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Unique Phenotypic Expression of Glucosephosphate Isomerase Deficiency

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INTRODUCTION

Defective glucosephosphate isomerase (GPI; D-glucose-6-phosphate ketol isomerase, E.C.5.3.1.9.) ranks high among the commonly encountered erythrocyte enzyme abnormalities associated with hereditary hemolytic anemia [1]. In every instance where appropriate studies have been made, the enzymatic deficiency has been shared to varying degrees by leukocytes [2–10] as well as by platelets [5, 8] and cultured fibroblasts [5]. Markedly decreased GPI activities in plasma [2, 10] presumably reflect deficiency in tissues not amenable to direct assays.

This inherited multisystem disorder possesses broad genetic polymorphism best demonstrated by the disparate electrophoretic patterns of residual erythrocyte GPI [2, 3, 7, 9, 11–13]. Normal zymograms have been observed with hemolysates from only two deficient subjects [4, 10]. Several variant patterns have also occurred in hematologically normal individuals surveyed at random [14, 15]. At least one such variant exhibited definite phyletic tendencies with a greater than 1% incidence among certain Asiatic Indians.

This report presents studies of a Mexican family afflicted with GPI deficiency of an apparently unique phenotype. Not only did residual GPI in hemolysates exhibit entirely normal electrophoretic partitioning, but the deficiency appeared to be confined to the erythrocyte population. Repeated leukocyte assays invariably returned normal activities in each family member regardless of the degree of erythrocyte GPI deficiency. Although other tissues could not be studied directly, plasma GPI was normally active by assay.

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CASE REPORT

The proband was a 14-year-old Mexican girl from Valle Hermoso, Tamaulipas, who had a history of jaundice and pallor dating from the neonatal period. During the last few years, her hemoglobin varied between 9 and 10 g/100 ml. Her chronic hemolytic process was punctuated at irregular intervals by frank crises associated with elevated total and indirect serum bilirubin and reticulocytosis markedly above her usual level of 15%–20%. Such episodes often accompanied upper respiratory tract infections and were unassociated with drug ingestion. Exposure to an aerosol insecticide was suspected to have induced an episode of acute hemolysis on one occasion, but glucose-6-phosphate dehydrogenase (G6PD) deficiency could not be demonstrated in the proband, parents, or siblings.

Family members, including five siblings, had never exhibited signs or symptoms of anemia. The parents of the proband were third cousins.

Physical examinations were normal except for fluctuating icterus. There was no detectable hepatic or splenic enlargement. Although her physical and mental development had appeared satisfactory, she has recently exhibited a slight growth retardation.

Laboratory studies revealed a Dacie type-I erythrocyte autohemolysis pattern with reduction of autohemolysis by addition of glucose. Osmotic fragility of incubated cells repeatedly was normal, as were hemoglobin electrophoresis, heat stability, and alkali denaturation. Tests for sickling were negative. Leukocyte counts ranged between 5,300 and 9,000/mm^3 with normal differential distributions. Peripheral blood smears exhibited slight to moderate anisopoikilocytosis and polychromatophilia, occasional basophilic stippling, and scattered spiculated microspherocytes.

MATERIALS AND METHODS

Heparinized blood specimens were refrigerated and sent by airmail from Mexico to Los Angeles, where studies were initiated within 36–48 hr of blood collection. Suspensions of saline-washed erythrocytes and leukocytes were prepared by differential sedimentation in either polyvinylpyrrolidone or Plasmagel. Measurements of GPI activities were performed on thrice frozen and thawed lysates using a modification [2] of the procedure described by Chapman et al. [16].

The assay system combined 25 μmol glycylglycine buffer (pH 8.1), 5 μmol fructose-6-phosphate (F6P), 1 μmol NADP, 1 U G6PD, and an aliquot of lysate equivalent to 3×10^6 erythrocytes or 4×10^4 leukocytes in a total cuvette volume of 3.0 ml. Rate of F6P isomerization to glucose-6-phosphate (G6P) was monitored by measuring absorbance changes at 340 nm as NADP was converted to NADPH during oxidation of G6P in an indicator system as follows:

$$\text{F6P} \xleftrightarrow{\text{GPI}} \text{G6P} \xrightarrow[\text{NADP NADPH}]{\text{G6PD}} \text{6-phosphogluconate.}$$

Units of GPI activity were defined as micromoles of pyridine nucleotide reduced per minute by 10^{10} cells at 37° C. Corrections were made for contributions to activity by leukocytes contaminating red cell preparations and for reagent-blank activities in systems devoid of lysate.

Starch gel electrophoresis of cellular GPI was performed according to the method of Detter et al. [14], and results were confirmed by Dr. E. R. Giblett, King County Central Blood Bank, Seattle, Washington. Hexosemonophosphate shunt activity in intact red cells was assessed in the laboratory of Drs. K. R. Tanaka and W. D. Davidson utilizing their previously described technique [17].

Assay procedures for other enzymes of glycolysis and of glutathione and nucleotide metabolism have been outlined in a previous report [18]. Methods described by Minakami

et al. [19] were used to measure concentrations of adenine nucleotides and of glycolytic intermediates in red cells, except that 2,3-diphosphoglycerate was assayed according to Krimsky [20]. Cellular concentrations of glutathione were determined as described by Beutler et al. [21].

RESULTS

Assays of glycolytic intermediates in proband peripheral blood revealed increased concentrations of G6P, 2,3-diphosphoglycerate, and lactate, which were, respectively, 4.7, 1.6, and 2.2 times greater than normal mean levels. F6P, fructose-1,6-diphosphate, glyceraldehyde-3-phosphate, and dihydroxyacetone phosphate were barely detectable (< 10 nmol/10^{10} erythrocytes). Concentrations of all other glycolytic intermediates, glutathione, and adenine nucleotides were within normal limits. No significant alterations from normal were noted in parental or sibling cells.

With one exception, activities of all glycolytic enzymes in proband erythrocytes were either normal or elevated commensurate with the degree of reticulocytosis. GPI alone was significantly deficient, ranging over the 4-year study period from 1.7 to 5.1 U and averaging 3.4 U (normal laboratory mean = 13.8 U, SD = 1.8 U). The mean value was only 21% of that derived from 33 cases with comparable reticulocytosis (10%–20%) due to causes other than GPI deficiency.

The GPI activity in both proband and control hemolysates was unaltered by thorough dialysis against water or against 0.05 M glycylglycine buffer, pH 8.0. No dialyzable inhibitors could be demonstrated in proband hemolysates. This was further supported by studies in which aliquots of parental or proband hemolysates were individually mixed with equal parts of normal control hemolysate and incubated at 37° C for 1 hr in the presence of 2-mercaptoethanol (7 mM), EDTA (0.5 mM), and NADP (10 mM). Following incubation, activities in the mixtures were identical to the sums of activities in the appropriate hemolysates incubated alone.

Intermediate degrees of GPI deficiency were repeatedly demonstrable in both parents and in four siblings, all of whom were devoid of clinical symptoms, anemia, or reticulocytosis. As shown in figure 1, the mother and father had mean erythrocyte GPI activities which were, respectively, 34% and 45% of the normal mean. Only one sibling appeared to have entirely normal red cell GPI activity; in four others, this ranged between 27% and 46% of the normal mean.

Despite the prevalence of erythrocyte GPI deficiency, leukocyte values in all family members closely approximated the normal mean (1800 U). Figure 1 also records averages of from four to five separate determinations for proband, mother, and father and at least two determinations on each sibling.

GPI in native plasma was normally active in the proband and her parents when compared to that of control blood shipped and processed simultaneously.

