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cation permeability
cell lysis
erythrocyte
glucose
hemolysis
ion transport
osmosis
potassium
sodium

A New Familial Disorder with Abnormal Erythrocyte Morphology and Increased Permeability of the Erythrocytes to Sodium and Potassium

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Extract

A newborn infant of Philippine parents was found to have a morphological abnormality of his erythrocytes consisting of an elliptical shape of the cells and one or more transverse slitlike areas of decreased density. These changes were also present in erythrocytes of the patient's father, a half-sister of the father, and four of the patient's six siblings. None of the affected family members had anemia or evidence of abnormal hemolysis, and erythrocyte survival by the radiochromium method was normal in three of the individuals studied. Erythrocytes from the affected family members had an increased degree of autohemolysis after incubation for 48 hr, but this was prevented almost entirely by addition of glucose. Glucose consumption *in vitro* by erythrocytes of the propositus occurred at a rate approximately 60% greater than that of normal controls. The intracellular sodium concentration of the erythrocytes was not different from that of erythrocytes from normal individuals, but a moderate decrease in intracellular potassium was found. When washed cells were incubated in a glucose-free medium, sodium gain and potassium loss were significantly greater than from cells of normal controls. When compared with normal values, efflux of radiosodium was increased during incubation of cells in glucose-containing medium. Erythrocytes from the affected individuals had decreased osmotic fragility, and osmometric measurements indicated a lesser degree of cell swelling in hypotonic solutions than occurred with cells from normal controls.

Speculation

The findings in this family demonstrate that increased erythrocyte cation permeability need not be associated with an accelerated rate of hemolysis. In these individuals the absence of hemolytic disease may reflect a capacity of the erythrocytes to compensate for increased cation permeability by an elevated rate of glycolysis-linked ion transport. This compensatory process would appear to be virtually unaffected by interaction of the erythrocytes of these individuals with the spleen.

Introduction

In recent years it has been established that a variety of hereditary hemolytic disorders are associated with altered permeability of the erythrocytes to sodium or potassium. Erythrocytes from individuals with β-thalassemia [18] and with pyruvate kinase deficiency [17] have been found to have excessive potassium permeability, presumably as a secondary manifestation of the

underlying biochemical defects in the cells. In hereditary spherocytosis [11] and in one form of elliptocytosis [21] sodium transport by the erythrocytes has been shown to be increased, apparently reflecting a primary abnormality of the red cell membrane [10, 12]. Recently it was shown that in at least two forms of congenital stomatocytosis increased permeability of erythrocytes to monovalent cations was present together with gross changes in erythrocyte electrolyte concentrations [20, 26].

It is apparent from studies of these various disorders that the degree of abnormality of erythrocyte cation transport may not necessarily correlate with the severity of the hemolytic process. In each of these disorders, however, some degree of shortening of erythrocyte survival occurs, and susceptibility of the erythrocytes to osmotic lysis *in vitro* is almost invariably increased.

The present report describes a disorder in which the erythrocytes of affected individuals are morphologically abnormal and have increased permeability to sodium and potassium. This erythrocyte abnormality was found in several members of a Philippine family and appeared not to be associated with an accelerated rate of hemolysis. The osmotic fragility of the erythrocytes was decreased as compared with normal, which serves to differentiate this abnormality from similar erythrocyte disorders.

Case Report

The propositus, *MB*, was born in June, 1967, at the University of Illinois Hospital. Placenta previa was diagnosed antepartum and the delivery was by cesarean section. At 8 hr of age the red cell packed volume was 39%, and over the succeeding 2 weeks it decreased to 21%. A concomitant increase in the concentration of bilirubin in the serum occurred reaching a maximum of 20 mg/100 ml of indirect bilirubin on the 7th day of life, decreasing thereafter. The mother's blood was type O, Rh-positive, and an immune anti-B isoagglutinin titer of 1:256 was obtained. The infant was type B, Rh-positive, but the direct antiglobulin test was negative, and isoimmune hemolytic disease could not be documented. In week 1 of life the infant took feedings poorly and was noted to have enlargement of the liver and spleen. Because of the findings of pyuria and Klebsiella bacteriuria, the infant was given a transfusion and antimicrobial therapy. Urographic studies were normal and the urine remained sterile thereafter.

