G-6-PD Tripler: A Unique Variant Associated with Chronic Hemolytic Disease

By Paul F. Engstrom and Ernest Beutler

Erythrocyte glucose-6-phosphate dehydrogenase (G-6-PD) deficiency causes a heterogeneous group of disorders in which mutations affecting the structure of the enzyme produce various clinical manifestations. These range from the totally asymptomatic state to the commonly reported drug-induced hemolytic anemia of Negroes, the neonatal jaundice frequently reported in Mediterranean peoples, and congenital nonspherocytic hemolytic disease (CNHD). In CNHD, 12 distinct variants of this enzyme have been characterized and differentiated, based on kinetic properties, pH optimum, thermal stability and electrophoretic mobility.

Many of the reported cases of CNHD due to G-6-PD deficiency are children who experience neonatal jaundice and a moderately severe chronic hemolytic anemia exacerbated by drugs. By contrast, our adult patient presented with a history of recurring jaundice unrelated to drug intake and a compensated hemolytic anemia. The biochemical studies of the enzyme from this unusual patient demonstrate the existence of a new variant: G-6-PD Tripler.

Materials and Methods

One hundred milliliters of the patient's blood collected in ACD formula A was shipped by air in a plastic bag in ice from Hawaii to Duarte for biochemical studies. G-6-PD was partially purified and the enzyme was characterized, using standard methods as recommended by the W.H.O. scientific group.

Clinical Findings

D.B., a 52-year-old Caucasian male, was evaluated on 9 December 1968 for abdominal pain, not associated with nausea or fever. There was no history of neonatal jaundice. While a prisoner of war in 1944, the patient suffered malnutrition and recurring episodes of jaundice which were attributed to hepatitis. In 1948 he had a cholecystectomy for chronic cholecystitis and cholelithiasis. Because of recurrent abdominal pain, fever and jaundice, a common duct exploration in 1952 uncovered choledocholithiasis. In 1963 recurrent abdominal pain necessitated a reexploration of the common duct and performance of a transduodenal sphincterotomy for stenosis of the sphincter of Oddi. Although the patient has a history of allergy to penicillin and to cholegraffin, he denied a reaction to other common medications and could not recall taking antimarial drugs. The patient has never been treated for anemia.
He has no children or siblings, and there was no family history of recurring jaundice or anemia.

Significant findings on physical examination included scleral icterus, mild right-upper-quadrant tenderness, and the absence of hepatosplenomegaly.

Initial laboratory data included: white blood cell count 13,200/mm$^3$, hemoglobin 13.3 Gm. per cent, hematocrit 38 per cent, reticulocyte count 3.8 per cent; total bilirubin 4.6 mg. per cent, serum alkaline phosphatase 4.1 Bodansky units, and total serum protein 6.2 Gm. per cent. The peripheral blood smear showed a normocytic, normochromic red cell population with occasional target cells and increased red cell basophilia. The following tests were normal: serum amylase, two-hour urine amylase, direct and indirect Coombs test, red cell osmotic fragility, examination of stool for occult blood, chest X-ray, abdominal film, and upper G. I. series.

The patient was treated with nasogastric suction and intravenous tetracycline. Within 72 hours the abdominal pain and evident icterus had cleared and the patient was discharged from the hospital.

Subsequent laboratory determinations included: hemoglobin 13.8 Gm. per cent, hematocrit 41 per cent, white blood cell count 6600/mm$^3$, reticulocyte count 4.8 per cent, total bilirubin 1.9 mg. per cent, a negative Heinz body preparation, a normal adult hemoglobin electrophoresis, absence of a precipitate upon heating the hemolysate to 50°C and a negative acid hemolysis test. A G-6-PD decolorization test (Sigma Corporation) showed no decolorization at the end of six hours, which is consistent with a severe deficiency of G-6-PD. The $^{51}$Cr-tagged red blood cell survival was calculated to be 15 days (normal 26–32 days). On 4 March 1969 blood was collected for enzyme assay and characterization.

