level has ranged between 9 and 10

From the Institute of Hematology, Chaim Sheba Medical Center, Tel Hashomer, and Tel-Aviv University Medical School, Israel.

Submitted November 20, 1975; accepted January 9, 1976.

Address for reprint requests: Dr. I. Ben-Bassat, Institute of Hematology, Chaim Sheba Medical Center, Tel Hashomer, Israel.

Dr. Bracha Ramot is Principal Investigator, Chief Scientist's Bureau, Ministry of Health.

© 1976 by Grune & Stratton, Inc.

Table 1. Hematologic and Laboratory Data on E. Kindred

	Mother	Father	Brother	Propositus	Brother
Hemoglobin (g/dl)	13.2	15.7	13.7	9.5	8.7
PVC	39	45.7	39.7	28.9	27.3
MCV (fl)	85	79	78	87	82
MCH (pg)	30.5	27.6	28.4	30.1	27.5
Reticulocytes (%)	1.6	0.8	1.5	10	7
Peripheral blood	Normal	Normal	Normal	Anisocytosis, polychromatophilia, basophilic stippling	
Autohemolysis (48h, - glu/+ glu, %)	2.2/1.4	—	3.9/2.2	6.6/4.5	6.4/3.6
Hemoglobin electrophoresis and heat stability	Normal	Normal	—	Normal	—
P5N activity (IU/g Hb)*	2.86	3.98	6.45	0.67	1.98

*Normal values: 7.38 ± 2.56 IU/g Hb.

g/dl, and he, too, has not had a blood transfusion. He had mild jaundice, palpable spleen and liver, both 3 cm below the costal margin, but otherwise his physical examination was normal. The eldest sibling and both parents were healthy, and routine hematologic and laboratory examinations were within normal limits.

Because the peripheral blood smear of both patients showed striking basophilic stippling of the red cells, the family was reexamined in 1975 after this morphological feature was described as typical of P5N deficiency.

RESULTS

The hematologic and relevant laboratory findings of the E. family are summarized in Table 1. The peripheral blood of both patients showed moderate anisocytosis, polychromatophilia, a few densely staining poikilocytes, and very prominent basophilic stippling. The number of stippled red cells varied from smear to smear and was best seen in slowly dried capillary blood smears, where almost 5% of the cells were stippled. No stippling was seen in blood stored in EDTA for more than 3 hr, while, in heparinized blood, stippling was seen even after 24 hr of storage. Hemoglobin electrophoresis was repeatedly normal, and no unstable hemoglobin could be found in any of the family members. The direct and indirect antiglobulin tests were also negative. Autohemolysis was slightly increased and poorly corrected by glucose. The activities of the glycolytic enzymes, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase, glutathione peroxidase, and adenylate kinase were assayed on several occasions and found to be within normal levels or increased to levels expected in reticulocyte-rich blood.

P5N activity of the propositus' red cells was 0.67-0.98 IU/g Hb, and that of her brother 1.98 IU/g Hb, about 11% and 27% of the mean activity in 32 normal controls: 7.38 ± 2.56 IU/g Hb. Reticulocyte-rich bloods had a higher activity ranging from 7 to 13 IU/g Hb, suggesting an age-dependent decline in the activity of this enzyme. This finding was explored further on age-fractionated, deficient red cells obtained from the propositus. The top fraction, representing 5% of the total red cell population and consisting mainly of reticulocytes, had P5N activity of only 1.5 IU/g Hb. The whole blood had an activity of 0.67 IU/g Hb and the 5% densest cells 0.23 IU/g Hb. The parents

had red cell P5N activities of about 40% and 54% of the mean normal, while the normal sibling had normal enzymatic activity (Table 1).

The ATP content of the deficient cells, as measured by routine enzymatic method, was only slightly elevated: 4.8 $\mu\text{mole/g Hb}$ (mean normal, 3.15 ± 1), while the reduced glutathione was clearly elevated to 9.26 $\mu\text{mole/g Hb}$ (mean normal: 6.52 ± 1.54).

Spectral analysis of deproteinized extracts of the family members' erythrocytes showed that only in the two patients was the maximum absorbance shifted to about 265 nm, whereas the normal peak was between 255 and 260 nm.

