Glucose-6-phosphate dehydrogenase deficiency and chronic haemolysis in an English family¹

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SYNOPSIS Three male members of an English family with chronic haemolytic anaemia due to glucose-6-phosphate dehydrogenase deficiency are reported. The disease was symptomless in one adult, but crises, caused either by increased haemolysis or failure of marrow compensation, occurred in two children. They were typically precipitated by trivial infection. Two normal female members of the family were obligatory heterozygotes. A hitherto undescribed 'slow' variant of the enzyme was identified electrophoretically.

The association of acute or chronic haemolysis and reduced levels of glucose-6-phosphate dehydrogenase (G6PD) in the red cell is well known, and a wide range of disorders resulting from it is recognized throughout the world (Carson and Frischer, 1966). Different clinical manifestations are associated with molecular variants of the enzyme, identifiable electrophoretically or by other characteristics. Acute haemolysis was first described following the introduction of the 8-aminoquinoline anti-malarials but many other drugs, infections, and broad beans (favism) can precipitate haemolysis in susceptible subjects. Transfusion studies showed that the tendency to haemolysis was a peculiarity of the red cells of the affected individual, and this peculiarity was later identified as deficiency of the enzyme G6PD, genetically determined and transmitted as a sex-linked intermediate character.

Very low levels of G6PD have been described in a small number of families, mostly of western European origin, associated with chronic haemolytic anaemia. A compensated haemolytic state beginning at birth is interrupted by crises of increased haemolysis associated with infection or drugs.

The exact incidence of G6PD deficiency in England is not known, but is probably very low.

Shortage of susceptible subjects and the uncommon occasion for the use of antimalarial drugs have made primaquine sensitivity a rarity, largely confined to immigrants. Three English families have been reported in whom favism was the only manifestation of G6PD deficiency (Davies, 1962; Brodribb and Worsam, 1961). In two English families, chronic haemolytic anaemia has been found to be associated with this deficiency (Blackburn and Lorber, 1963; Bowdler and Pranker, 1964). We report a further family in whom an electrophoretically distinct enzyme variant was identified.

Case Reports

CASE III 5

A boy, of English descent, presented in October 1965, aged 12, with severe anaemia of acute onset. There was a vague history of preceding upper respiratory tract infection treated with penicillin. He was admitted with complaints of headache, tiredness, and dizziness, and on examination was very pale and febrile (102°F). The spleen was not palpable. Laboratory investigations gave the following results: haemoglobin level 5.6 g/100 ml, packed cell volume 16%, and normal red cell morphology; a white blood count of 5,600/c mm with a

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Fig. 1 Family tree.

normal differential, reticulocytes less than 1%, platelets 200,000/c mm; a direct Coombs test gave a negative result, there was normal osmotic fragility; no cold agglutinins were detected, and the serum bilirubin was 1.5 mg/100 ml. The bone marrow showed intense normoblastic erythropoietic activity arrested at the intermediate normoblast stage; leucopoiesis and megakaryocyte activity were normal, as was starch gel electrophoresis of haemoglobin. Throat swabs grew commensal organisms and blood cultures were negative. Treatment with whole blood transfusion effected a dramatic improvement in the patient's condition, and he was discharged home 10 days after admission. Follow-up studies revealed slightly lowered haemoglobin levels (10.6-12.4 g/100 ml), a reticulocytosis (8-12%), and raised serum bilirubin (0.7-1.5 mg/100 ml). Three months after the initial investigations the red cell G6PD was found to be reduced.

The patient had had no previous episodes of jaundice, but at the age of 4 months, he had been admitted to hospital with bronchitis. Haemoglobin was found to be 8.6 g/100 ml. No further investigations were carried out, though in retrospect this may have been the first manifestation of disease.

In January 1967 the patient had a haemolytic crisis, associated with a sore throat and penicillin therapy. Haemoglobin fell to 8.3 g/100 ml with reticulocytosis up to 15%. Since that time he has had four milder haemolytic episodes, usually associated with upper respiratory tract infections.

CASE III 7

A brother of the previous patient presented in June 1967, aged 5, with acute haemolytic

anaemia. Abdominal pain, headache, and rigors began 24 hours before admission and he was noted to be pale and slightly yellow. He was given 150 mg aspirin after the onset of symptoms; he had taken aspirin on many previous occasions without ill effect. On examination he was febrile (102°F), pale and slightly jaundiced. The spleen was palpable, and laboratory investigations showed the haemoglobin level to be 8.4 g/100 ml, with packed cell volume 25%, reticulocytes 13% rising to a maximum of 29% on the third day of admission, and bilirubin level 2.2 mg/100 ml.

Because of a steadily falling haemoglobin, he was transfused with whole blood with satisfactory improvement. Subsequent follow-up studies confirmed a persistent reticulocytosis and elevated serum bilirubin levels, and three months after the initial investigations, the red cell G6PD was found to be reduced.

Family Study

The family tree is shown in Figure 1. One further case of chronic haemolytic anaemia was found: case II 1, a maternal uncle (aged 38) of the first patient (case III 5), who had never been jaundiced or required blood transfusion.

This family study illustrates the features of chronic non-spherocytic haemolytic anaemia, the rarest manifestation of G6PD deficiency. In three male members of the family, a compensated haemolytic state was demonstrated. In two children, there were crises of severe anaemia, due usually to increased haemolysis but on one occasion (the first episode in case III 5) to inability of the marrow to compensate for the increased red cell breakdown.

The crises seemed commonly to be precipitated by upper respiratory tract infection, and this is a feature of many reported cases (Shahidi and Diamond, 1959; Zinkham and Lenhard, 1959; Kirkman and Riley, 1961). By contrast, drugs are uncommon precipitating factors. Our second patient (case III 7) was given 150 mg aspirin, although it appeared that haemolysis was already occurring at the time. In negroes, this dose would be insufficient to cause haemolysis (Beutler, 1959; Kellermeyer, Tarlov, Brewer, Carson, and Alving, 1962), but in chronic haemolytic anaemia a smaller dose is likely to be required since G6PD levels are lower. In individual cases it is often difficult to decide whether haemolysis is precipitated by infection or by the drugs used for its treatment. Fikrig, Chun, and Watson (1966) reported a case in which sulphafurazole apparently caused haemolysis, but the drug was later given without ill effect. Bousser, Christol, Boivin, Mallassenet, and Lods (1963) described a haemolytic crisis precipitated by aspirin but this was prescribed for a high fever.

Case No.	Haemoglobin (g/100 ml)	Reticulocytes (%)	Bilirubin (mg/100 ml)	Red Cell Half Life (days) ¹
I 1	16.0	2	—	—
I 2	14.6	2	—	—
II 1	13.1	7	1.4	11.5
II 2	15.2	2	—	—
II 3	16.6	2	—	—
II 4	12.6	5	0.6	28.0
II 6	13.9	—	—	—
III 1	12.1	2	0.5	—
III 2	13.6	2	—	—
III 3	16.1	2	—	—
III 4	16.1	1	—	—
III 5	13.3	16	1.6	7.5
III 6	13.9	1	—	—
III 7	11.5	13	2.2	—
III 9	15.2	1	—	—
III 10	11.4	1	—	—
III 11	10.8	—	—	—

Table I Haematological data

¹Normal red cell half life = 28-32 days.

Case No.	G6PD (units/min/ml packed red cells) ¹	Glutathione Reductase (units/min/ml packed red cells)	6-Phosphogluconate Dehydrogenase (units/min/ml packed red cells)	Reduced Glutathione (mg/100 ml whole blood)
I 1	3.15	1.05	1.35	—
I 2	2.70	1.95	1.65	—
II 1	0.45	1.35	2.55	11.3
II 3	3.37	1.80	1.50	22.5
II 4	2.25	1.35	2.10	24.5
II 6	2.70	1.20	1.95	33.4
III 1	2.85	0.90	—	—
III 4	3.40	3.00	1.65	22.0
III 5	0.15	3.75	2.70	1.6
III 6	3.30	1.20	1.65	—
III 7	0.90	1.65	2.85	13.8
III 9	2.50	—	—	—
III 10	2.60	—	—	—
III 11	3.45	1.65	2.10	21.3
Normal range	2.99 ± 0.52 n = 73	1.66 ± 0.51 n = 19	1.80 ± 0.29 n = 9	23.6 ± 4.0 n = 9

Table II Enzyme data

¹1 unit of enzyme activity is defined as a change of optical density of 1.0 at 340 mμ. G6PD estimations in cases II 2, III 2, and III 3 were carried out in a different laboratory; the results fell within the normal range for that laboratory.

Study of the family tree (Fig. 1) reveals three affected males, two brothers (III 5 and III 7) and their maternal uncle (II 1); these findings are consistent with the known sex-linked intermediate inheritance of the deficiency. In addition there are two obligatory heterozygotes. The mother (II 4) of the affected siblings (III 5 and III 7) had a slightly lowered G6PD level; reticulocytosis noted at the time of the study was probably due to menorrhagia, and red cell survival time was normal. The daughter (III 1) of the affected male (II 1) had a normal amount of G6PD. The observed variation in enzyme levels found in heterozygotes in this condition may be explained by X inactivation according to the Lyon hypothesis (Lyon, 1961).

Chronic haemolysis in these patients is presumably related to the constantly lowered levels of reduced glutathione: Jacob and Jandl (1962) showed that reduced glutathione has a protective action on the red cell surface, and G6PD is

required for the maintenance of glutathione in the reduced state. A deficiency of G6PD is the end result of two possible situations, either a reduced rate of synthesis of a normal enzyme, or a normal rate of synthesis of an abnormal enzyme, either stable, but with low specific activity, or unstable. The clinical manifestations of the deficiency therefore depend upon the level of enzyme activity and the properties of the enzyme. There is evidence that another factor, possibly genetically determined, operates in families with favism (Stamatoyannopoulos, Fraser, Motulsky, Fessas, Akrivakis, and Papayannopoulou, 1966) and the discovery of a family with chronic haemolysis and G6PD mediterranean raises the possibility that other factors may operate in this condition (Beutler, Mathai, and Smith, 1968).

