G6PD Deficiency and Chronic Hemolysis: Four New Mutants—Relationships Between Clinical Syndrome and Enzyme Kinetics

By Mario C. Rattazzi, Laurence M. Corash, George E. van Zanen, Ernst R. Jaffé, and Sergio Piomelli

G6PD deficiency of the common type (Gd' and Gd' Mediterannean) results in extremely mild chronic hemolysis. In contrast, 65 males (from 47 unrelated families) have been reported with a different syndrome of severe chronic hemolysis associated with a superficially similar deficiency in the activity of G6PD. Five new such patients (from four unrelated families) are reported. Biochemical characterization of the erythrocyte G6PD from these patients indicates that these four mutant enzymes are different from each other and from previously reported variants. These new mutants have tentatively been named G6PD New York, G6PD Englewood, G6PD Rotterdam and G6PD Den Haag. The congenital nonspherocytic hemolytic anemias associated with G6PD deficiency appear to be an extremely heterogeneous group from the point of view of biochemical kinetics. The relationships between the clinical syndrome and the various biochemical enzyme characteristics are discussed in the light of the information presently available.

A deficiency in the activity of the enzyme glucose-6-phosphate dehydrogenase (G6PD: D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) was first discovered by Carson et al. in 1956 in the erythrocytes of individuals who had experienced acute hemolysis after exposure to primaquine. Since then it has become apparent that the defect is sex-linked and that it might result from a number of genetically different mutants at the...
same locus. Several million individuals in various parts of the world are believed to be carriers of one or another defective mutant and live clinically unaffected, unless they are exposed to certain drugs or ingest fava beans. The high incidence of this inborn error of metabolism has been attributed to its protective effect against \textit{Plasmodium falciparum} malaria on the basis of its geographical distribution and the recent demonstration of decreased parasite concentration in enzyme-deficient red cells.

In 1958, Newton et al. first described three boys in whom deficiency of G6PD in the erythrocytes was associated with an unusual and totally different clinical syndrome. In their words: “These patients present the hematologic picture of a chronic nonspherocytic hemolytic anemia (CNSHA) with or without specific drug exposure, even though chemical studies on their erythrocytes are comparable to patients that show no anemia or increased hemolysis except when exposed to the specific drugs and fava beans.” Since their original brief report at least 65 similar cases from 47 unrelated families have been described. The clinician and the biochemist have been baffled by the different clinical pictures stemming from an apparently similar biochemical defect.

The purpose of this report is to describe four different families in whom male members have G6PD deficiency of the erythrocytes associated with chronic hemolysis. The biochemical characteristics of three of these mutant enzymes (tentatively named G6PD New York, G6PD Englewood, and G6PD Rotterdam) are presented and compared with those of analogous mutants described in the literature. (The fourth mutant, G6PD Den Haag, proved too unstable for a complete characterization.) Some hypotheses are discussed that might explain the apparent clinical contradictions in terms of peculiar kinetic abnormalities of the mutant enzymes.

**Patients**

Five patients from four unrelated families were studied. The hematological data at time of study are summarized in Table 1. Summary of case histories and family data follow.

**G6PD New York**

C.V. is the son of a black American father and Southern-Italian mother. The mother originates from the province of Potenza, a mountainous area where G6PD deficiency of the Mediterranean type is uncommon. An older sister is hematologically normal and has normal GGPD activity. The patient was extremely jaundiced in the first few hours of life and was exchange-transfused first at 12 hr and again at 36 hr. He was noticed to be pale and jaundiced at 13 yr of life but received no transfusions. At 23 yr he was referred to the New York University Medical Center, where a diagnosis of erythrocyte G6PD deficiency was established. Extensive hematological studies were consistent with an extremely young

<table>
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<th>Table 1.—Hematological Data at Time of Study</th>
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<tr>
<td>Patient</td>
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</tr>
<tr>
<td>New York</td>
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<tr>
<td>Englewood</td>
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<tr>
<td>Rotterdam</td>
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<td>Den Haag (L.)</td>
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<td>Den Haag (H.)</td>
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erythrocyte population, but no other defect was detectable except for G6PD deficiency of the erythrocytes and leukocytes. $^{51}$Cr survival revealed a T 3/4 of 2.5 days. The patient has since then required transfusions, coincident with two upper respiratory infections and varicella. In these circumstances, he presented with hemoglobin 3–4 g/100 ml and a decreased reticulocyte count. Characterization studies were performed at age 3. Physical examination revealed a well-developed 4-yr-old child, with liver 3 cm below the costal margin and no splenomegaly; there was scleral icterus; the serum bilirubin ranged between 2 and 4 mg/100 ml.

**G6PD Englewood**

A.R. is a 30-yr-old male, the first son of North-Italian parents. A younger brother and sister are hematologically normal and have normal erythrocyte G6PD activity. The patient was noted to be anemic and jaundiced at birth. He received over 30 transfusions during the first 18 yr of life and was hospitalized several times with hemoglobin levels of 5–6 g/100 ml coincident with minor respiratory infections. Splenomegaly was noted at 3 yr of age and cholelithiasis at age 18; he underwent splenectomy and cholecystectomy at age 19. After surgery his hemoglobin level has ranged between 9 and 12 g/100 ml, he has not required transfusions, and serum bilirubin ranges between 1.4 and 2.0 mg/100 ml. At age 24 a diagnosis of G6PD deficiency was established at Englewood Hospital and the Bronx Municipal Hospital Center. Extensive hematological and biochemical studies provided data consistent with an extremely young erythrocyte population. No other defect was detectable, except for G6PD deficiency of the erythrocytes and leukocytes. Characterization studies were performed at age 28. He appeared a well-developed male, with intelligence quotient well below average. Physical findings included pallor and abdominal surgical scars.

**G6PD Den Haag**

This family of Dutch descent has two boys, whose histories are reported below, and one girl, who is hematologically normal and has normal G6PD activity of the erythrocytes. L.A. is the older of the two brothers. He was found to be anemic and jaundiced at birth and he received two transfusions. At 3 mo of age a diagnosis of G6PD deficiency was established by Dr. Oort of the Netherland Red Cross Laboratory in Amsterdam. Since then he has been followed at the Sophia Children's Hospital in Rotterdam with persistent anemia and slight jaundice. He tolerates his anemia well and has not required transfusions. On physical examination he appeared a well-developed 8-yr-old boy with slightly enlarged liver (2 cm below costal margin) and spleen (1 cm below costal margin).

Characterization studies were performed in patient L.A. at age 8 and in patient H.A. at age 2.

**G6PD Rotterdam**

Patient P.V.S. is the only child of a family of Dutch descent. He was noted to be jaundiced at birth, but he received no exchange transfusion. He was first admitted to the hospital at the age of 10 mo with marked anemia. He has experienced episodes of acute anemia coincident with ingestion of aspirin, infections, administration of Felixmas extract, and ingestion of fava beans. He was transfused only once during one of these episodes. In the interval he was followed in the outpatient service of the Sophia Children's Hospital, Rotterdam, until his present age (15 yr). Characterization studies were performed at

*This patient is P IV 12 of Dr. Oort's doctoral thesis.37
age 14. On physical examination he appeared a well-developed 15-yr-old male without any special findings.

**MATERIALS AND METHODS**

**Partial Purification of G6PD**

This was performed at +4°C according to the first step of the procedure described by Rattazzi. The erythrocytes were washed three times in saline and suspended in 1.5 vol of buffer 1 (5 mM Na-phosphate buffer, pH 6.4, containing, in final concentration, 1 mM EDTA, 1 mM β-mercaptoethanol, 0.1 mM disopropyl fluorophosphate, and 0.02 mM NADP). Chloroform (0.2 vol) was added, the suspension was vigorously mixed for 1 min, and cellular debris was removed by centrifugation at 31,000 g for 30 min. DEAE-Sephadex A-50 (beam form), equilibrated with buffer 1, was added to the hemolysate until no G6PD activity was detectable in the supernatant solution. The suspension was then poured into a glass column and washed free of hemoglobin and 6-phosphogluconate dehydrogenase with buffer 2 (prepared by addition of NaCl to buffer 1 to a final concentration of 0.05 M and readjustment of pH to 6.4). G6PD was then eluted from the column with small volumes of buffer 3 (prepared by addition of NaCl to buffer 1 to a final concentration of 0.25 M and readjustment of the pH to 6.4). The preparations were dialyzed free of excess NADP and NaCl against buffer 4 (50 mM Tris-HCl buffer, pH 8, containing 0.27 mM EDTA, 7 mM β-mercaptoethanol, and 0.01 mM NADP). They were used for biochemical characterization within 12 hr of preparation. A 200-fold purification was usually obtained; the partially purified preparations had no detectable 6-phosphogluconate dehydrogenase activity. A sample of normal blood was always processed at the same time and used as a control in the characterization studies. This purification procedure is essentially identical to the second method recommended by the World Health Organization, except that DEAE-Sephadex A-50 was used instead of DEAE-cellulose to obtain a better yield. Paired characterizations of normal G6PD, G6PD A-, G6PD A+ and G6PD B Mediterranean purified by both techniques gave identical results, comparable to those reported in the literature.