The mother of this child had had seven previous pregnancies and six normal deliveries. None of the children had neonatal icterus, anemia, or hepatosplenomegaly. Both of the parents were descended from Philippine families; neither was known to have anemia or jaundice, and in neither could the liver or spleen be palpated.

Methods

Hematological measurements were performed by standard methods. Autohemolysis and osmotic fragility tests were carried out with sterile defibrinated blood as described by Dacie [5]. Informed consent was obtained in accordance with the Declaration of Helsinki.

For erythrocyte incubation studies blood was collected in heparin and centrifuged at room temperature; the plasma and buffy coat were removed by aspiration and the cells were washed twice with NKP solution (sodium, 0.150 M; potassium, 0.005 M; phosphate, 0.030 M; with the remaining anion as chloride, pH 7.4). The cells were suspended in a quantity of incubation medium to achieve a final packed cell volume of approximately 35%. The cell suspensions were incubated in Erlenmeyer flasks open to room air in a metabolic shaker maintained at 37°. Glucose, when added, was at a final concentration of 1 mg/ml. Ouabain [28] was diluted to a final concentration of 3.4×10^{-5} M.

Erythrocyte glucose consumption was measured at hourly intervals over a 4-hr incubation period. The samples were assayed by a semiautomated microprocedure employing a glucose-oxidase method [1].

For measurements of intracellular sodium and potassium, the cation concentrations were determined with a flame photometer with lithium internal standardization [29]. Intracellular potassium concentrations were assayed indirectly by measurement of the potassium concentrations of the total cell suspensions and of the supernatant fractions after sedimentation of the cells by centrifugation. The intracellular potassium concentrations were calculated from these values and corrected for the packed cell volumes of the cell suspensions. For determination of intracellular sodium concentrations the cells were added to a large volume of magnesium chloride, 0.1 M, at 4°, and centrifuged briefly. The supernatants were removed by aspiration, and the cells were washed twice with the cold magnesium chloride solution by repeated suspension and centrifugation. The washed cells were resuspended in the magnesium solution, and the packed cell volumes and sodium concentrations were measured.