To detect possible qualitative abnormalities of the defective enzyme, measurements were made of pH optima, Michaelis constants (K_m) for F6P, electrophoretic mobility, and thermostability. Hemolysates from the proband and her accompanying normal control were assayed for GPI activity at 0.5 U intervals between pH 6.0 and 9.0. Both showed minimal activity below pH 7.0, sharply increasing to peaks be-

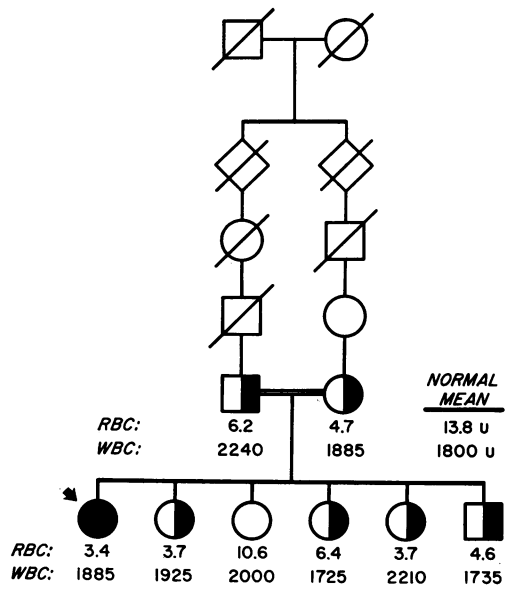

FIG. 1.—Pedigree showing mean GPI activities in erythrocytes (RBC) and leukocytes (WBC) of family members. Each value for erythrocytes is the mean of from three to 10 separate determinations. Values for leukocytes are means of from two to five determinations. Half-closed symbols signify heterozygous GPI deficiency; proband is indicated by an arrow. Members of the first 4 generations were unavailable for study.

tween 8.0 and 8.5 with variably graduated declines at more alkaline levels. Michaelis constants were determined from linear coordinate curves plotting GPI activities at eight different concentrations of F6P between 0.033 and 1.7 mM. Average K_m values for proband, mother, and father (0.07, 0.09 and 0.08 mM, respectively) did not differ significantly from the normal control (0.085 mM). The K_m for GPI in proband leukocytes (0.095 mM F6P) was also similar to control values (0.08 mM).

Starch gel electrophoresis of proband and parental hemolysates demonstrated reduced intensities of GPI bands compared to normal controls, but there were no detectable alterations in relative migration rates of the individual major and minor components.

Enzyme stability was tested at 4° C and at 48° C. Activity measurements were performed daily on proband, parental, and control erythrocytes stored at 4° C in isotonic saline without substrates or other additives. Activity decay slopes throughout a 10-day period were identical in all.

Thermostability of buffered (0.5 mM glycylglycine, pH 8.1) hemolysates at 48° C was evaluated in the presence of 2-mercaptoethanol (7 mM), EDTA (0.5 mM), and NADP (10 mM) according to the method described by Blume et al. [7]. As shown in figure 2, proband GPI exhibited marked lability, falling below 50% of initial activity after an average of only 10 min and reaching virtually negligible levels shortly thereafter. By contrast, normal control GPI remained well above 50%

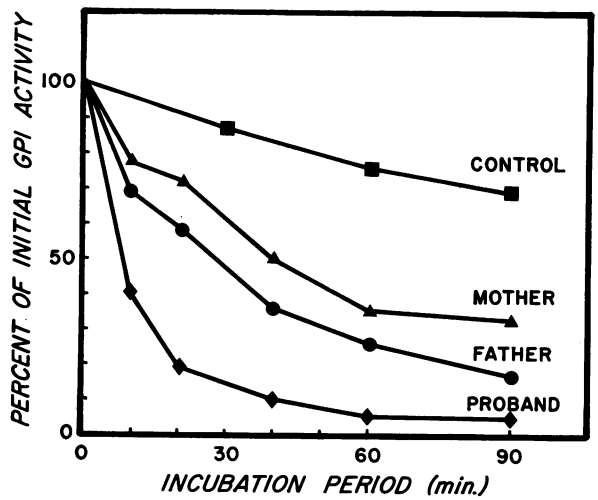

FIG. 2.—Thermostability of erythrocyte GPI in crude hemolysates at 48° C. Each point is the mean of three separate determinations.

of initial activity throughout the 90-min test period. One sister without deficient activity was entirely normal, but both parents exhibited intermediate levels of sensitivity to thermal inactivation.

