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EXTREME HEMOLYSIS AND RED-CELL DISTORTION IN ERYTHROCYTE PYRUVATE KINASE DEFICIENCY*

I. Morphology, Erythrokinetics and Family Enzyme Studies

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BOSTON

BIZARRE morphologic abnormalities of the erythrocytes are generally not considered a prominent feature of congenital nonspherocytic hemolytic anemias.[1,2] We have observed a child with a severe congenital nonspherocytic hemolytic anemia associated with a deficiency of erythrocyte pyruvate kinase in whom morphologic abnormalities of the red cells were profound. The bizarre red-cell abnormality was in some respects similar to that observed in the syndrome of acanthocytosis associated with absence of serum beta-lipoprotein.[3] Of additional interest was the finding that the mother of the patient was not only a heterozygote for the pyruvate kinase (PK) deficiency but was also a carrier of the erythrocyte glucose-6-phosphate dehydrogenase (G6PD) deficiency. The hematologic findings in this family and their implications form the basis of this report.

CASE REPORT

The patient, a boy now 5 years and 8 months of age, was first admitted to the Children's Hospital Medical Center on October 16, 1961, at the age of 3 years and 7 months for investigation of chronic anemia.

He had been born at term after a normal pregnancy. Jaundice was noted on the 1st day of life. The mother was blood Type O and Rh positive, and the infant Type B and Rh positive; the direct Coombs test was negative. Because of hyperbilirubinemia and anemia an exchange transfusion was performed on the 4th day of life. During the 1st 3 weeks of life the child required 2 transfusions of packed red cells to maintain the hemoglobin above 5 gm. per 100 ml. Before the 2d transfusion the hemoglobin had been recorded as 2.0 gm. per 100 ml. Mild jaundice persisted throughout the 1st 2 months of life. At 2 months of age he received a 3d transfusion and required 6 more transfusions at the rate of 1 per month during the first 9 months of life to maintain the hemoglobin above 4 gm. per 100 ml.

At 5 months of age significant splenomegaly was first detected. One month later he was given prednisone, and he received this medication for 6 weeks without any change in transfusion requirements. At 8 months of age an occasional spherocyte was noted in peripheral blood smears, and a slight increase in red-cell osmotic fragility was detected. At the age of 9 months a splenectomy was performed. After this operation the patient maintained a hemoglobin level between 6.0 and 8.5 gm. per 100 ml. and required no further transfusions until 2 years, 8 months, of age. At that time 2 blood transfusions were required to correct a severe anemia that developed during the course of treatment of an ear infection with tetracycline and triacetyloleandomycin. On antibiotic therapy very dark urine that was benzidine positive developed. Since that illness the patient has been well although slight jaundice has been noted intermittently, particularly during infections of the upper respiratory tract.

He sat alone at 7 months, walked at 18 months and spoke several words by 2 years of age. The parents stated that he had always been unsteady on his feet and that his speech had been difficult to understand.

The patient was 1 of 5 children. His siblings were all well, and there was no history of anemia or jaundice in any member of the immediate family. The father was of Italian, French and Scotch, and the mother of French, Irish and German ancestry.

Physical examination showed a small, thin, pale and shy boy who weighed 12.2 kg. (below the 3d percentile), and was 96.5 cm. tall (10th percentile); he was in no distress. Significant physical findings were as follows: slight scleral icterus, alternating strabismus of the right eye, hepatomegaly (liver edge palpable 4 cm. below the right costal margin) and a well healed scar in the left upper quadrant of the abdomen. Neurologic examination revealed generalized hypotonia, slight ataxia and mild athetosis. The fundi were normal.

The hemoglobin was 6.6 gm. per 100 ml., with a hematocrit of 25 per cent, red-cell count of 2,630,000, reticulocyte count of 47 per cent, mean corpuscular volume of 95 cubic microns, mean corpuscular hemoglobin of 25 micromicrogm. and mean corpuscular hemoglobin concentration of 26.4 per cent. The platelet count was 666,000, and the white-cell count 10,750, with 23 per cent neutrophils, 1 per cent band forms, 1 per cent metamyelocytes, 12

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per cent eosinophils, 2 per cent basophils, 53 per cent lymphocytes and 8 per cent monocytes and 1 nucleated red blood cell per 100 white cells.

During the 1st admission the hemoglobin ranged between 6.6 and 7.7 gm. per 100 ml., and the reticulocyte count from 41 to 65 per cent.

Examination of a Wright-stained smear of the peripheral blood (Fig. 1) revealed the presence of contracted cells with pseudopod-like horns projecting from multiple sites. Approximately 80 per cent of the cells were of this form. In addition some target cells, macrocytes, an occasional spherocyte and Howell–Jolly bodies were observed. Siderocytes Pappenheimer or Heinz bodies were not present. Despite the fact that these bizarre forms were noted on repeated dry films a staining artifact could not be excluded; therefore, wet preparations were examined. These oddly prickled cells were also observed in wet preparations examined within 5 seconds of the time the blood left the patient's finger (Fig. 2). In addition some of the red cells assumed a large balloon shape, with a few prickles on their surface. At no time was rouleau formation observed. Additional morphologic studies were performed, as described below.