Efflux of ^{22}Na from erythrocytes was studied essen-

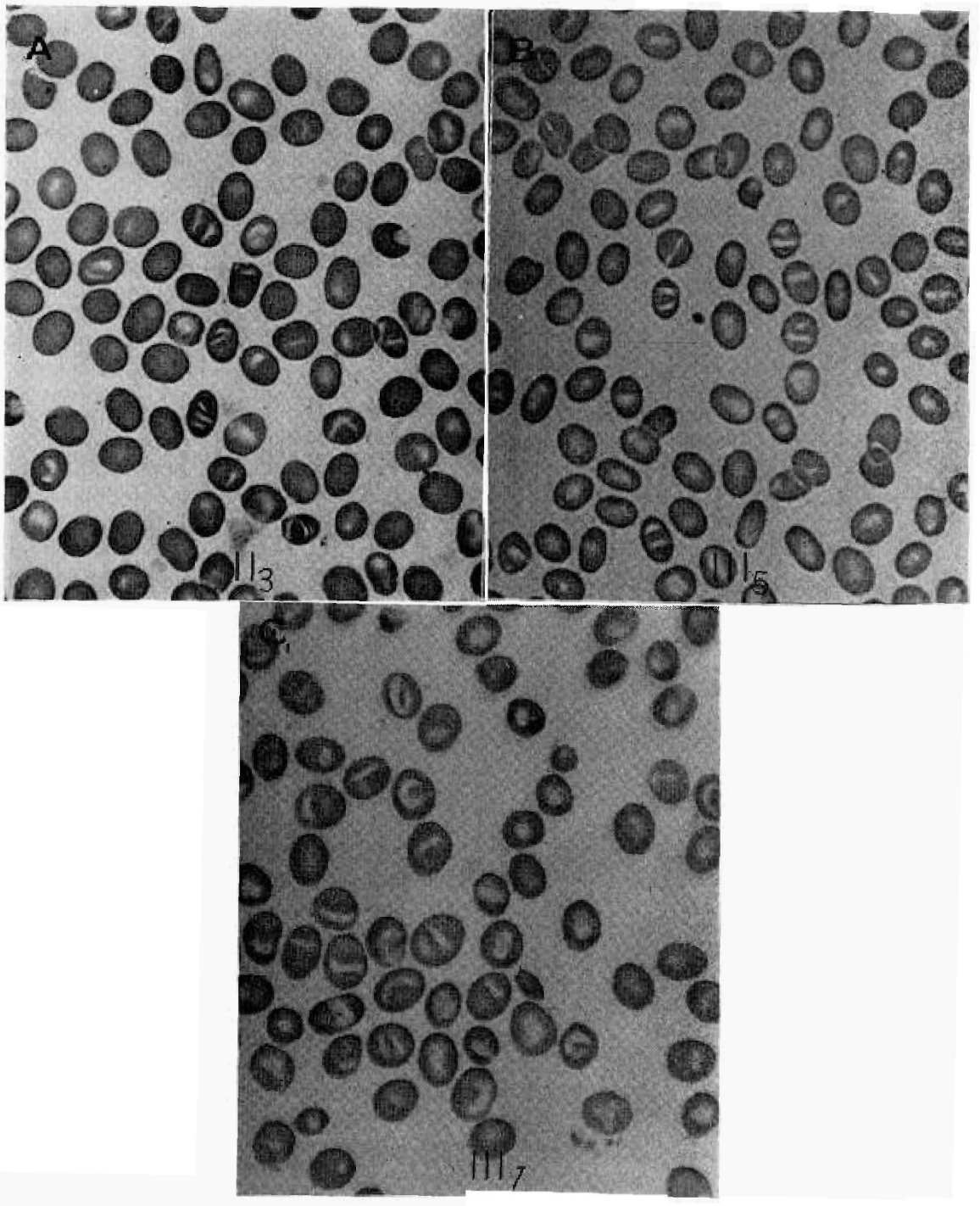


Fig. 1. Peripheral blood smears of three affected family members.

tially as described by Jacob and Jandl [11]. Whole blood (15–20 ml) was incubated at 37° for 1–2 hr with 10 μCi of sodium chloride-^{22}Na [30]. The cells were washed twice with NKP solution at room temperature and resuspended to a packed cell volume of approximately 35%. Samples were removed for measurement of radioactivity and of the packed cell volumes, and the cell suspensions were reincubated at 37°. Further samples were removed at intervals during the incubation period, and intracellular radioactivity was calcu-

lated for each time point from the radioactivity measured in aliquots of the total cell suspension and in the cell-free supernatant solution.

For osmometry measurements fresh whole blood samples were diluted 1:10,000 in a buffered isotonic solution [31] and in varying dilutions of this buffer made with distilled water. Mean corpuscular volumes of the erythrocytes were determined directly by the use of a particle counter [32] with a mean corpuscular volume calculator.

Erythrocyte chromium survival studies were performed after reinjection of the patient's own labeled cells. Blood samples were taken 45 min after the injection and daily thereafter for 9 days. The radioactivity of the samples was measured with a well-type counter, and decay slopes were calculated from the radioactivity values by the method of least squares.

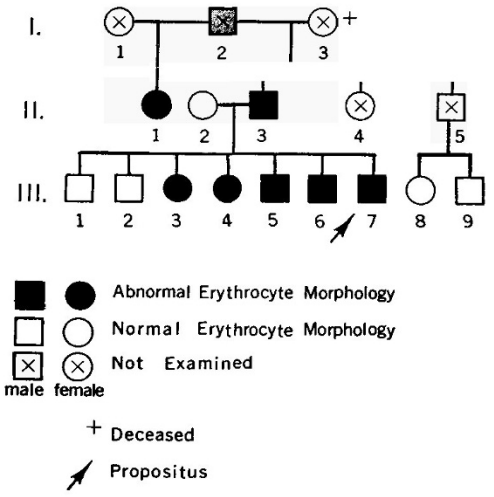


Fig. 2. Family pedigree.

Studies of the propositus and his parents were carried out in Chicago, and those of the other family members in Quezon City. At the time that the electrolyte and glycolysis determinations were made, only the propositus was available for study. All of these determinations were performed when the child was beyond 1 year of age.

Results

Erthrocyte Morphology and Hematologic Studies

Stained blood smears from the propositus and from several family members showed characteristic morphological abnormalities of the erythrocytes (Fig. 1). Many of the cells were elliptical in shape, and some had slitlike areas of central pallor similar to cells which were described in stomatocytosis [14]. The most distinctive feature was the presence of elliptical cells containing two or three transverse slits of decreased density, suggestive of the presence of multiple infoldings of the red cell membrane.

These morphological abnormalities of the erythrocytes were found in four of the patient's siblings as well as in his father (Fig. 2). The mother's blood smear appeared normal. A half-sister of the father was also found to have the abnormal erythroycte morphology, and it was presumed that the grandfather (pedigree no. I_2) also has this disorder, although it was not possible to examine his blood smear. From these observations it appears that this disorder is inherited as an autosomal dominant trait.

Hematological values from the parents and siblings of the propositus are presented in Table I. None of the individuals with abnormal erythrocyte morphology

Table I. Hematological data

Subjects	Age, yr	Hemoglobin, g/100 ml	Packed cell volume, %	Erythrocyte count, $\times 10^6/mm^3$	Reticulocytes, %	Erythrocyte ^{51}Cr $t_{1/2}$, days	Autohemolysis	
							Without glucose	Glucose added
							%/48 hr	
Abnormal erythrocyte morphology								
II-3	42	15.0	42		0.2	25.1	14.9	0.94
III-3	11	12.0	44	4.51	0.5	34.5	8.3	0.52
III-4	10	12.2	42	4.21	0.5		9.7	<0.20
III-5	9	11.4	40	4.09	0.2		13.3	0.76
III-6	7	11.4	39	3.95	0.7	24.5	4.6	<0.20
III-7	1	12.2	34	4.00	0.9		15.8	1.18
Normal erythrocyte morphology								
II-2	40	12.5	39	4.01	1.1		1.3	0.43
III-1	14	11.2	39	4.03	0.7		3.7	0.43
III-2	13	11.9	40	4.12	0.3		4.3	0.40
Normal values							0.4–4.5	0.3–0.7

showed evidence of anemia or hemolytic disease and no differences were apparent between affected and unaffected family members in regard to hemoglobin value, packed cell volume, erythrocyte count, and percentage of reticulocytes. Erythrocyte survival studies using radioactive chromium were performed in three of the affected individuals and results were essentially normal, supporting the impression that an accelerated rate of hemolysis was not present. In autohemolysis studies of the family members, the erythrocytes underwent an abnormal degree of hemolysis after 48 hr in those individuals having abnormal cell morphology (Table I). Addition of glucose to the blood of these individuals had a protective effect and resulted in nearly normal autohemolysis values.

Glucose Consumption

Consumption of glucose by erythrocytes of the propositus occurred at a rate approximately 60% greater than that of cells from normal controls (Fig. 3). In the presence of ouabain, glucose consumption was approximately 35% greater than in the corresponding control, suggesting that a major portion of the enhanced glycolysis in these cells may be linked to the ouabain-sensitive cation transport adenosine triphosphatase mechanism [23]. Erythrocyte chemical studies including Heinz body preparations, assay of glucose 6-phosphate dehydrogenase activity, and glutathione stability tests were normal (Table II), and no evidence was obtained to indicate the presence of an abnormal hemoglobin.