To determine whether a similar enzyme lability was reflected in vivo by pronounced differences in GPI activities between young and old cells, density-gradient fractionations of erythrocytes were performed using isotonic bovine serum albumin [22]. Adequacy of fractionation was assessed by determination of pyruvate kinase and G6PD activities and reticulocyte counts in the youngest (most buoyant) and the oldest (most dense) cell fractions, each of which comprised 10%–20% of the total red cell population in the sample. No deviations from normal control GPI decay rates were found with either proband or maternal erythrocytes. This contrasts with findings in the comprehensive studies of another GPI deficiency reported by Arnold et al. [23].

Studies with intact erythrocytes demonstrated metabolic alterations which occurred as a consequence of defective hexosephosphate isomerization. Proband cells in Krebs-Ringer bicarbonate buffer responded readily to methylene blue stimulation by increasing pentosephosphate shunt activity to twice the levels of those of parents and accompanying control. These investigations were conducted by Drs. K. R. Tanaka and W. D. Davidson employing their technique [17] of measuring the rates of $^{14}CO_2$ evolution from glucose labeled in the first carbon position. With glucose labeled in the second position, however, $^{14}CO_2$ production is dependent upon conversion of shunt-derived pentosephosphate to F6P, isomerization to G6P, and recycling the latter through the shunt pathway. Under these conditions, proband cells were able to increase $^{14}CO_2$ evolution by only 10%–20% of the levels achieved by control cells following stimulation by 10^{-6} M methylene blue. Parental cells behaved like those of the proband, but with an increased concentration (2.5 ×

10^{-6} M) of methylene blue, parental responses were directly intermediate between those of proband and control. Such impaired capacity to recycle F6P through the pentosephosphate pathway is a logical consequence of defective isomerization to G6P and has been observed with previous patients with GPI deficiency [2, 3, 6].

Intact erythrocytes incubated with 10 mM glucose or mannose in isotonic phosphate produced lactate at rates which further emphasized the metabolic impairment resulting from GPI deficiency (table 1). Despite the high metabolic capacities expected of a young red cell population, proband erythrocytes were able to convert glucose to lactate at only half the rate of normal control cells. Mannose, however, may be phosphorylated readily by hexokinase and subsequently converted to F6P by a separate isomerase [24], thereby bypassing the GPI step. Proband cells, relatively rich in mannosephosphate isomerase, utilized this substrate almost twice as effectively as control cells. Maternal erythrocytes in which GPI activity was about half-normal nonetheless converted glucose to lactate at a rate comparable to control cells.

TABLE 1

RATES OF LACTATE PRODUCTION BY INTACT ERYTHROCYTES IN ISOTONIC PHOSPHATE BUFFER* AT 37° C

SUBSTRATE	LACTATE FORMATION (nmol/min/10^{10} Erythrocytes)		
	Control	Proband	Mother
Glucose	78	42	63
Mannose	49	79	43

* pH 7.4.

DISCUSSION

In many respects, the proband in this kindred was indistinguishable from other reported cases of hereditary deficiency of erythrocyte GPI. Her clinical course was characterized by lifelong anemia with the nonspecific hematologic sequelae of chronic hemolysis and by acute exacerbations under the stress of intercurrent infections. Quantitatively, erythrocyte GPI assayed at 20% of normal mean in vitro activity but demonstrated normal kinetics and pH activity curve and, as in other cases, was abnormally sensitive to thermal inactivation. Intracellular G6P was increased, whereas concentrations of glycolytic intermediates just distal to the partial enzymatic block were concurrently decreased. Severe impairment of F6P/G6P interconversion and hence recycling of F6P through the oxidative shunt pathway was clearly demonstrable, and mannose was much more effectively converted to lactate than was glucose. The heterozygous state in parents and siblings was devoid of overt hematologic manifestations but was easily definable by partial perturbations in many of the foregoing measurements.