The globin chain ratio of the propositus' reticulocytes showed an imbalanced α/β ratio of 1.5, which was significantly higher than the normal ratio of 0.97 ± 0.07 .⁸

DISCUSSION

P5N deficiency associated with hereditary hemolytic anemia has been reported so far, including the present report, in seven kindreds affecting nine cases.^{1-3,9} We report our family mainly to draw attention to the morphological hallmark of this enzymatic deficiency, i.e., increased basophilic stippling. This finding, especially in the absence of hypochromia and microcytosis, should prompt a spectral analysis of the red cell extracts or, preferably, P5N assay. It should be noted that in blood stored in EDTA for more than 3 hr, basophilic stippling was not observed, probably due to a requirement of Mg^{2+} or Ca^{2+} ions for the formation of the ribosomal aggregates. A similar observation has been reported in lead poisoning.¹⁰ Therefore, unless smears made from slowly dried capillary or heparinized blood are screened, this important clue can be overlooked.¹¹

The clinical picture of the affected cases, on the other hand, is not diagnostic. All have mild to moderate hemolytic anemia, rarely necessitating blood transfusions. Splenectomy has been performed in at least three cases,^{1,2,9} but has not resulted in significant clinical amelioration.

The biochemical characteristic of this hemolytic syndrome is a deficiency of a uniquely specific nucleotidase utilizing only uridine or cytidine ribonucleoside (or deoxynucleoside) 5'-monophosphates.¹² It seems that the normal maturing reticulocyte degrades ribosomes and RNA to 5'-pyrimidine nucleotides which are then dephosphorylated by a specific pyrimidine nucleotidase so as not to compromise the important adenine nucleotide pool. In the deficient reticulocytes, ribosomes are not degraded normally, giving rise to aggregates seen in the peripheral blood smear as basophilic stippling.

The results of the globin synthesis study in the propositus' reticulocytes showed an imbalanced α/β ratio of 1.5. This finding could be due to either an excess of α chains or a deficiency of β chains. The lack of hypochromia would favor the former possibility. It is conceivable that the excess of pyrimidine nucleotides somehow favors the synthesis of α chains, but this observation needs confirmation in more cases.

The genetics of this syndrome can now be established as having an autosomal recessive mode of inheritance. As the first cases were all females, an X chromosome-linked disease, lethal in male hemizygotes, could have been postulated.

This thesis is most unlikely, as there are at least three deficient males known (nine and present report). Heterozygotes, as was also previously reported,¹ can be identified by enzymatic assays. The activity of P5N in the father as well as in the mother, was about half the normal level, also consistent with an autosomal linkage.

REFERENCES

1. Valentine WN, Fink K, Paglia DE, Harris SR, Adams WS: Hereditary hemolytic anemia with human erythrocyte pyrimidine 5'-nucleotidase deficiency. *J Clin Invest* 54:866, 1974
2. Valentine WN, Anderson HM, Paglia DE, Jaffé ER, Konrad PN, Harris SR: Studies on human erythrocyte nucleotide metabolism. II. Non-spherocytic hemolytic anemia, high red cell ATP, and ribosephosphate pyrophosphokinase (RPK, EC 2.7.6.1) deficiency. *Blood* 39:674, 1972
3. Valentine WN, Bennett JM, Krivit W, Konrad PN, Lowman JT, Paglia DE, Wakem CJ: Nonspherocytic haemolytic anaemia with increased red cell adenine nucleotides, glutathione and basophilic stippling and ribosephosphate pyrophosphokinase (RPK) deficiency: Studies on two new kindreds. *Br J Haematol* 24:157, 1973
4. Beutler E: *Red Cell Metabolism, A Manual of Biochemical Methods*. New York, Grune & Stratton, 1971
5. Brok F, Ramot B, Zwang E, Danon D: Enzyme activities in human red blood cells of different age groups. *Israel J Med Sci* 2:291, 1966
6. Clegg JB, Naughton MA, Weatherall DJ: An improved method for the characterization of human hemoglobin mutant: Identification of Glu⁹⁵ hemoglobin N (Baltimore). *Nature* 207:945, 1965
7. Shchory M, Ramot B: Globin chain synthesis in the marrow and reticulocytes of beta thalassemia, hemoglobin H disease and beta delta thalassemia. *Blood* 40:105, 1972
8. Ben-Bassat I, Mozel M, Ramot B: Globin synthesis in iron deficiency anemia. *Blood* 44:551, 1974
9. Rochant H, Dreyfus B, Rosa R, Boiron M: First case of pyrimidine 5'-nucleotidase deficiency in a male. *International Society of Haematology, European and African Third Meeting, London, August 24-28, 1975, Abstract 1:19*
10. White JM, Selhi HS: Lead and the red cell. *Br J Haematol* 30:133, 1975
11. Jensen WN, Moreno GD, Bessis MC: An electron microscopic description of basophilic stippling in red cells. *Blood* 25:933, 1965
12. Paglia DE, Valentine WN: Characteristics of pyrimidine-specific 5'-nucleotidase in normal human erythrocytes. *American Society of Hematology Seventeenth Annual Meeting, Atlanta, Georgia, December 7-10, 1974, Abstract 189*