The Coombs test was negative. Hemoglobin electrophoresis demonstrated no abnormal hemoglobin with a fetal hemoglobin of 3.8 per cent (normal for the patient's age, less than 2.0 per cent); serum haptoglobin was absent. The serum iron was 60 microgm., and the serum unsaturated iron-binding capacity 318 microgm. per 100 ml. The serum vitamin B_{12} was 412 micromicrogm. (normal), and the serum folic acid 3.4 millimicrogm. per milliliter (indeterminate range). The serum bilirubin was 0.2 mg. per 100 ml. direct and 1.7 mg. total; serum electrolytes and osmolarity were normal. The red cells had a decreased osmotic fragility before incubation. After 24 hours of incubation 20 to 25 per cent of cells showed increased osmotic fragility. The sedimentation rate was 0 mm. per hour. The acid hemolysis test was negative on 2 occasions.

Aspiration of the bone marrow revealed marked erythroid hyperplasia. None of the nucleated red-cell precursors showed the abnormal morphology that was observed in the mature erythrocytes (Fig. 3).

Because of the similarity between the appearance of the patient's red cells and those observed in the syndrome of acanthocytosis the following studies were done: serum cholesterol (250 mg. and 178 mg. per 100 ml.) and serum protein (5.7 gm., with 4.05 gm. of albumin, 0.11 gm. of $alpha_1$ globulin, 0.40 gm. of $alpha_2$ globulin, 0.51 gm. of beta globulin and 0.63 gm. of gamma globulin per 100 ml.). The serum beta lipoprotein was present in normal quantities both on starch-gel electrophoresis and by gas chromatography. There was 29 per cent excretion of D-xylose in a 5-hour collection period. The patient's diet contained 50 gm. of fat per day, and he excreted 5.7 gm. during a 3-day collection. An oral glucose tolerance test was negative. Vitamin A absorption was normal. Biopsy of the duodenum revealed normal mucosa. A urinary amino acid chromatogram was normal except for the presence of increased amounts of beta aminoisobutyric acid (observed in 5 per cent of the normal population and in patients with increased tissue breakdown).[4] All these studies gave normal results and excluded the diagnosis of acanthocytosis with absent beta lipoprotein.

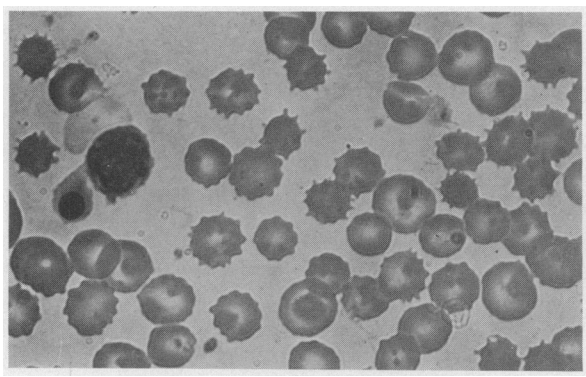

FIGURE 1. *Peripheral Smear of Blood from M.P. (Note the Mixed Population of Irregularly Contracted Cells and "Balloon" Forms with Irregular Surface Projections).*

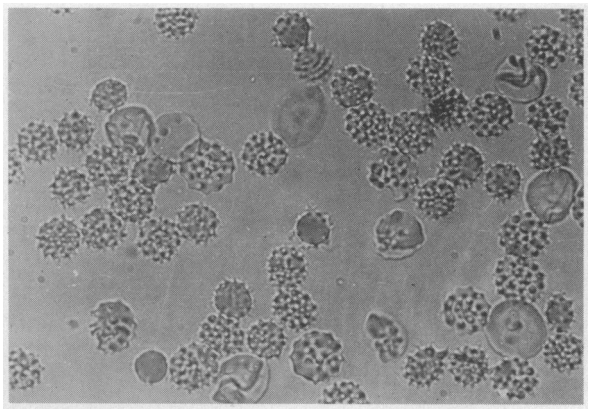

FIGURE 2. *Wet Preparation of Blood from M.P. One drop of blood was mixed with 1 drop of 0.9 per cent saline solution to emphasize the morphologic defect, which was evident as soon as the cells could be examined (five seconds).*

On 2 separate occasions the patient's red cells and plasma phospholipids and fatty acids were analyzed by gas chromatography by Dr. Robert Geyer and Dr. David Pollack at the Harvard School of Public Health. No significant differences from normal controls could be observed.

X-ray films of the skull, extremities, abdomen, kidneys and upper gastrointestinal tract were all normal. There was no definite evidence of an intramedullary expansile process in the skull or long bones.