### Biochemical Findings

Results of biochemical characterization of G-6-PD Tripler are compared with the normal in Table 1 and in Fig. 1. Although there was only partial deficiency of enzyme activity, the enzyme had marked thermal instability at 46°C after 20 minutes. Electrophoretically the enzyme was slow in all systems studied: Its mobility was 90 per cent of the common B variant in potassium phosphate buffer pH 7.0, 97 per cent of B in the tris-hydrochloride system, and 97 per cent of B in EDTA-borate-tris (EBT) buffer. The pH optimum curve is slightly biphasic but remains within the normal range.

### Discussion

The biochemical evaluation of the enzyme confirms the fact that this is a unique mutant of G-6-PD. In contrast to the marked deficiency of G-6-PD activity in the red cells in most other patients with CNHD studied, our patient has approximately 50 per cent activity. However, this enzyme is very labile to heat. If this reflects in vivo lability of the enzyme, this may account for the fact that this patient has a shortened red cell lifespan. The heat instability also accounts for the discrepancy between the dye decolorization test which

<table>
<thead>
<tr>
<th>Enzyme Activity (Per Cent of Normal)</th>
<th>Electrophoretic Mobility (Per Cent of B in KPO$_4$ Buffer)</th>
<th>Thermal Lability $^{20}$° at $^{46}$°C (Per Cent Activity Lost)</th>
<th>Km G6P ($\mu$M)</th>
<th>2d G6P Utilization (Per Cent of G6P)</th>
<th>Deamino NADP Utilization (Per Cent of NADP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal:</td>
<td>75–125</td>
<td>100</td>
<td>20</td>
<td>40–60</td>
<td>57–63</td>
</tr>
<tr>
<td>Patient:</td>
<td>40</td>
<td>90</td>
<td>98</td>
<td>30</td>
<td>3.7</td>
</tr>
</tbody>
</table>
requires incubation at 37°C and the enzyme assay. Except for G-6-PD Alhambra, the slow electrophoretic mobility is an unusual characteristic for variants associated with CNHD. The affinity for its substance glucose-6-phosphate (G-6-P) is increased, such as is found in the Mediterranean, Hong Kong, and Ashdod variants. The utilization of 2-deoxyglucose-6-phosphate is normal. Although some individual differences between G-6-PD Tripler and other variants are too small to be clearly significant, there appears to be no reason to doubt that this enzyme is unique on the basis of all of the findings.

The clinical picture of our patient is also unique. His compensated hemolytic anemia escaped detection for over 20 years of multiple hospitalizations for recurrent biliary tract disease. There was no history of neonatal jaundice and the patient denied episodes which suggest acute hemolysis secondary to infections or exposure to oxidating drugs. However, there is no evidence to suggest a cause for the hemolytic anemia apart from the relative G-6-PD deficiency.

This patient has no other family members who could be studied in order to delineate the genetics of this variant. The marked diversity of G-6-PD enzyme types which occur in chronic hemolytic disease and the lack of family history suggest that these mutant enzymes may arise spontaneously. Therefore, any unexplained or recurrent hemolytic anemia occurring in patients of any age or nationality must be screened carefully for G-6-PD deficiency.

**SUMMARY**

A 52-year-old Caucasian was hospitalized for recurring jaundice. Laboratory tests established a compensated hemolytic anemia due to partial erythrocyte G-6-PD deficiency. Biochemical characterization of the affected enzyme uncovered a unique variant: G-6-PD Tripler. Its significant properties include...
G-6-PD TRIPLET

marked thermal instability, an electrophoretic mobility slower than the normal B variant in all systems studied, and slightly increased affinity for the substrate G-6-P.

REFERENCES

G-6-PD Tripler: A Unique Variant Associated with Chronic Hemolytic Disease

PAUL F. ENGSTROM, Major and ERNEST BEUTLER

Updated information and services can be found at:
http://www.bloodjournal.org/content/36/1/10.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml