OCCURRENCE AND CLINICAL IMPLICATIONS OF RED-CELL GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN SOUTH AFRICAN RACIAL GROUPS*

RALPH E. BERNSTEIN, M.Sc., M.B., B.CH. (RAND), D.C.P. (LOND.)

Electrolyte and Metabolic Research Unit, South African Institute for Medical Research, Johannesburg

Aromatic drugs commonly used in medical practice and certain vegetable extracts may precipitate an acute, severe, self-limiting haemolysis — drug-induced haemolytic anaemia.[1,2] One form, favism, caused by ingestion of broad beans *(Vicia fava)* or inhalation of the pollen, has been known since the times of antiquity. The philosopher Pythagoras was aware of its dangers to certain individuals, but it has taken nearly 2,500 years for the reasons for his prohibition to be understood.[3] Favism is a well-documented haematological disorder of some frequency in Mediterranean races,[4,5] but rare elsewhere.[6]

Severe haemolysis was observed in healthy individuals, chiefly Indians and American Negroes and very rarely in Caucasians, who had been given primaquine to suppress malaria during World War II. This syndrome has since been extensively investigated as 'primaquine sensitive' haemolytic anaemia.[7] *Some* Negro infants who had been wrapped in clothes that had been stored in naphthalene (moth balls), or who had accidentally eaten moth balls, suffered a similar acute haemolysis.[8]

Up to 50% of the circulating red cells may be destroyed, with a fatal outcome from the effects of the massive haemolysis. On the other hand, recovery commonly occurs despite continuance of the haemolytic agent; this is due to the fact that the haemolytic action is on the older, circulating red cells, and results from an acceleration of the normal ageing process.[9]

These and other drug-induced haemolytic anaemias were originally described as separate entities. However, in the past 10 years, investigators from the USA,[7,10,11] Italy,[4] Greece and Israel[12] have shown that primaquine sensitivity, favism and allied phenotypic conditions have a common origin, viz. an inherited defect in the intrinsic metabolism of the mature red cell. The primary metabolic abnormality is a deficiency of glucose-6-phosphate dehydrogenase (G6Pd). The importance of this enzyme resides in its ability to generate reduced triphosphopyridine nucleotide (TPNH), a co-enzyme essential for the reduction of methaemoglobin to haemoglobin, and to keep reduced glutathione (GSH) and the sulphydryl (-SH) groups of red-cell proteins in a reduced state (Fig. 1). Sulphydryl compounds protect protein, including haemoglobin, from the processes of oxidation and denaturation, which are thought to be responsible for injury to the red-cell membrane with resultant haemolysis. In the absence of G6Pd, drugs capable of oxidant action stress this metabolic path and precipitate haemolysis of red cells. The secondary effects that occur in G6Pd-deficient erythrocytes, viz. a

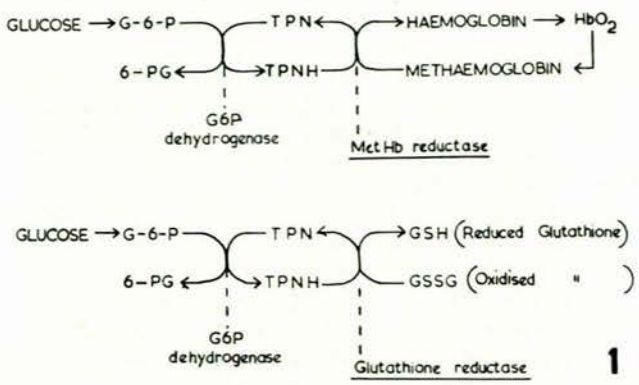

Fig. 1. Oxidative path of glycolysis in red cells with its relationship to glutathione and methaemoglobin reduction.

low level of GSH with marked instability of GSH, and increased Heinz-body formation on incubation with chemicals (e.g. primaquine, acetylphenylhydrazine) *in vitro* may be tested for.

Sensitive individuals are now known to suffer haemolysis when exposed to an extensive list of drugs that includes antipyretics (phenacetin, phenazone, acetylsalicylic acid[13]), antituberculotics (isoniazid, p-aminosalicylic acid), antimalarials (primaquine, pamaquine), antileprotics (dapsone, sulfoxone), bacteriostatics [nitrofurantoin ('furadantin'),[14] sulphonamides], and various aromatic chemicals such as the phenylhydrazines, phenothiazines, vitamin K, naphthalene and the fava bean principle.

The G6Pd deficiency is hereditary, with the genetic defect located on the X chromosome.[15] The male is, therefore, either normal or mutant. The female, with two X chromosomes, may be homozygous normal or homozygous mutant or heterozygous; in the latter case, intermediate degrees of the enzymatic defect are present with little adverse effect on exposure to drugs.

The defect is widespread throughout the world, but with a variable racial incidence. Reports to date indicate its rarity in North American and North European Whites,[7] Chinese,[7] South Americans[7] and Australian aborigines;[16] a moderate incidence in the American Negro[7,17] and certain Mediterranean races;[5,17] and higher incidences, up to 40%, in certain regions of the Middle East.[12,18]

Since the first report of a 10% incidence of the G6Pd deficiency in the American Negro, there have been studies in Africa, with indications of considerable variability. Incidences of 15 - 25% have been found in West Africa,[19,20] while an occurrence of 15 - 28% has been reported for coast and lake tribes in East Africa, compared with 1·7 - 2·9% for tribes in the Eastern Highlands.[21] Examination of 373 normal adult male South African Bantu mine labourers for glutathione instability revealed an incidence of 2·7%,[22] while other workers[22] reported 10 'sensitives' in 310 South African Bantu

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tested. It was therefore thought worth while to undertake a demographic survey of this genetic defect for the major racial groups of South Africa. The study was mainly confined to males, since they exhibit the deficiency in full.

MATERIAL

Samples were obtained mainly from subjects presenting at the SAIMR, Western Province and Natal Blood Transfusion Services. The following groups were examined:

1. Adult male Bantu (543) from the Witwatersrand gold mines, chosen at random. The tribal distribution was: Xhosa 24% (31%), Zulu 15% (26%), Msutu 11% (6%), Sechuana 11% (7·5%), Transvaal Shangaan 11% (5%), Pedi 6% (9·5%) and Pondo 6% (11%). The percentages in brackets represent the language affiliation for all South African Bantu; the various linguistic groups are thus reasonably represented.

2. Adult male (251) and adult female (253) Bantu residing in urban townships in the vicinity of Johannesburg.

3. Coloured subjects (211) of mixed ethnic origin, 134 from Johannesburg and 77 from Cape Town.

4. Male Cape Malays (51) from Cape Town.

5. Adult male Indians (200); samples from 150 of these were collected in Howick, Estcourt and Durban (Natal), 54% coming from Tamil-speaking Hindus and the rest from Urdu-speaking Moslems, generally a superior economic group. The remainder of the samples were collected in Johannesburg.

6. Male Europeans (250) bled at the SAIMR; 62% were of Afrikaans stock and 38% were of British origin. Smaller numbers of other ethnic groups (Greeks, Italians, Portuguese, Jews) were also examined.

Blood was collected and refrigerated in tubes containing 2·5 mg. (250 units) of heparin per 10 ml. of blood for all Johannesburg samples; analyses were completed within 24 hrs. All other specimens were taken in acid-citrate-dextrose (ACD), 1·25 ml. per 5 ml. of blood, and sent to the laboratory by air or train in refrigerated containers. Most of the analyses were completed the day after sampling.

METHODS

Various methods were employed to detect the GSH-unstable and G6Pd-deficient red cells:

(*a*) *Red-cell GSH.* A modification of the Thompson and Watson nitroprusside method[24] was used.[25]

(*b*) *Glutathione stability test.* The Beutler incubation procedure[26] was used. Initially, the blood was incubated with 5 mg. of phenylhydrazine (PH) per ml. of blood for 2 hrs. at 37°C. When acetylphenylhydrazine (APH) became available, 5 mg. of APH per ml. of blood were then used. Comparison of the GSH values after incubation with both agents for 133 consecutive samples showed that, while individual results differed, the average percentage stability was similar. The results have, therefore, been presented together.