The patient was readmitted twice to the Children's Hospital Medical Center (on October 1, 1962, and March 18, 1963) for further studies. It was during the 3d admission that he was found to be deficient in the erythrocyte glycolytic enzyme pyruvate kinase and family studies were initiated.

During the 2 years of observation he has remained in relatively good health and has required no transfusions. The hemoglobin has varied between 5.8 and 8.5 gm. per 100 ml., and the reticulocyte count between 34 and 94 per cent, with a mean of approximately 60 per cent. As many as 102 nucleated red blood cells per 100 white cells were counted although the range was usually between 5 and 10 nucleated red blood cells per 100 white cells.

SPECIAL STUDIES

Materials and Methods

Routine hematologic studies were performed by standard methods. Red-cell counts were performed in triplicate in the Coulter electronic blood-cell counter.

Assays of enzyme activity, determinations of glutathione and osmotic fragilities and autohemolysis tests were performed on heparinized blood.

Assays of the activities of erythrocyte glucose-6-phosphate dehydrogenase, pyruvate kinase, phosphoglycerate kinase, acid phosphatase and glutathione stability were performed by methods previously cited.[5]

Fresh heparinized blood was transported under refrigerated conditions to Los Angeles, California, and through the courtesy of Drs. Arthur Schneider and William Valentine, the following erythrocyte

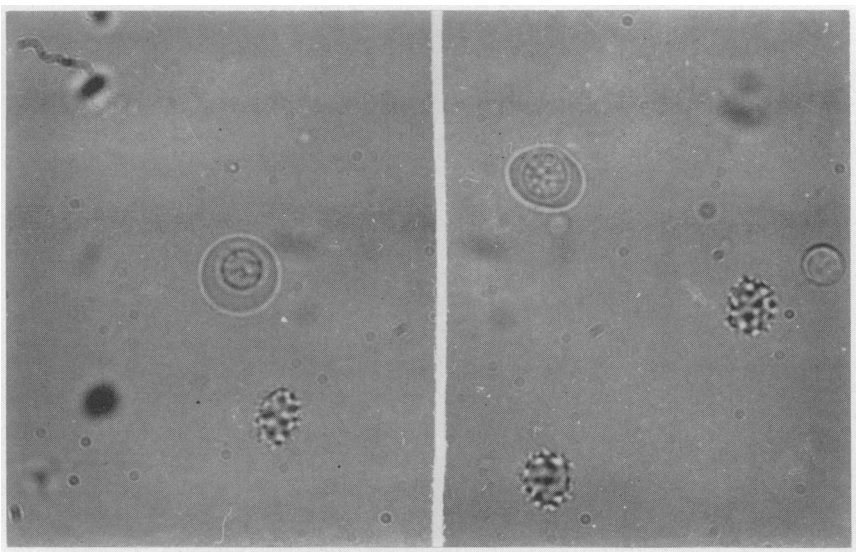

FIGURE 3. *Results When 1 Drop of Bone Marrow from M.P. was Mixed with 1 Drop of 0.9 per Cent Saline Solution (Two Fields X100).*
Note the contrast between irregularly contracted erythrocytes and ordinary-appearing normoblasts.

enzyme activities were assayed by methods also previously cited[6]: hexokinase, phosphohexose isomerase, phosphofructokinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, triose phosphate isomerase, phosphoglyceric kinase, phosphoglyceric mutase, enolase, pyruvate kinase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase and glutathione reductase.

Starch-gel electrophoresis of hemolysates for glucose-6-phosphate dehydrogenase activity was performed by the method of Boyer et al.[7]

Autohemolysis tests were performed on heparinized blood under sterile conditions; 2-ml. aliquots of blood were placed in pyrex tubes, 125 by 15 mm.. and glucose (final concentration, 0.026 M) or adenosine triphosphate* (final concentration, 0.02 M) was added to appropriate tubes. After twenty-four hours of incubation at 37°C. the tubes were gently rotated to resuspend the cells. Gentle rotation was repeated at the termination of the test after forty-eight hours. The tubes were then centrifuged, and 0.02 ml. of plasma was aspirated for determination of the plasma hemoglobin by the method of Crosby and Furth.[8] The blood was then vigorously agitated to ensure uniform mixing before microhematocrit and total hemoglobin were determined. The percentage of autohemolysis was calculated from the formula of Young et al.[9]

Incubated and unincubated osmotic fragilities were performed by means of the procedure outlined by Dacie.[10]

Studies of red-cell survival were performed with the radioactive sodium chromate ($Na_2Cr^{51}O_4$) method of Read et al.[11] To determine the intrinsic survival of the erythrocytes the labeled cells were transfused into normal adult recipients. The crossmatch was carried out with the antiglobulin technic. In vivo scanning of hepatic, cardiac and splenic radioactivity was performed with a thallium sodium iodide (NaITh) crystal and single-channel pulse-height analyzer. Two-milliliter samples of blood and plasma were counted in a dual-channel pulse-height analyzer. The highest counting error (ratio of 2 standard deviations of a total count to the net count rate) was 3 per cent.