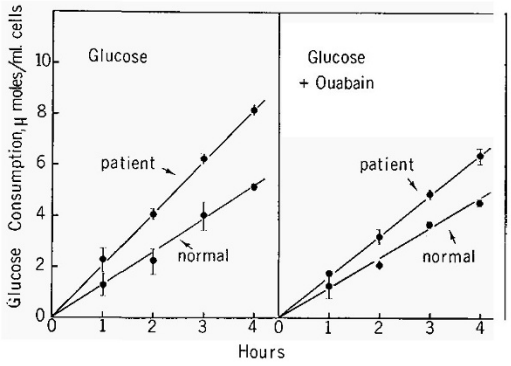


Fig. 3. Glucose consumption by erythrocytes. Each value represents the mean and range for three normal controls and for two determinations with cells from the propositus.

Table II. Chemical studies of erythrocytes of *patient MB*

	Patient	Normal value or control	Ref
Heinz body preparation	Negative	Negative	[3]
Glucose 6-phosphate dehydrogenase, units/g hemoglobin	8.6	5-10	[27]
Glutathione stability test (acetyl phenylhydrazine incubation), mg/100 ml	40.6	46.9, 54.3	[4]
Fetal hemoglobin, %	1.1	<2	[25]
Hemoglobin electrophoresis	AA	—	[9, 13]
Hemoglobin A_2, %	2.75	1.88 ± 1.54	[19]
Hemoglobin heat stability test	Negative	Negative	[6]
Oxygen dissociation P_{50}, mm O_2	26.8	26.0	[8]

Table III. Incubation studies of cells in glucose-free medium[1]

	Before incubation	Change after incubation	Change after incubation with ouabain
Sodium			
Controls	6.6 ± 0.3	+1.5 $P < 0.001$	+3.7 $P < 0.001$
Patient	6.8 ± 0.3	+6.0	+9.1
Potassium			
Controls	101.5 ± 1.7	−2.8 $P < 0.02$	−4.3 $P < 0.01$
Patient	94.6 ± 3.0	−7.1	−9.3

[1] Cation concentrations are expressed as intracellular concentrations in milliequivalents per liter of cells. The values represent the means of four to six determinations and the standard errors. Cell incubations were carried out for 210 min.

Erythrocyte Cation Studies

The finding that the addition of glucose had a protective effect on the autohemolysis of cells from the affected individuals, together with the observation of elevated rates of glycolysis which were partially sensitive to ouabain, suggested that this erythrocyte disorder might be associated with an elevated rate of cation transport linked to the increased glycolysis, as has been demonstrated with the erythrocytes in hereditary spherocytosis [11].

Erythrocytes from the propositus had an intracellular sodium concentration which was not different from that of normal controls. The intracellular potassium concentration, however, was moderately decreased (Table III). Incubation of the cells in the absence of glucose resulted in an increased net gain of intracellular sodium and loss of potassium, as compared with similarly incubated control cells. The abnormal permeability of the cells to sodium and potassium was further enhanced by the addition of ouabain (Table III).

Efflux of ^{22}Na was measured in cells which were incubated in glucose-containing medium. Under the incubation conditions employed, erythrocytes from normal individuals demonstrated a sodium 50% turnover time of approximately 135 min; in erythrocytes from the patient, the rate of sodium efflux was significantly greater, with 50% of the intracellular sodium being released to the medium by 85 min (Fig. 4).

Osmotic Fragility and Osmometry Studies

Osmotic fragility studies of fresh defibrinated blood demonstrated that erythrocytes of the affected individ-

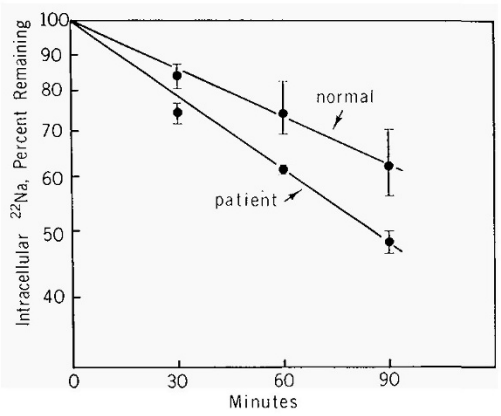
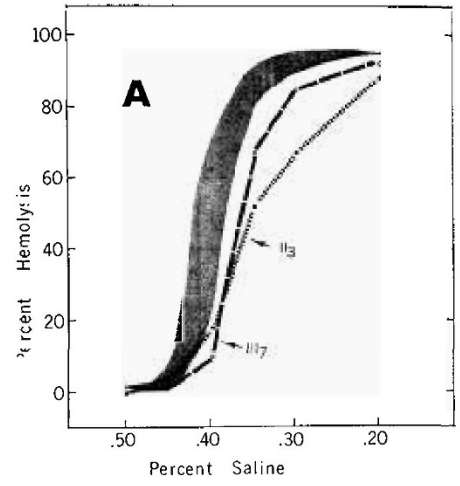


Fig. 4. Efflux of ^{22}Na from erythrocytes. Each value represents the mean and range for three normal controls and for two determinations with cells from the propositus.



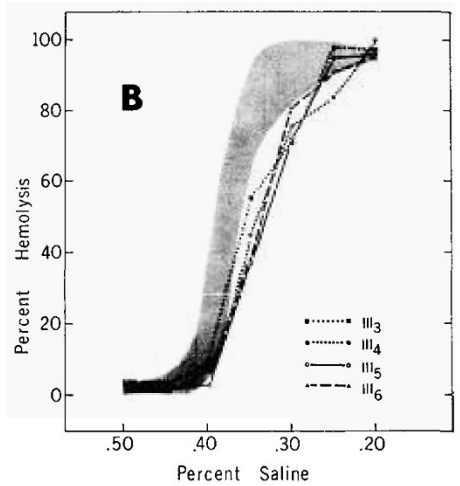


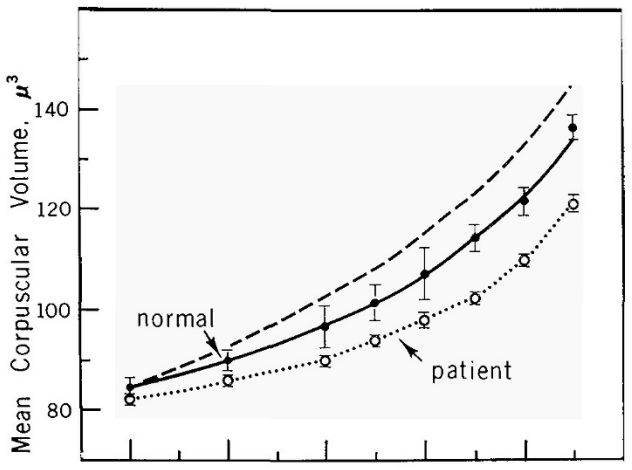
Fig. 5. Osmotic fragility of fresh defibrinated blood. The shaded areas represent the mean ± 2 SD from four to six normal controls. *A:* Patients studied in Chicago. *B:* Patients studied in Quezon City.

uals were more resistant than normal cells to lysis in hypotonic media (Fig. 5). When sterile blood was incubated at 37° for 24 hr, the osmotic fragility was no longer found to differ from that of normal cells similarly incubated. Incubation of blood for 24 hr at 5°, which was observed by Miller and co-workers to increase markedly the osmotic fragility of erythrocytes in one form of stomatocytosis [15], produced no significant change in the osmotic fragility of the erythrocytes in two of the affected individuals.

Osmometric measurements were performed with fresh whole blood. Erythrocyte mean corpuscular volumes of cells from the propositus and from normal individuals did not differ when the cells were suspended in isotonic medium. With increasingly hypotonic media, cells from the patient showed a progressive decrease in degree of swelling as compared with the normal control cells (Fig. 6).