Despite these similarities to other GPI deficiencies, this case represented a distinct phenotype (designated GPI Valle Hermoso) on the basis of normal electrophoretic patterns and apparent confinement of the enzymopathy to erythrocytes. In only two previous instances have normal zymograms been reported [4, 10, 25]. Both of these were associated with significantly decreased leukocyte activities, and at least one, GPI Matsumoto, exhibited decreased plasma activity as well [10, 25]. The latter was further differentiable by an acidic shift of its pH optimum curve. Leukocytes of all members of the kindred reported here contained entirely normal levels of GPI activity regardless of the degree of erythrocyte deficiency. This finding has not been observed in any other kindred so studied. In addition, normal GPI activities in plasma suggested that other tissues may also have been spared the deficiency.

This apparent exclusion of leukocytes from the deficiency state may be explained on a genetic or a molecular basis. The former would require separate genetic control for erythrocyte and leukocyte GPI and perhaps for other tissues as well. This, however, would conflict with compelling evidence against existence of such tissue-specific isozymes. Detter et al. [14] reported virtual identity among primary GPI electrophoretic components from normal erythrocytes, leukocytes, and platelets, as well as those from plasma, saliva, and other tissues. Payne et al. [26] utilized cellulose-acetate electrophoresis and isoelectric focusing to demonstrate the common identity of GPI from normal erythrocytes, heart, brain, liver, and kidney. Where GPI deficiency has existed without demonstrable electrophoretic anomaly [10], the residual enzyme in erythrocytes, leukocytes, and plasma has also exhibited normal migration rates [25].

Perhaps the most cogent argument against GPI variants among diverse tissues is presented by GPI Narita [9]. Nakashima et al. [25] found that for each family member in this kindred the GPI zymograms of leukocytes and plasma were indistinguishable from the abnormal erythrocyte pattern. Further, the altered GPI zymogram from proband erythrocytes was identical with those obtained from extracts of spleen, liver, and muscle biopsies, strongly supporting common genetic control over the structural character of GPI from these tissues.

Molecular alteration of the enzyme seems a more plausible explanation for the unique occurrence of normal leukocyte and plasma activities in company with erythrocyte deficiency. In this kindred, both parents had red cell GPI activities and thermal inactivation curves which were directly intermediate between proband and normal controls, and all shared identical zymograms. Coupled with a history of distant consanguinity, this strongly suggests homozygous inheritance by the proband of a single mutant allele coding for markedly unstable GPI with normal electrophoretic properties. If the mutant isozyme remained catalytically effective despite the alteration which affected its stability, it might easily be replenished by those cells capable of continued protein synthesis, yet lost irrevocably to mature erythrocytes. Such a combination of isozyme properties might be analogous to those associated with the A(—) variant of G6PD, which is characterized by diminished activity, decreased in vivo stability, and electrophoretic migration identical with that of the A isozyme.

SUMMARY

Studies of a Mexican kindred present evidence for a unique phenotype of erythrocyte glucosephosphate isomerase, GPI Valle Hermoso. The proband was apparently the homozygous recipient of a mutant autosomal allele governing production of an isozyme characterized by decreased activity, marked thermal instability, normal kinetics and pH optimum, and normal starch gel electrophoretic patterns. Unlike previously known cases, leukocyte and plasma GPI activities were unimpaired. This suggested that the structural alteration primarily induced enzyme instability without drastically curtailing catalytic effectiveness, thereby allowing compensation by cells capable of continued protein synthesis. Age-related losses of GPI, however, were not evident by density-gradient fractionation of affected erythrocytes.

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