RED CELL PHOSPHOGLYCERATE KINASE DEFICIENCY*

A new cause of non-spherocytic hemolytic anemia.

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Hereditary non-spherocytic hemolytic anemia caused by abnormalities of the following glycolytic enzymes of red blood cells has been described: glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, hexokinase, phosphohexose isomerase, aldolase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, 2,3-diphosphoglycerate mutase, pyruvate kinase and glutathione reductase (Carson and Frischer; Keitt; Valentine et al.). A hitherto unreported abnormality of a red cell glycolytic enzyme, phosphoglycerate kinase deficiency, resulting in non-spherocytic hemolytic anemia is described.

Methods

Activities of red cell glycolytic enzymes were measured by the following methods: glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Zinkham et al.), hexokinase and phosphohexose isomerase (Brewer et al.), phosphohexokinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and phosphoglyceromutase (Koutras, et al.), pyruvate kinase (Tanaka et al.), triosephosphate isomerase (Schneider et al.), enolase (Bock et al.), glutathione instability (Beutler), 2,3-diphosphoglycerate mutase and 2,3-diphosphoglycerate content (Schröter and Heyden). These determinations were performed on the whole red cell population. In addition, determinations of PK[#] and PGK were also made on red cells separated into reticulocyte-rich and reticulocyte-poor fractions by a modification of the method of Brewer and Powell substituting 30% bovine albumin for plasma. Hemoglobin A_2 was quantitated according to Chernoff. Red cell survival was determined by use of ^{51}Cr. Hematologic data, including osmotic fragility and autohemolysis, were obtained according to methods described by Cartwright.

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[#]Abbreviations: PGK-phosphoglycerate kinase, PK=pyruvate kinase, ATP=adenosine triphosphate, RBC=red blood cell, WBC=white blood cell.

Results

The patient, a 63-year-old Caucasian female, gave a history of life-long anemia documented for the past 20 years. A diagnosis of non-spherocytic hemolytic anemia was made 2 years ago at another hospital on the basis of the following findings: normochromic, normocytic anemia with a hematocrit varying between 16-27%, reticulocytosis ranging from 6-11%, serum bilirubin 1.2 mg% total with 0.3 mg% conjugated, ^{51}Cr apparent red cell half-survival 12.5 days. Two bone marrow examinations revealed erythroid hyperplasia. Repeated lupus erythematosus preparations, fluorescent-antibody determinations, and tests for paroxysmal nocturnal hemoglobinuria were all negative. After she was unsuccessfully treated with steroids and pyridoxine the spleen was removed. This organ was considerably enlarged, weighed 610 g and showed marked hyperemia and focal extramedullary hematopoiesis. The hemolytic process was not significantly altered by the operation.

Two years later she was hospitalized at the City of Memphis Hospitals for pneumonia. The peripheral blood findings were similar to those found during the previous hospitalization except for a leukocytosis up to 25,000/cu mm and up to 75 nucleated red cells/100 WBC. The red cells showed a tendency to macrocytosis and a moderate number of oval, burr, thorn, and target cells were found. Howell-Jolly bodies and stippling were present. Some of these changes were undoubtedly due to the previous splenectomy. After recovery from the acute infection the hemolytic anemia was restudied. A bone marrow examination again revealed erythroid hyperplasia. Hemoglobin electrophoresis was normal. Fetal hemoglobin comprised less than 2% and hemoglobin A_2 2.6% of the total hemoglobin. Serum proteins, including electrophoresis, and a Coomb's test were normal. Incubated red cell fragility revealed an initial hemolysis at .65% saline and complete hemolysis at .10% saline (control 0.55% - 0.35%). The number of cells sensitive to hypotonic saline hemolysis was small, the patient's curve crossing that of the control at 18% hemolysis. Serum bilirubin again was elevated and ^{51}Cr red cell half-survival was found to be 12 days. The autohemolysis test resulted in 28% hemolysis after 48 hours incubation. This was not reduced by addition of glucose (33% hemolysis), but was reduced to 2.5% by addition of ATP (Dacie Type II). Therefore, red cell enzymes were studied. The results are given in Table I.

All red cell enzymes measured showed values well above normal levels established for this laboratory except for PGK. This increase in enzymatic activity was interpreted to result from the young age of the red cells. The defect of PGK activity became more apparent when young and old cells were separated and tested. Relatively old cells (1.5% reticulocytes) had 7.3 units

TABLE I.

Activity of red cell enzymes.

Enzyme	Units* per 10^{10} RBC	
	Patient	Normal Range
Glucose 6-phosphate dehydrogenase	3.5	1.6-2.7
6-phosphogluconate dehydrogenase	2.2	1.1-1.7
Hexokinase	0.48	0.17-0.38
Phosphohexose isomerase	35.0	3.4-14.9
Phosphohexokinase	7.7	1.2-2.5
Aldolase	1.3	0.3-0.9
Triosephosphate isomerase	218	85-136
Glyceraldehyde-3-phosphate dehydrogenase	65	15-26
2,3-diphosphoglycerate mutase	2.0	1.1
Phosphoglycerate kinase	17.5	18.8-27
Phosphoglycerate mutase	21.6	7.6-12.4
Enolase	12.8	2.0-3.9
Pyruvate kinase	14.3	1.0-3.7

*An enzyme unit is defined as that amount required to decompose one μMole of substrate per minute at 37° C.

of PGK activity per 10^{10} RBC, whereas the relatively young cells (12.3% reticulocytes) gave a value of 29 units per 10^{10} RBC. Storage of the red cells in ACD for one month at 4° C did not reduce enzyme activity. Since certain variants of glucose 6-phosphate dehydrogenase have been shown to be heat-labile (Kirkman *et al.*) the hemolysate from the patient was incubated at 37° C for 3 hours. No loss of PGK activity was observed. 2,3-diphosphoglycerate content was 4.4 μM per 10^{10} RBC (normal 3.9-5.2). White cell PGK activity was found to be normal, i.e., 233 units per 10^{10} WBC (control 242 units per 10^{10} WBC).

Discussion

In this patient the diagnosis of non-spherocytic hemolytic anemia is well established and its presence has been documented for the last 20 years. Unfortunately there are no living members of the family and the hereditary nature of this defect cannot be proven. The history of anemia in the mother and two of the patient's siblings only suggests this possibility.

Nevertheless, there is evidence that her red cells are deficient in PGK. As shown in the autohemolysis test, the hemolysis is markedly reduced by addition of ATP. Only two enzymes of the glycolytic cycle result in ATP formation, namely PK and PGK. The activity of the former is greatly increased even when tested at low levels of substrate (phosphoenolpyruvate) (Paglia et al.). PGK activity, however, is just below normal values for this enzyme when measured in the whole cell population containing a preponderance of young cells. In the older red cell population this enzyme is markedly reduced. The substrate affinity as expressed in the K_m value, however, is normal. This suggests reduced enzyme levels rather than a structurally altered enzyme. Cells deficient in PGK would be unable to effect a net gain in ATP through glycolysis, although they could reform ATP by means of PK, bypassing PGK via 2,3-diphosphoglycerate. This shunt is active in the cells of this patient as shown by the normal levels of 2,3-diphosphoglycerate. Addition of ATP to such cells will decrease hemolysis in vitro as shown in PK deficiency although they will not be helped by glucose (Tanaka et al.). As in glucose 6-phosphate dehydrogenase deficiency of the Negro type, white cell PGK in this patient was normal.

Summary

Phosphoglycerate kinase deficiency, a hitherto unreported red cell enzyme defect, has been demonstrated in a patient with non-spherocytic hemolytic anemia. It is suggested that the enzyme deficiency is the cause of the anemia.

References

Beutler, E. J., J. Lab. Clin. Med. 49, 85 (1957).

Bock, H. E., Karges, O., Löhr, J. W. and Waller, H. D., Klin. Wschnschr. 36, 56 (1958).

Brewer, C. J., Powell, R. D., Swanson, S. H. and Alving, A. S., J. Lab. Clin. Med. 64, 601 (1964).

Carson, P. E. and Frischer, H., Amer. J. Med. 41, 744 (1966).

Cartwright, G. E., Diagnostic Laboratory Hematology, Grune & Stratton, New York (1963).

Chernoff, A., Ann. N. Y. Acad. Sci. 119, 557 (1964).

Keitt, A. S., Amer. J. Med. 41, 762 (1966).

Kirkman, H. N., Rosenthal, I. M., Simon, E. R., Carson, P. E. and Brinson, A. G., J. Lab. Clin. Med. 63, 715 (1964).

Koutras, G. A., Hattori, M., Schneider, A. S., Ebaugh, F. G., Jr. and Valentine, W. N., J. Clin. Invest. 43, 323 (1964).

Paglia, D. E., Valentine, W. N., Baughan, M. A., Miller, D. R., Reed, C. F. and McIntyre, O. R., American Society of Hematology, 10th Annual Meeting, Abstract # 59 (1967).

Schneider, A. S., Valentine, W. N., Hattori, M. and Heins, H., New Engl. J. Med. 272, 229 (1965).

Schröter, W. and Heyden, H., Biochem. Zeitschrift. 341, 387 (1965).

Tanaka, K., Valentine, W. N. and Miwa, S., Blood. 19, 268 (1962).

Valentine, W. N., Oski, F. A., Paglia, D. E., Baughan, M. A., Schneider, A. S. and Naiman, J. L., New Engl. J. Med. 276, 1 (1967).

Zinkham, W. H., Lenhard, R. E. and Childs, B., Bull. of Johns Hopkins Hospital. 102, 169 (1958).