**Assay of G6PD**

The activity of the enzyme was measured at 20°C according to Clock and McLean and expressed in μM of NADPH formed per minute (International Units) per gram of hemoglobin in the hemolysates, or per milligram of protein in the partially purified preparations. Protein concentrations were determined according to Warburg and Christian.

**Biochemical Characterization of G6PD**

Electrophoresis was performed on starch gel at pH 8.6 (Kirman et al.) and on cellulose acetate gel (Cellogel) at pH 7.5 (Rattazzi et al.).

Thermal stability, pH-activity curves and rates of utilization of the substrate analogues galactose-6P and 2-deoxyglucose-6-P were determined on samples freed of (NH₄)₂SO₄ as described.

Michaelis constants for G6P and NADP were calculated by least-squares fit from the plot of [S]/V vs. [S]. Eight different concentrations of either substrate were used, G6P ranging from 10 to 200 μM, NADP from 1.5 to 100 μM. True substrate concentrations were determined enzymatically with yeast G6PD. The amount of NADP present in the dialyzed samples was in the same range as the lowermost concentrations used for the determination of KmNADP. This was obviated in the following ways: (1) the cuvettes containing enzyme, buffer, and G6P were preincubated at 25°C until the spontaneous rate of the reaction decreased to zero (10-15 min), then NADP in the desired concentration was added to start the reaction, or (2) immediately before the determination of KmNADP, the enzyme preparations were passed through a small column of Sephadex G-25 equilibrated with buffer 4 minus NADP, or (3) the resulting final concentration of NADP was accounted for in the calculation. Essentially identical results were obtained with the three different methods.
Table 2.—Biochemical Characteristics of G6PD Variants Associated With Severe Chronic Hemolysis

<table>
<thead>
<tr>
<th>Variant Name</th>
<th>Ref. No.</th>
<th>RBC Activity ( % of normal )</th>
<th>Electrophoretic Mobility ( % of normal )</th>
<th>Michaelis Constant ( NADP ) ( nM )</th>
<th>Michaelis Constant ( G6P ) ( nM )</th>
<th>2d G6P Utilization ( % of G6P )</th>
<th>pH Optima</th>
<th>Thermal Stability</th>
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<td>100</td>
<td>20</td>
<td>127–200</td>
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<td>8–8.2</td>
<td>Labile</td>
</tr>
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<td>4</td>
<td>105</td>
<td>-</td>
<td>182</td>
<td>-</td>
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<td>Very labile</td>
</tr>
<tr>
<td>Beaujon</td>
<td>44</td>
<td>0</td>
<td>105</td>
<td>-</td>
<td>87–118</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>70</td>
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<td>13</td>
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<td>9–26</td>
<td>100</td>
<td>31–37</td>
<td>58–76</td>
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<td>100</td>
<td>5</td>
<td>58</td>
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<td>5</td>
<td>100</td>
<td>4–6</td>
<td>40–60</td>
<td>3.7</td>
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</tr>
<tr>
<td>Ohio</td>
<td>45</td>
<td>2–16</td>
<td>110</td>
<td>St. incr.</td>
<td>51. incr.</td>
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<td>3</td>
<td>105</td>
<td>6</td>
<td>48–60</td>
<td>2.4</td>
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</tr>
<tr>
<td>Tripler</td>
<td>36</td>
<td>40</td>
<td>90</td>
<td>-</td>
<td>30</td>
<td>3.7</td>
<td>Labile</td>
<td></td>
</tr>
<tr>
<td>Alhambra</td>
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<td>10–25</td>
<td>95</td>
<td>2.6</td>
<td>55</td>
<td>2</td>
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<td>Reduced</td>
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<tr>
<td>Dren Haag</td>
<td>*</td>
<td>1.7–3</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Ext. labile</td>
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<tr>
<td>Englewood</td>
<td>*</td>
<td>0.5</td>
<td>100</td>
<td>0.5</td>
<td>56</td>
<td>29.6</td>
<td>6.5 + 10</td>
<td>Labile</td>
</tr>
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<td>*</td>
<td>0.6</td>
<td>100</td>
<td>3</td>
<td>51</td>
<td>15.7</td>
<td>7.5</td>
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</tr>
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<td>Hong Kong</td>
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<td>106</td>
<td>St. red.</td>
<td>50% normal</td>
<td>Incr.</td>
<td>Bimodal</td>
<td>Normal</td>
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<tr>
<td>Rotterdam</td>
<td>*</td>
<td>1.9</td>
<td>95</td>
<td>3.3</td>
<td>23</td>
<td>6</td>
<td>7 + 9.5</td>
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<tr>
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<td>†</td>
<td>100</td>
<td>100</td>
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<td>†</td>
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<td>7 + 10</td>
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<td>A–</td>
<td>†</td>
<td>&lt; 10</td>
<td>105</td>
<td>3–4.5</td>
<td>35–65</td>
<td>&lt; 3</td>
<td>Truncate</td>
<td>Normal</td>
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* This paper.
† Values in this laboratory, in agreement with values reported in Reference 43.
RESULTS

Biochemical Studies

The biochemical characteristics of the G6PD variants described in this paper are summarized in Table 2. The other known variants associated with CNSHA for which adequate biochemical data are available are also listed for comparison. The variants have been divided into three groups. Group 1 includes variants with grossly altered kinetic characteristics; Group 2 comprises variants with minimally altered or normal kinetics, but showing greatly decreased thermal stability and Group 3 includes variants with normal or minimally altered kinetics and normal thermal stability.

G6PD Englewood and G6PD New York fall into Group 2. G6PD Englewood is different from all the others in this group, because of the very low $K_m^\text{NADP}$, the increased rate of utilization of 2-deoxy G6P, and the bimodal pH activity curve (Fig. 1). It resembles very closely G6PD Mediterranean (which is not associated with CNSHA) but for its $K_m^\text{G6P}$ which is in the normal range (thus higher than that of G6PD Mediterranean) and the more markedly biphasic pH curve.

G6PD New York, on the other hand, can be distinguished from the other variants in the group by the extremely low activity in erythrocytes, the increased affinity for 2d-G6P, and the abnormal pH activity curve (Fig. 1). G6PD Den Haag was found to have an activity of less than 3% in the hemolysate, and to lose this activity completely in the hemolysate after 6 hr at $+4^\circ C$. The partially purified preparation, which had given a yield of 0.1 to 0.3 unit from 30 ml of blood in the patients with the other variants, proved completely inactive on two attempts. Electrophoretic mobility in a crude hemolysate was normal. Thus, the only characteristics known for this variant are very low erythrocyte activity, extreme lability even in the cold, and normal electrophoretic mobility. This variant, though listed with Group 2, may thus belong to either Group 1 or Group 2.

G6PD Rotterdam, the fourth variant studied, is listed in Group 3, comprising variants with nearly normal kinetics and normal thermal stability. Of the variants described in the literature, only G6PD Hong Kong falls in this category. G6PD Hong Kong and Rotterdam are very similar in kinetic characteristics, and resemble each other also in the bimodal pH activity curve (Fig. 1) and slightly increased 2d G6P utilization. They differ, however, in their electrophoretic mobility, slower than normal for G6PD Rotterdam, faster than normal for G6PD Hong Kong. The thermal stability of G6PD Rotterdam was found to be normal.

DISCUSSION

Chronic Hemolysis in G6PD Deficiency

In the common types of G6PD deficiency (Gd(-) Mediterranean and Gd(-) A-) severe hemolysis occurs only after exposure to offending agents. Several observations, however, indicate that very mild, compensated hemolysis is present in the steady state as well. A significant reticulocytosis was reported in both Gd(-) Mediterranean and Gd(-) A- males. In addition, increased
activity of the age-dependent enzyme pyruvate kinase was observed in the erythrocytes of these individuals, and similarly increased hexokinase activity was reported in the erythrocytes of Gd(−) A− males. Independent measurements of erythrocyte survival estimated their average life-span in both Gd(−) A− and Gd(−) Mediterranean males at approximately 100 days (a 17% reduction over the normal of 120 days). The clinical impact of an effect of this magnitude is such that hematological examination of a single individual yields values within the normal limit, but significant differences can be appreciated when larger groups are studied.