In addition to the patient and his mother (Mrs. P.) studies of red-cell survival were also performed on 1 female carrier of the PK deficiency (Mrs. V.), the mother of a previously reported boy with PK deficiency (Case 2 of previous series[5]), and on a Caucasian female carrier of the G6PD deficiency (Mrs. H.). This woman had a son with congenital nonspherocytic hemolytic anemia associated with marked G6PD deficiency.

*Adenosine triphosphate (ATP), Mann Research Laboratories.

Results

Pyruvate kinase assays. In our laboratory normal values for erythrocyte PK activity range from 1.35 to 2.01 units per 10^{10} erythrocytes, with a mean value of 1.79 units (Table 1). The patient's values on multiple determinations varied between 0.20 and 0.31 unit. Drs. Schneider and Valentine could detect no PK activity in his erythrocytes whereas control samples simultaneously shipped to Los Angeles had normal PK activity.

The patient's mother, father and 3 of 4 siblings had PK values ranging between 0.68 and 0.90 unit. These values are intermediate between those of normal controls and those of patients with clinical disease and suggest that these members of the family are heterozygous for the pyruvate kinase deficiency. The patient's paternal grandmother, maternal aunt and maternal uncle were also found to

TABLE 1. *Results of Enzyme Assays in Members of the Patient's Family.*

DESIGNATION IN FAMILY TREE*	RELATION	PYRUVATE KINASE	G6PD
		units/10¹⁰ red cells*†	*units/100 ml. red cells*
III-3	Propositus	0.20-0.31‡	325-380
III-1	Sibling	0.73§	188
III-2	Sibling	1.90§	202
III-4	Sibling	0.90§	245
III-5	Sibling	0.77	251
II-1	Father	0.78§	260
II-2	Mother	0.69§	117-130§
II-3	Maternal aunt	0.80§	142§
II-4	Maternal aunt	1.70	223
II-5	Maternal aunt	1.89	228
II-6	Maternal uncle	1.78	212
II-7	Maternal uncle	0.88§	242
II-9	Maternal uncle	1.58	230
I-1	Paternal grandfather	1.41	251
I-2	Paternal grandmother	0.68§	221
I-3	Paternal great uncle	1.87	244
I-4	Maternal grandfather (deceased)	—	—
I-5	Maternal grandmother	1.47	159§
Normal values		1.35-2.01	180-300

*See Fig. 6.

†1 unit of pyruvate equals activity resulting in conversion of 1 micromole of DPNH to DPN/min. by 10^{10} erythrocytes under assay conditions of experiment.

‡Indicates deficient state (homozygous).

§Indicates carrier state (heterozygous).

have erythrocyte PK values in the intermediate or heterozygote range (Table 1 and Fig. 4).

Glucose-6-phosphate dehydrogenase assays. The patient was repeatedly demonstrated to have G6PD values above the upper limits of normal. The patient's mother, maternal aunt and maternal grandmother were all found to have erythrocyte G6PD values in the intermediate range. This suggests that these 3 women are carriers of G6PD deficiency (Table 1 and Fig. 4). The patient's mother and his maternal aunt appeared to be heterozygous for both the G6PD and PK deficiency traits.

In an attempt to rule out the possibility that the patient's extremely young red-cell population was masking the presence of a G6PD deficiency

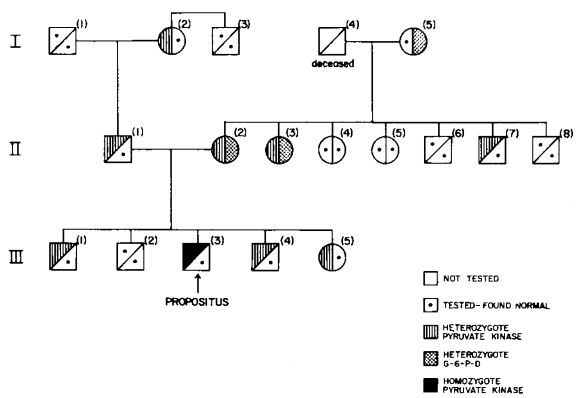

FIGURE 4. *Family Tree of M.P., Showing the Red-Cell Activities of G6PD and PK.*

TABLE 2. *Results of Autohemolysis Tests.*

SUBJECT & DIAGNOSIS	CONTROL	GLUCOSE (0.026 M)	ATP (0.02 M)
	%	%	%
Normal persons (20 subjects)	0.59 (0.27-1.25)	0.11 (0.07-0.21)	0.38 (0.11-0.91)
Propositus (PK deficiency)	11.10	14.70	10.30
Patient's mother (PK carrier plus G6PD carrier)	1.63	0.23	0.64
Mrs. V. (PK carrier)	0.19	0.10	0.15
Mrs. H. (G6PD carrier)	0.45	0.06	0.28

the white cells were assayed for the presence of this enzyme. They were found to be normal. In addition, incubation of the red cells with primaquine phosphate (2.2×10^{-3} M) at 37°C. for two hours produced only a rare Heinz body, and the patient had normal glutathione stability in the presence of acetylphenhydrazine. Starch-gel electrophoresis of his hemolysate revealed that his G6PD had an electrophoretic mobility identical to that of 4 normal Caucasian controls.