Discussion

The abnormal morphological characteristics of the erythrocytes in this disorder include an elliptical shape of the cells and the presence of transverse slitlike areas of decreased density. The findings in the affected family members indicate that this disorder is clearly distinct from any of the reported forms of congenital stomatocytosis [14, 15, 26, 17]. Despite a superficial resemblance of this abnormality to the nonhemolytic form of familial elliptocytosis, a number of factors serve to differentiate these disorders: in nonhemolytic elliptocytosis slitlike areas of central pallor in the



erythrocytes are not present, autohemolysis is usually normal, and osmotic fragility is normal both with fresh and with incubated blood [7]. Iron deficiency and thalassemia could be excluded by usual criteria.

The morphological abnormality of the erythrocytes which is present in this disorder is accompanied by an increased permeability of the erythrocytes to both sodium and potassium. This permeability defect would appear to account for the increased extent of erythrocyte autohemolysis *in vitro* which was observed with cells from the affected individuals. Although direct comparisons were not made between cells from these patients and from patients with hereditary spherocytosis, from reported studies of autohemolysis and electrolyte permeability properties of erythrocytes in hereditary spherocytosis [2, 11, 24] these abnormalities appear to be quantitatively similar in the two disorders. A further point of similarity is the apparent capacity for essentially complete compensation *in vitro* for the cation permeability defect. It has been demonstrated that erythrocytes in hereditary spherocytosis are able to compensate for a defect in sodium permeability by augmented sodium transport, which is accompanied by an increased rate of glycolysis [11] and adenosine triphosphate production [16]. In the disorder presented in this report the increased rate of glycolysis by the erythrocytes and the accelerated efflux of intracellular sodium under glycolytic conditions probably reflect a similar compensatory process.

In patients with hereditary spherocytosis the spleen plays a critical role in the accelerated destruction of red cells, as evidenced by the prolongation of erythrocyte survival following splenectomy; this occurs despite the absence of any measurable change in the membrane permeability defect of the erythrocytes. It has been proposed that the mechanism by which the spleen promotes hemolysis in hereditary spherocytosis is related to erythrocyte stasis in the splenic sinusoids, which leads to depletion of an adequate supply of exogenous glucose. With ensuing loss of the capacity of the cell to compensate for the rapid influx of sodium, lysis of the cell follows [10, 12]. In contrast to the increased rate of hemolysis which occurs in hereditary spherocytosis, however, in the present disorder erythrocyte survival appears to be normal. In view of the increased cation permeability of the erythrocytes of these individuals, the finding of normal erythrocyte survival may imply that compensatory cation transport mechanisms are adequate to prevent intracellular electrolyte imbalance, and that interaction of the erythrocytes with the spleen is not sufficient to compromise these compensatory processes. Whether splenic sequestration of the erythrocytes in hereditary spherocytosis is abnormally increased specifically because of the spherical erythrocyte shape has not yet been settled, but this may account for the deleterious interaction between the erythrocytes and the spleen in this disorder.

Further notable features of the erythrocytes from the individuals described in this report are the resistance to osmotic swelling in hypotonic solutions and the decreased osmotic fragility. It is possible that these properties of the erythrocytes may contribute to the stability of the cells and may serve to protect against premature cell lysis if adequate exogenous glucose becomes temporarily unavailable.

Summary

Several members of a Phillipine family were found to have an erythrocyte abnormality characterized by morphological changes and by increased permeability of the cells to sodium and potassium. The erythrocytes apparently compensated for the cation permeability defect for sodium by increased sodium transport, which was linked to an elevated rate of glucose consumption. In contrast to other disorders in which erythrocyte cation permeability has been shown to be increased, in the present disorder erythrocyte survival studies were normal. Osmotic fragility and swelling of the erythrocytes in hypotonic medium were decreased in cells from affected family members and may reflect a protective mechanism which helps to prevent premature cell lysis.

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