(*c*) *Heinz-body formation in vitro.* Blood was incubated with buffered 0·1% APH for 4 hrs. The suspension was then mixed with crystal-violet solution and a coverslip preparation was examined under oil immersion for the number of Heinz bodies formed per red cell.[27] Difficulties in examining such slides led to the development of the following technique:[28] Blood was incubated with 0·25% APH for 1 hr., then spun and the supernatant removed. Crystal violet was added to the resuspended red cells for 15 - 30 minutes and the fluid was removed as completely as possible after spinning. A smear was made. Examination under phase-oil immersion (bright field also satisfactory) showed brilliant green-blue Heinz bodies, and the slide was permanent.

(*d*) *Glucose-6-phosphate dehydrogenase.* The quantitative spectrophotometric assay[29] was modified to give optimal conditions for red-cell haemolysates. The test consisted of adding glucose-6-phosphate (G6P) as substrate and triphosphopyridine nucleotide (TPN) as co-enzyme to the red cell haemolysate, and estimating the increase in optical density at 340 $m\mu$ brought about by the reduction of TPN (Fig. 1). The unit activity of the enzyme was expressed as the change in optical density of 1 per minute per gram of haemoglobin.

(*e*) *Visual dye screening test.* Motulsky[17] adapted the quantitative G6Pd assay as a screening method for red-cell G6Pd by incorporating brilliant cresyl blue (BCB). The dye underwent decolorization in the presence of TPNH formed (which depended on the activity of G6Pd): TPNH + BCB → TPN + leuco - BCB (colourless), and the time for decolorization was inversely proportional to the enzyme activity. The results reported here were based on a modified test using particular brands of dye (Sigma; National Aniline; Anachemia).[30] Other brands, e.g. B.D.H., Coleman-Bell, Gurr, etc., required longer decolorization times for normal enzyme activity.

RESULTS

GSH and G6Pd in Stored Red Cells

Red-cell GSH was found to decrease on storage, either in heparin or ACD. However, the fall in GSH content was slight in the first 48 hrs. in refrigerated samples.

Thereafter, the decline was more evident and had decreased to about 60% of the original levels in 3 - 5 days. The G6Pd of red cells stored at 4°C. was unaltered for periods of 7 - 14 days, but decreased fairly rapidly in haemolysates, even if deep-frozen.

Glutathione Levels in Red Cells

Over 1,600 estimations of basal GSH were made (Table I). The average values for each racial group (those regarded as normal by the GSH stability test and G6Pd assay) showed little difference between the races. Table II details values from the literature of a very similar order.

TABLE I. GSH VALUES AND STABILITY IN THE RED CELLS OF SOUTH AFRICAN RACIAL GROUPS

Subjects	*Sex*	*No. of normals*	*GSH (mg./100 ml. red cells)*		*No. of sensitives*
			No APH	*With APH*	
Bantu	F	251	74±13	61±10	2
Bantu	M	757	66±11	52±10	37
Bantu	M		51±11	23± 5	37
Coloured ..	M	156	69±12	56±11	3
Malays ..	M	50	64±10	53±11	1
Indians ..	M	149	63± 8	51± 9	1
Europeans ..	M	250	68±10	60±11	0

Glutathione Stability

Incubation with PH or APH produced a decrease of red-cell GSH. In the majority this was small, averaging some 8 - 15 mg. per 100 ml. in the different groups (Table I). The stability of GSH after incubation with PH, was 76% for some 600 samples (Table II), and the same effect was obtained with APH. Reports from the literature confirmed the slight action of these hydrazines for the concentrations used in normal red cells (Table II).

In a proportion, the GSH had fallen precipitously after incubation. Such subjects were initially classified as

TABLE II. NORMAL HUMAN RED-CELL GSH LEVELS AND STABILITY

Author	No.	GSH (mg./100 ml. red cells)	GSH stability %
Beutler (1957) ..	153	67±15	96 (APH)
Shahidi (1958) ..	18	81±11	69± 7 (APH)
Gross (1958) ..	76	49±12	89±10 (APH)
Childs (1958) ..	328	53—84	
Thompson (1952)	21	31*	
Bernstein (1960)	600	69±11	76±12 (PH)

* Per 100 ml. whole blood.

'sensitives' on Beutler's criterion[26] — a GSH level after incubation of less than 30 mg. per 100 ml. For the first 460 stability tests performed on mine Bantu, there were 25 sensitives, of whom 22 were shown to be G6Pd-deficient quantitatively. The results of this assay *(vide infra)* indicated that GSH levels after incubation of 25 mg. per 100 ml. or less had slight or nil G6Pd activity, while GSH levels between 25 and 35 mg. per 100 ml. might or might not be G6Pd deficient. Additional tests (Heinz-body formation, quantitative G6Pd assay and later, the brilliant cresyl blue screening test) were therefore performed on all subjects with post-incubation levels of GSH of less than 35 mg. per 100 ml. Subsequently, Beutler[31] recommended that all subjects with GSH levels below 20 mg. per 100 ml. after incubation should be regarded as sensitives. On this basis, only 8 of the above 25 would be so classed. Slight differences in the method of performing the stability test may be responsible for these discrepancies.

The GSH results on all male Bantu are presented in Fig. 2. There were 41 subjects with GSH $<$ 30 mg. per 100 ml. after incubation, of which 4 were found to have G6Pd within normal limits. The average GSH values before and after incubation for 757 normals and 37 sensitives (4·7% incidence) are shown in Table I and Fig. 2.

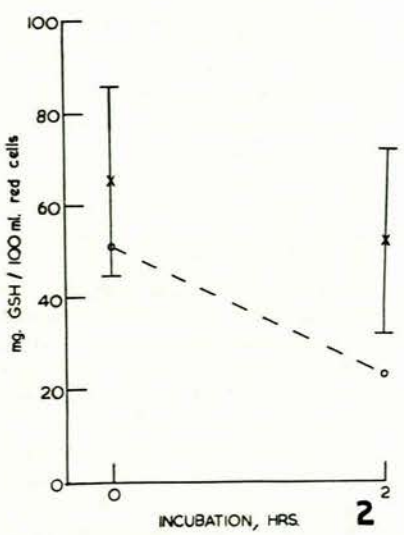

Fig. 2. Reduced glutathione (GSH) in red cells of 757 male Bantu (x) and 37 sensitives (o) before and after incubation with phenylhydrazines. The normal range is shown by the vertical lines.

The average pre-incubation GSH level for sensitives was 51 mg. per 100 ml., a value significantly different from the normals; however, there was overlap of the values, so that the basal level was not useful as a test for sensitivity. After incubation, the average GSH for sensitives was 23 mg. per 100 ml., less than half that for the normals.

Heinz-body Incubation Test

All samples having a post-incubation GSH value of less than 35 mg. per 100 ml., together with normal post-incubation GSH samples between 35 and 60 mg. per 100 ml. as controls, were tested. Smears showing a single large Heinz body in the majority of cells (Fig. 3A) were classified as normals; less than 25% of red cells had 5 or more Heinz bodies per cell. All had normal G6Pd activity. Slides with more than 50% of red cells having 5 or more Heinz bodies per cell were of enzyme-deficient cells (Fig. 3B). Samples having 25 - 50% of erythrocytes with 5 or more Heinz bodies corresponded to the borderline cases of the glutathione stability test.

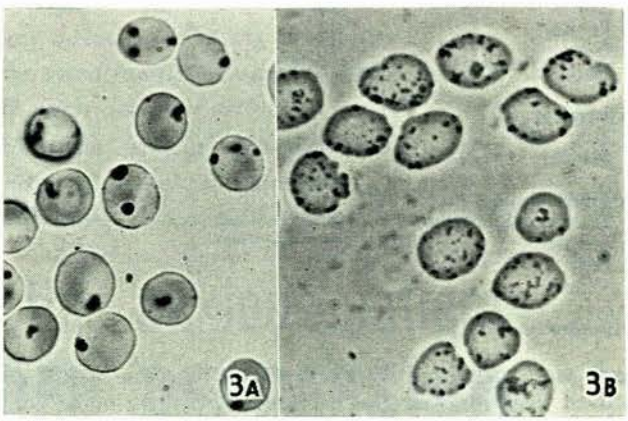

Fig. 3. Heinz-body incubation test. A. Normal blood showing usually one large Heinz body per cell. B. 'Sensitive' blood with G6Pd-deficient and GSH-unstable red cells.

Glucose-6-phosphate Dehydrogenase Activity

All specimens having a GSH value less than 35 mg. per 100 ml. on incubation, together with 25 normals by the GSH stability test, were assayed. The results fell into two distinct series, those having an activity of 9 - 16 units/minute/G. of haemoglobin and regarded as normal, and those with activities of 0 - 2 units/minute/G. of haemoglobin and classed as sensitives. The G6Pd activity of the sensitives was less than 10% of the normals.

Visual Screening Test

Toward the end of this survey, the visual screening test was used for 208 male Bantu samples from Table I, together with a further 50 Indian and 52 Coloured specimens. Decolorization times were 30 - 75 (usually 30 - 50) minutes for those regarded as normal by the quantitative G6Pd assay. Enzyme-deficient red cells took more than 2 hrs. to decolorize. No false reactions were found.

Incidence of Defect

Table I details the number of sensitives for each group. In addition, one sensitive was found in 50 Indian samples and no sensitives in 52 Coloured samples tested by the brilliant cresyl blue screening test. No sensitives were detected in the small groups of other ethnic European groups tested. Only the Bantu showed more than a 2% incidence. The tribal origins are detailed in Table III, indicating a range of incidence up to 10%.

Clinical

Nine Bantu patients with acute haemolytic anaemia, where no apparent cause was found and other diagnostic

TABLE III. GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN SOUTH AFRICAN MALE BANTU

Subjects	*No. tested*	*Sensitives*	*%*
Southern group:			
Xhosa	184	7	3·8
Pondo	44	0	
Baca	30	0	
Hlubi	17	1	
Central group:			
Zulu	116	5	4·3
Msutu	85	4	4·7
Northern group:			
Transvaal Shangaan ..	81	8	9·9
Sechuana	85	6	7·0
Pedi	45	3	6·7
Venda	9	1	
Ndebele	12	1	
Miscellaneous	49	1	

tests were negative, were examined by these tests. All proved negative, and the haemolysis was attributed to a direct toxic action of some unknown chemical; in one case, an auto-immune mechanism was thought responsible, but the chemical could not be identified.[38]

DISCUSSION

A battery of tests is available for the detection of persons genetically susceptible to drugs and for the study of cases of drug-induced haemolytic anaemia. The quantitative G6Pd assay was used as a direct measure of the defect; it clearly differentiated normals from sensitives, but required expensive apparatus and chemicals. The GSH stability test has been extensively used as an indicator of G6Pd activity. However, factors are apparent that affect basal GSH and G6Pd levels differently. The GSH levels of red cells are influenced by the intake of protein, carbohydrate and vitamin-B complex, while thyroid and certain steroid hormones are inhibitory to G6Pd activity. Further, in the GSH incubation test, the solution of the dried APH and exposure of the red cells to its action during incubation may not be uniform, and may be a source of difficulty in interpreting the intermediate results. The qualitative Heinz body and brilliant cresyl blue tests were also unable to differentiate the borderline cases. Thus the GSH stability, Heinz body and visual dye tests should be used as screening tests, and the borderline tests assayed by a quantitative G6Pd test.

The survey of some 1,800 persons comprising the major South African races revealed a low incidence of G6Pd deficiency in all groups except the Bantu. The defect is a rarity in Caucasians, e.g. only 5 cases of enzyme-deficient haemolytic anaemia have been reported from Great Britain since 1955. It is to be noted that South African Jews, being descended from European Jews, showed absence of the defect; in Israel, Oriental Jews showed a high incidence, with a virtual absence for Jews from Europe.[12] For Mediterranean races, the defect is widespread in Greece, with incidences up to 3%,[5] while in Italy there is a negligible occurrence on the mainland, but a 5 - 35% incidence in Sicily, Sardinia and parts of Southern Italy.[17] The presence of this inborn error has been reported from Portugal;[17] however, no sensitives were found in 80 recent Portuguese immigrants examined.