Other red-cell enzymes. Drs. Schneider and Valentine found that all other erythrocyte enzymes were present in this patient in normal or in increased quantities, as observed in other patients with PK deficiency.[6,12]

Autohemolysis tests (Table 2). The propositus was found to have a marked increase in autohemolysis. The autohemolysis was slightly accentuated by addition of glucose and unimproved by the addition of ATP.

The mother of the propositus, the carrier of both the G6PD and PK traits, also had a positive autohemolysis test. This test was improved but not completely corrected by the addition of glucose but was corrected by the addition of ATP. In sharp contrast to the findings in the patient's mother was the presence of normal autohemolysis tests in the women who were either heterozygous for the G6PD or PK traits alone.

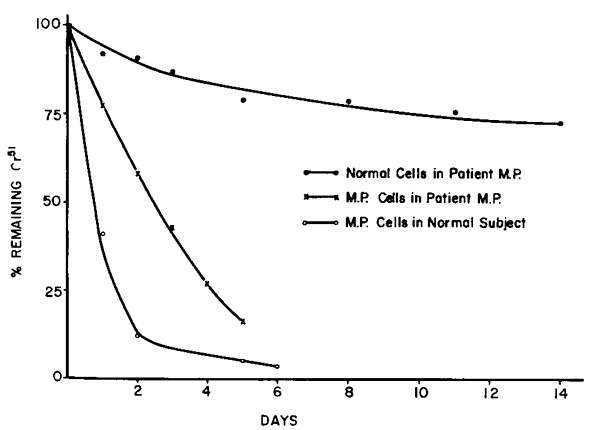

FIGURE 5. *Survival of M.P. Erythrocytes in M.P. (Splenectomized) and in a Normal Recipient (Nonsplenectomized), with Survival of Normal Erythrocytes in M.P.*

TABLE 3. *Hematologic Data on PK and G6PD Heterozygotes and Patient's Mother.*

SUBJECT	HEMOGLOBIN	RETICULOCYTES	SERUM IRON	SERUM IRON-BINDING CAPACITY	ERYTHROCYTE G6PD	ERYTHROCYTE PK
	gm./100 ml.	*%*	*microgm./100 ml.*	*microgm./100 ml.*	*units/100 ml. red cells*	*units/100 ml. red cells*
Mrs. P. (heterozygote for G6PD & PK)	12.0-13.3	1.8, 2.2, 2.7, 3.4	138	200	117.0-130.0	0.69
Mrs. V. (heterozygote for PK)	13.8-14.7	0.1, 0.8	146	294	239.0	0.96
Mrs. H. (heterozygote for G6PD)	13.2-15.0	1.4, 1.5, 2.4, 2.8	144	320	94.5-105.0	1.48

Hematologic data on pyruvate kinase carrier, G6PD carrier and the patient's mother (Table 3). The patient's mother had hemoglobin values ranging between 12.0 and 13.3 gm. per 100 ml. during the two-year period of observation, and her reticulocyte count was usually mildly elevated (1.8 to 3.4 per cent). The G6PD carrier (Mrs. H.) had slight elevations of her reticulocyte count (2.4 per cent and 2.8 per cent) whereas the PK carrier was hematologically normal. All 3 women had normal serum iron concentrations and serum iron-binding capacities.

Morphologic observations. Varying concentrations of the patient's red cells were studied in wet preparations. The suspending fluids were autologous plasma and varying concentrations of saline. The results were recorded by photomicrography and microcinematography.

The bizarre spiculated erythrocytes were present in mixtures of blood and autologous plasma as well as in mixtures of blood and 0.9 per cent saline solution; macrocytes comprised approximately 20 per cent of the cell population, and the small spiculated forms made up the remaining 80 per cent. The spicules were present in finger-prick blood samples that were immediately mixed in 4 per cent formalin. The cells imbibed enough water at 0.63 per cent saline dilution to abolish the spiculated appearance, but the cell surface appeared coarsely irregular. In addition when the blood was mixed with 0.9 per cent saline solution at a low concentration of cells (less than 10,000 per cubic millimeter) the spiculated effect was absent although the cell surface remained irregular.

Microcinematography confirmed the microscopical findings. The impression was gained that the spiculated forms eventually became balloon forms, but this was not precisely confirmed.

The red-cell survival curves of the propositus were striking (Fig. 5). Autologous cells in the splenectomized propositus disappeared rapidly, with a half-time of two days. Propositus cells transfused into a normal recipient whose spleen was intact disappeared with a half-time of one day whereas normal cells transfused into the propositus survived normally.