Methods

ENZYME ASSAYS

All enzyme assays were conducted at 25°C. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (6PGD) were assayed by the methods of Kornberg and Horecker (1955), and Horecker and Smyrniotis (1955). Glutathione reductase was assayed by the method of Racker (1955).

GLUTATHIONE ESTIMATION

Reduced glutathione was estimated by the method of Beutler (1957) and expressed as milligrams per 100 millilitres whole blood.

PREPARATION OF HAEMOGLOBIN-FREE EXTRACT

Ten ml red cells from a normal control and 25 ml red cells from the G6PD-deficient patient II 1 were freed from haemoglobin by the method of Kirkman (1962). Modifications included the use of 1.0 mM EDTA in all buffers and lysing solutions, and the use of DEAE-Sephadex A-50 instead of DEAE-cellulose in the batch technique. The haemoglobin-free A 50 eluate was concentrated to about 1/20 of the original red cell volume by vacuum dialysis against 50 mM potassium phosphate buffer, pH 7.0, containing 1.0 mM EDTA, 2 μM NADP, 2 mM ε-amino caproic acid, and 0.1% v/v β-mercaptoethanol. These protective agents were also added to the eluate. Further purification by ammonium sulphate fractionation was unsuccessful, probably due to lability of the enzyme.

HORIZONTAL STARCH GEL ELECTROPHORESIS

This was carried out as by Fildes and Parr (1963).

NADP, 0.5 mg per cent w/v, was added to the gel before degassing and to the cathode vessel buffer. The gel was run at 4°C for 18 hours at 4.5 volts per cm of gel.

After slicing horizontally, one side of the gel was stained for G6PD and the other for 6PGD, both being visualized as purple bands of formazan at the site of enzyme action. Enzyme mobilities were expressed as percentages of the normal B type G6PD mobility which was taken as 100% on each run.

MICHAELIS CONSTANT (KM) FOR GLUCOSE PHOSPHATE

The following assay conditions were used: 0.05 M Tris-HCl buffer, pH 7.6, 0.016 M magnesium chloride, 0.1 mM NADP, 0.25-0.0625 mM glucose-6-phosphate, and enzyme extract, 0.5 ml from patient II 1, and 0.05 ml from a control patient. The K_m for glucose-6-phosphate was calculated from the negative intercept on the 1/S axis of the Lineweaver-Burke plot.

Other haematological studies were performed using the methods of Dacie and Lewis (1963).

Results

The results of haematological studies, enzyme assays, and glutathione estimations are shown in Tables I and II. The determinations on affected members of the family were carried out in the chronic phase, at the same time as the determinations on the other members of the family, and at least three months after the last haemolytic episode or blood transfusion.

The result of starch gel electrophoresis is shown in Figure 2. In the patient's extract, the G6PD shows 71% of the normal mobility of the B variant. Dilution of the normal extract to the level of activity in the deficient extract did not alter the enzyme mobility. No G6PD band could be seen in the patient's whole haemolysate. In a normal whole haemolysate, the G6PD band appears at the same position as in the normal partially purified extract.

In a normal whole haemolysate, and in both normal and G6PD-deficient extracts, the 6-phosphogluconate dehydrogenase shows 58% of the normal G6PD mobility. This is considerably slower than the variant G6PD described here.

Under the stated conditions the normal G6PD had a K_m for glucose-6-phosphate of 37 μ molar, while that of the enzyme from the deficient subject II 1 was 123 μ molar, showing a threefold increase. However, these results cannot be taken as conclusive proof of a lowered affinity of abnormal enzyme towards glucose-6-phosphate, owing to the contamination of

Fig. 2 G6PD electrophoresis showing (left to right) whole haemolysate from a normal control, haemoglobin-free extract from case II 1, and haemoglobin-free extract from a normal control.

both samples with 6-phosphogluconate dehydrogenase which was not corrected for in these assays.

White cell G6PD was found to be 2.7 expressed in units per unit of 6-phosphogluconate dehydrogenase activity compared with a level of 3.7 in a normal control. However, no G6PD band could be seen on an electrophoretic strip using a white cell extract.

Discussion

A number of enzyme variants have been characterized in patients with chronic haemolysis and G6PD deficiency. Beutler *et al* (1966) reviewed nine variants occurring in populations of western european origin, and Wong, Shieh, and Hsia (1965) described two found among the Chinese. The present studies show that the disease in our cases is associated with an enzyme variant with a very slow electrophoretic mobility which appears to differ significantly from those previously described. We suggest that this variant should be referred to as 'G6PD Fulham' in accordance with the recommendations of the World Health Organization (1967).

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