In contrast, patients with CNSHA associated with G6PD deficiency show, at all times, clear-cut evidence of severe chronic hemolysis. The salient features of this syndrome consist (in the 70 cases described in the literature including this report) of hemoglobin concentrations between 6 and 14.8 g/100 ml and reticulocyte counts between 4 and 34%. Erythrocyte life-span, measured in 16 patients with 51Cr, revealed T ½ ranging between 2 and 17 days. Neonatal jaundice was reported in 26 patients; hyperbilirubinemia (up to 6 mg/100 ml) was frequently observed. Splenomegaly is rare and splenectomy does not usually result in clinical improvement, although it appears to have been beneficial in one of our patients (G6PD Englewood). There seems to be a positive correlation between age and hemoglobin levels. It has been suggested that this is the result of increased red cell production with puberty.
A separate category of G6PD-deficient individuals should probably include several patients reported in the literature with normal or only slightly reduced hemoglobin levels, normal or only slightly elevated reticulocyte counts, and a clinical history of episodes of acute hemolysis, alternating with long periods of hematological normality. A few individuals in this group were found to have a reduced erythrocyte survival (as estimated through $^{51}$Cr T%) disproportionate to the reticulocytosis.

An additional difficulty in establishing the exact role of G6PD deficiency in chronic hemolysis is the high incidence of one common type or the other in certain ethnic groups. In members of these populations with chronic hemolysis due to other causes, the G6PD deficiency could be just coincidental finding.

Different types of G6PD deficiency of the erythrocyte result therefore in an almost continuous spectrum of hemolytic syndromes from the clinically silent carrier of the common type to the extreme chronic hemolysis of most CNSHA patients.

Biochemical Aspects of G6PD Deficiency and Clinical Hemolysis

Of the several G6PD variants described to date at least two (G6PD A and G6P Hektoen) have been shown to result from a single amino acid substitution. It has been postulated by inference that this might be the case for the majority of the variants described. If this generalization is indeed correct, the differences in kinetic characteristics of mutant enzymes would indicate that some amino acid substitutions exert a greater influence than others on the function of the molecule. The exact nature of the abnormality that results in a defective erythrocyte (and in CNSHA) is, however, not clear. Some of the variants associated with CNSHA exhibit grossly altered kinetic characteristics that would justify the defective function, but for other types the relationship is not obvious. It is indeed difficult to find a common denominator for the clinical manifestations among the biochemical characteristics of these variants, at least as determined by current techniques (Table 2).

Although the majority of these variants have a very low erythrocyte activity, some of them actually have in the standard assay higher activity than the clinically silent G6PD Mediterranean and A−.

Curves of pH activity and electrophoretic mobility and utilization rates for substrate analogues seem to have little or no relationship with CNSHA. They may reflect, however, the nature of the amino acid substitution and its effect on the substrate binding site.

The great majority of mutant enzymes associated with CNSHA show an abnormal thermal lability in vitro, and it is commonly held that this may reflect a more rapid decay in vivo of the enzyme. However, the simple relationship lability in vitro = lability in vivo = CNSHA is contradicted by the findings of variants that are stable in vitro and labile in vivo (G6PD A−) or labile in vitro as well as in vivo (G6PD Mediterranean), but not associated with CNSHA; and, on the other hand, by the findings of variants associated with CNSHA that are stable in vitro (G6PD Hong Kong, Rotterdam). It is indeed possible that in the method usually employed a relatively high concentration of NADP (10 μM) might obscure a small but physiologically sig-
significant in vitro lability of apparently stable variants, like G6PD Rotterdam. However, the absence of severe chronic hemolysis in the case of the unstable G6PD Mediterranean still contradicts the physiological significance of instability itself.

An attractive biochemical hypothesis has been put forward by Kirkman, after he observed a $K_{\text{m}}$ of 200 $\mu$M in Gd(ohlhausen) and of 20 $\mu$M in Gd(Mediterrenean) (the normal enzyme has a $K_{\text{m}}$ to 35-64 $\mu$M). Since the intracellular concentration of G6P is 40-90 $\mu$M/ml of erythrocytes, he suggested that the efficiency of the enzyme rather than its absolute level of activity was of physiological significance.60 As an estimate of efficiency he used the ratio $V_{\text{max}}/K_{\text{m}}$, where $V_{\text{max}}$ is the maximum velocity as a percentage of normal and $K_{\text{m}}$ is the Michaelis Menten constant for G6P, in $\mu$M. The hypothesis cannot however account for chronic hemolysis in all the mutants presently known, since at least 5 out of 22 CNSFA associated variants have a ratio $V_{\text{max}}/K_{\text{m}}$ more favorable than that of G6PD Mediterranean. Furthermore, in G6PD Mediterranean, measurable activity is present only in reticulocytes, and mature erythrocytes apparently devoid of G6PD activity (as this is detected by the standard assay system) survive in the circulation for as long as 100 days, despite a ratio $V_{\text{max}}/K_{\text{m}}$ approaching zero.

Recent data indicate that the intracellular efficiency of the enzyme might be even more critically influenced by its affinity for the other substrate (NADP). Omachi et al. have shown that (with the precautions used by Burch et al. with rabbit erythrocytes) in human erythrocytes the concentration of NADP is actually of the order of 1 $\mu$M. Thus, within the erythrocyte, G6PD is far from being saturated with NADP. At this low concentration of NADP the monomeric state of the enzyme is favored, while at higher concentration of NADP the enzyme is in the dimeric form. In accord with these findings it has recently been shown that the affinity for NADP of the functionally normal enzymes G6PD B and G6PD A does not follow hyperbolic (Michaelis-Menten-like) kinetics, but is described by a sigmoid-shaped curve. This phenomenon suggests a cooperative homotropic allosteric effect, resulting from conformational changes of the molecule associated with the NADP-dependent monomer–dimer equilibrium. Two dissociation constants can be derived from the sigmoid-shaped saturation curve. Of these only the constant at higher concentrations of NADP can be estimated by determination of the $K_{\text{m}}$ in the usual way. No information is obtained as to the dissociation constant at lower concentration of NADP, which is of real physiological significance. At the concentration of NADP of the order of 1 $\mu$M as present in the erythrocyte in the steady state, G6PD functions only at the extremely low activity of its subactive monomer. When the intracellular concentration of NADP is increased by oxidizing agents the monomer–dimer equilibrium is disrupted in the dimeric form, with a rapid increase of NADPH generation through the cooperative effect. Only 7% of the glucose metabolized by normal erythrocytes flows through the pentose shunt; thus in the normal erythrocyte G6PD is working at less than 0.1% of its potential activity. In Gd(Mediterrenean) erythrocytes, glucose utilization is normal, but only 1.5% is shunted through the pentose pathway; therefore, although potential G6PD
activity is less than 1% of normal, actual function of this pathway is 20% of normal.\textsuperscript{72} These data support the speculation that in G6PD Mediterranean the function of the monomer is relatively unaffected, while the function (or the formation) of the dimer at higher concentrations of NADP might be impaired. Thus, these erythrocytes might be capable of sustaining NADPH levels above a threshold below which chronic hemolysis ensues, but fail to increase NADPH regeneration in situations when intracellular NADP rises. Although no comparable data are available for CNSHA-associated variants, it is conceivable that at low concentration of NADP the activity of the monomeric form of these enzymes might be reduced below the "chronic hemolysis threshold." At higher concentrations of NADP some of these mutants might tend to remain in a monomeric subactive state, whereas others might show a marked cooperative effect, thus explaining the findings of levels of activity higher than in G6PD Mediterranean in the standard assay system. Differently shaped curves of NADP affinity can be easily envisioned if one postulates that the amino acid mutation for the common type of deficient variant affects the association and/or cooperative effect, whereas the substitution for CNSHA-associated variants affects the activity even of the monomeric form. Experiments are in progress to elucidate these hypotheses.

It is, of course, possible that the defect in the enzyme in the CNSHA associated mutants might be located in a part of the molecule responsible for a hitherto unknown essential function of the enzyme or in a molecular subunit shared with some other protein necessary for erythrocyte survival. Indirect support for the latter hypothesis is provided by the observation that NADPH-dependent methemoglobin reductase is decreased in Gd(−) \textsuperscript{74} and Gd(−) Mediterranean erythrocytes, but has been found normal in the two patients with Gd(−) Englewood and New York and in one patient with Gd(−) Alhambra.\textsuperscript{32} The possibility of common subunit between G6PD and TPNH dependent methemoglobin reductase has in fact been previously suggested by Beutler.\textsuperscript{75}

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G6PD Deficiency and Chronic Hemolysis: Four New Mutants—Relationships Between Clinical Syndrome and Enzyme Kinetics

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