In a more detailed study (Fig. 6) red cells from the propositus were transfused into a normal recipient who had never received previous transfusions. Frequent blood samples were analyzed for radioactivity, and monitoring for hepatic, splenic and cardiac radioactivity was performed. In this study the red cells disappeared very rapidly with initial splenic sequestration. The cells then seemed to cease accumulating in the spleen and were finally rapidly sequestered and destroyed in the liver. The Cr^{51} erythrocyte half-time was eight hours.

Studies of the survival of the red cells of the mother (Mrs. P.) who is doubly heterozygous for G6PD and PK carrier states, of an unrelated Caucasian female with PK trait (Mrs. V.) and of another Caucasian female with G6PD trait (Mrs. H.) are summarized in Figure 7. In each case the cells were transfused into a normal recipient, and survival of the radioactivity followed for seventy-one days, or until 10 per cent or less remained in the blood. It should be noted that the doubly heterozygous PK-deficient plus G6PD-deficient cells and the singly heterozygous G6PD-deficient cells had a short survival.* The heterozygous PK-deficient cells had a normal life-span.

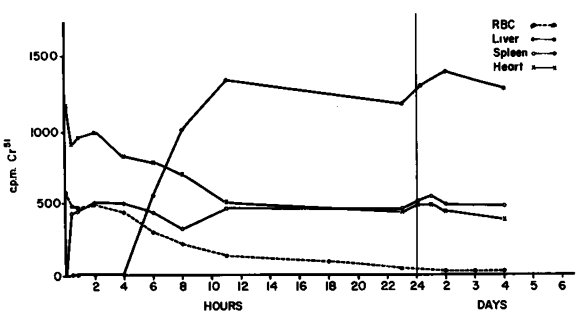

FIGURE 6. *Survival and Organ Sequestration of M.P. Erythrocytes in a Normal Subject.*
The Cr^{51} radioactivities of the liver and spleen are corrected for blood flow.

DISCUSSION

In 1961 Valentine, Tanaka and Miwa[12] first demonstrated that a deficiency of the erythrocyte glycolytic enzyme pyruvate kinase was associated with one form of congenital nonspherocytic hemolytic anemia. Since the original report 17 cases of congenital nonspherocytic hemolytic anemia with a deficiency of pyruvate kinase have been reported in the English literature.[5,6,13,14]

The disease is inherited as an autosomal reces-

*There was apparent immune destruction of the G6PD-trait cells in the last week of the study although extensive blood grouping found none of the usual offending minor blood groups to be different in the donor and recipient. No antibody was found in repeated Coombs tests. The short survival was confirmed by assessment of an autologous Cr^{51} survival curve in the G6PD-trait subject.

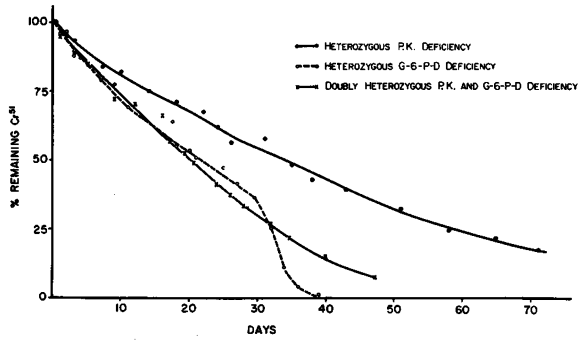

FIGURE 7. *Cr^{51} Survival of Homotransfused Enzyme-Deficient Erythrocytes.*

Heterozygous PK-deficient cells survive normally whereas singly heterozygous G6PD-deficient and doubly heterozygous G6PD-deficient and PK-deficient cells have short intervals.

sive and demonstrates great variation in its clinical severity. Jaundice and anemia are often present in infancy, and hyperbilirubinemia in the newborn period may require exchange transfusion. Splenomegaly has been present in all patients. The anemia has often been profound, and many of the patients have required multiple transfusions. In contrast with the results observed in hereditary spherocytosis splenectomy in pyruvate kinase deficiency has not completely corrected the anemia or the reticulocytosis although it has markedly reduced transfusion requirements in several patients.

The autohemolysis test has been positive in 16 of 17 patients. It has not been corrected by the addition of glucose but has been corrected by the addition of ATP. Maximum reticulocyte counts have ranged from 5.2 to 45 per cent.

The disease has been characterized morphologically by the presence of normochromic macrocytes. The macrocytosis has been attributed to the presence of increased numbers of reticulocytes. Small numbers of spherocytes,[5] an occasional "tailed poikilocyte,"[5] elongated oval forms[13] or irregularly contracted cells[5,6,14] have also been observed. De Gruchy et al.[2] also reported some irregularly contracted cells in their patients with congenital nonspherocytic hemolytic anemias. Target cells, Howell–Jolly bodies, Pappenheimer bodies and siderocytes have been present in the patients who have had splenectomies.

Our patient shares many of the clinical features observed in other patients with pyruvate kinase deficiency. A severe hemolytic process was present at birth, and hyperbilirubinemia required treatment with exchange transfusion. Kernicterus may have occurred because the patient now demonstrates athetosis and ataxia. The severe hemolytic process required multiple transfusions during the early months of life. Splenomegaly was noted at five months of age, and splenectomy, performed at nine months of age, dramatically reduced the need for further transfusions although a marked hemolytic process continues.

The patient differs from the others with pyruvate kinase deficiency who have been described in the severity of the hemolytic process. In addition, the failure of ATP to correct the autohemolysis test and the presence of an almost complete population of morphologically bizarre erythrocytes make him unique among patients with pyruvate kinase deficiency. His maximum reticulocyte count of 94 per cent is approximately twice as high as any other noted in the literature and is far in excess of any that we have ever observed. The survival of his red cells in a nonsplenectomized person is markedly curtailed. His calculated effective hemoglobin production somewhat exceeds previous maximum estimates.[15]*

A morphological abnormality that is somewhat similar to that of patient M.P. has been observed in the syndrome of acanthocytosis.[3,16-19] The erythrocytes in this condition have multiple, irregularly spaced, pseudopod-like projections which give the cells a crenated appearance. Singer et al.[16] named these cells acanthocytes from the Greek word acanthos for thorn or sharp point. Rouleau formation does not occur in acanthocytosis, and the red-cell sedimentation rate is therefore low. These red cells have a decreased osmotic fragility and a shortened survival when injected into a normal recipient. A mild hemolytic anemia appears to be an inconstant feature of the disease. In the patient described above serum beta lipoprotein was not absent, nor did he have low serum cholesterol, steatorrhea, jejunal mucosal abnormalities, or retinitis pigmentosa, all said to be characteristics of the syndrome of acanthocytosis. It is believed that the slight ataxia may have resulted from mild kernicterus during the newborn period.

Preliminary evidence[20] suggests that the morphologic abnormalities observed in this patient are the result of impaired ATP stability and abnormal transport of potassium across the red-cell membrane. This abnormality is accompanied by a block in the glycolytic pathway. Previous work[5] demonstrated a decrease in red-cell ATP that was more rapid than normal during sterile incubation of erythrocytes in patients with pyruvate kinase deficiency. The extent to which the red cells assume the morphologic abnormality in dry smears may well be a function of the severity of the lesion of the red-cell pump and the ATP deficiency. The spiculated cells are also apt to be observed in smears in greater profusion in splenectomized patients with this disorder. Re-examination of the blood in 1 previously reported case (Case 2 of Oski and Diamond[5]) and that of another patient with PK deficiency reveals large numbers of the crenated forms in wet preparations. The "balloon forms" are not as evident, however, and the

*On the basis of a blood volume of 70 ml. per kilogram of body weight a hemoglobin concentration of 6.6 gm. per 100 ml. and a mean cell life of four and three-tenths days, the hemoglobin production is 12.6 gm. per day; the expected hemoglobin production would be approximately 1 gm. per day.

rate of hemolysis not as severe. Children splenectomized for other causes also have a few such cells in peripheral smears.

Of equal interest in this patient were the results of family enzyme investigations. The family study again supports the conclusions originally drawn by Valentine et al.[6] and confirmed by others[5,13] that the defect in pyruvate kinase deficiency is inherited as an autosomal recessive, the patient inheriting the trait from both parents. The heterozygous state is not associated with hematologic disease whereas the homozygous state is associated with a hemolytic anemia of variable severity. The carrier state, as indicated by intermediate values for enzyme activity, was demonstrated in both the patient's parents. The trait appeared to be inherited on the paternal side of the family from the paternal grandmother, a woman of Scotch ancestry. It is assumed that the pyruvate kinase carrier state was carried on the maternal side of the patient's family in the now deceased maternal grandfather, a man of French-English ancestry, in view of the fact that the maternal grandmother was normal but had 3 children who were heterozygotes. There appeared to be no relation between haptoglobin type or blood groups and the pyruvate kinase carrier state.

A heterozygous state for the G6PD deficiency in addition to a heterozygous state for the PK deficiency was detected in the mother and maternal aunt of the propositus. These two women appeared to have inherited their G6PD-deficiency trait from their mother, a woman of French-German ancestry.

The mother of the propositus was found to have a mild hemolytic process as reflected by her slight reticulocytosis, abnormal autohemolysis test and a shortened survival of Cr^{51}-labeled red-cells. Whether these abnormalities reflected the interaction of two nonallelic genes, the gene for G6PD deficiency being carried on the X chromosome and the gene for PK deficiency being carried on an autosomal chromosome, is not certain. Interaction is possibly suggested by the fact that the female with G6PD carrier state had a short red-cell survival with a normal autohemolysis test and that the female with PK carrier state had a normal autohemolysis test and a normal red-cell survival whereas both autohemolysis and survival were abnormal in the mother of the propositus.

Heterozygotes for the PK deficiency have previously been found to be hematologically normal. Slight elevations of in vitro autohemolysis tests and minor abnormalities of erythrocyte ATP stability have occasionally been detected in such persons.[5]

Little information is available on red-cell lifespan in Caucasian heterozygotes for G6PD deficiency. Newton and Bass[21] reported that the mother of their Caucasian patient with a congenital hemolytic anemia due to G6PD deficiency had a normal red-cell survival. It is apparent from the work of Davidson, Nitowsky and Childs[22] that G6PD heterozygous females may differ markedly in the percentage of their cells that may be G6PD deficient. Since heterozygotes maintain a mosaic of normal and of G6PD deficient erythrocytes a shortening of the red-cell survival could be expected to occur in some women with a high percentage of deficient cells. The deficient cells might have disappeared rapidly, and the normal cells more slowly. This should result in a biphasic disappearance of Cr^{51}-labeled cells. We did not observe such a pattern either in the heterozygous G6PD-deficient female or in the doubly heterozygous female.

We have no evidence that the child is a carrier of G6PD deficiency and that this combined deficiency state might be responsible for his severe hemolytic process and bizarre red-cell morphology. Caucasians with erythrocyte G6PD deficiency generally demonstrate this deficiency in their leukocytes as well — patient M.P. did not.

In conclusion it appears that the homozygous deficiency state for erythrocyte pyruvate kinase may be associated with hemolytic anemias of variable severity. Morphologic abnormalities may be observed in this form of congenital nonspherocytic hemolytic anemia, and this morphologic abnormality probably reflects the disturbance in intracellular metabolism.

The combination of the heterozygous state for both G6PD and PK deficiency has resulted in a mild hemolytic anemia and abnormal autohemolysis test. This is not the result of the PK trait alone, but the possibility that it is due to the G6PD trait alone cannot be excluded.

Summary

The history of a boy with a severe hemolytic anemia associated with homozygous pyruvate kinase deficiency is presented. Marked morphologic abnormalities of the erythrocytes were present and were somewhat similar to the abnormalities observed in the syndrome of acanthocytosis, but the patient had normal serum beta lipoproteins.

The patient's mother was found to be a carrier of both the pyruvate kinase trait and the glucose-6-phosphate-dehydrogenase-deficiency trait. She had a mild reticulocytosis and a shortened red-cell survival.

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POLYMYXIN B AND COLISTIN*

A Critical Comparison

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GRAM-negative bacilli, in particular pseudomonas species, are of increasing concern to the clinician as infectious and therapeutic problems.[1,2] Accordingly, polymyxin B has grown to singular importance as its utility to the treatment of systemic infections caused by pseudomonas species has been appreciated and exploited.

In 1961 colistin was introduced as an agent that supposedly retained the antibacterial merit of polymyxin B in a polypeptide compound of lesser inherent nephrotoxic liability. From the outset, the striking pharmacologic and chemical similarities of colistin to the entire polymyxin group of antibiotics, including polymyxin B, were recognized.[3-6] However, critical comparison of these agents, taking into account the chemical modifications available for therapy, has not been carried out. In particular, we have found no reports dealing with the methanesulfonate derivative of polymyxin B.

This report presents the results of side-by-side comparisons of equimolar quantities of the sulfate and methanesulfonate derivatives of polymyxin B and colistin for the following characteristics; in vitro antibacterial activity against pseudomonas species isolated from human beings and acute lethal toxicity in Swiss albino mice (by intramuscular as well as by intravenous and intraperitoneal administration).

MATERIALS AND METHODS

In Vitro Susceptibility Testing

Fifty strains of pseudomonas species, isolated from clinical specimens submitted to the Clinical Microbiology Laboratory of the Salt Lake County General Hospital, were studied. Each isolate was stored until tested at −22°C. after overnight incubation in semisolid agar (commercial tryptic digest of casein-papaic digest of soybean-broth base, without glucose, combined with 0.4 per cent agar) in screw-capped culture tubes 13 by 100 mm.

Solutions of polymyxin B sulfate, colistin sulfate, polymyxin B methanesulfonate and colistin methanesulfonate§ were prepared in synthetic protein-free broth culture medium.[7] Working solutions provided concentrations (as the free base equivalent) of each agent of 1.0, 0.10, 0.05 and 0.01 micromole per milliliter. One-tenth-milliliter aliquots of these working dilutions were stored until use at −22°C. in plastic, snap-top tubes, 12 by 75 mm.

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§Polymyxin B sulfate was obtained commercially as Aerosporin brand, Lot 108W, from Burroughs Wellcome and Company, Tuckahoe, New York; polymyxin B methanesulfonate, Lot 18-057-02-EPD, was supplied by Dr. Robert R. Riggio, Charles Pfizer and Company, Incorporated, New York City; colistin sulfate, Lot W1978, and colistin methanesulfonate (especially compounded without dibucaine), Lot 0161-25, were obtained from the Warner-Lambert Research Institute, Morris Plains, New Jersey (research affiliate of Warner-Chilcott Laboratories).