There were 2 sensitives in the 200 Indians tested. The low incidence is worthy of comment. South African Indians are descendants of immigrants coming mainly from South India (Madras area) 60 years ago. A higher incidence is reported for Indians from other regions, e.g. 5% for Bengalese,[32] 3·3% for Singapore Indians,[33] and 27 of 200 Punjabis suffering from haemolytic anaemia on taking primaquine.[34]

The Cape Malays are a group that have preserved their customs since coming from the Dutch East Indies in the 17th century, but have some racial admixture with other South African groups. The number tested was small, with 1 sensitive in 51 subjects. An incidence of similar order has been reported from Singapore, viz. 0·65% in 155 Malayans tested.[33]

The overall incidence in 794 male Bantu was 4·7%. In certain tribes the incidence was higher, up to 10%. These differences in gene frequency may be geographical rather than tribal, since the central and northern tribes had higher incidences than those for southern areas (Transkei). Selected groups are being studied further in this respect. The lower incidence of 1·4% in Coloured subjects probably reflects their mixed racial origin.

Haemolytic reactions to drugs may occur from (1) hypersensitivity to a commonly used drug; (2) a drug with a direct toxic action — this may be taken without the doctor's knowledge; (3) rarely, an auto-immune mechanism where the patient develops an antibody to a drug — red-cell complex;[35] and (4) a drug in an enzyme-deficient subject.

Many industrial workers are exposed to various chemicals. Despite the fact that a haematological survey of industrial workers with enzyme-deficient erythrocytes did not reveal any increased haemolysis,[36] the possibility of an industrial hazard is being examined for Bantu and other groups with G6Pd deficiency.

Treatment of diseases in South Africa frequently requires the use of drugs (e.g. vitamin-K analogues in the newborn, sulphonamides and nitrofurans for infections, sulphones for leprosy, isoniazid and p-aminosalicylic acid for tuberculosis, etc.) that are known to induce haemolysis in enzyme-deficient subjects. The occurrence of haemolytic anaemia attributable to the ingestion of unknown chemicals, drugs and herbal remedies, is of some frequency in the Bantu, but the precipitating cause is rarely ascertained. A haemolytic episode of unknown origin in a Bantu subject, whose tribe is known to have a high incidence of G6Pd deficiency, should lead to tests for the exclusion of enzyme-deficient red cells as the basic cause.

With the influx of immigrants from Mediterranean countries, both favism, from the use of the broad bean, pea and other plants, and drug-induced haemolysis, may be precipitated in sensitive subjects, since the incidence of enzyme deficiency is appreciable in peoples from certain Mediterranean regions. From Australia, some 20 haemolytic episodes in 15 children of Mediterranean racial extraction have recently been reviewed.[37] The precipitating agent was usually the broad bean, but sulphonamide,

phenacetin, chloramphenicol and a vitamin-K analogue were thought to be responsible in other cases. *The dangers of drug-induced haemolysis should thus be borne in mind in certain races in South Africa.*

SUMMARY

A sex-linked inherited deficiency of glucose-6-phosphate dehydrogenase in red cells with associated instability of glutathione is responsible for an acute, severe self-limiting haemolytic anaemia on exposure to certain aromatic drugs and vegetable substances. The possible mechanism of haemolysis is outlined, and laboratory tests for the detection of the defect are indicated. The reduced glutathione stability, Heinz-body incubation and brilliant cresyl blue tests were used as screening tests to detect the deficiency, with the quantitative glucose-6-phosphate dehydrogenase assay for deficient and borderline cases.

In 1,800 samples examined from the major South African racial groups, the incidence was low or negligible for Europeans, Coloureds, Malays and Indians. The enzyme defect averaged 4·7% in the Bantu, ranging from 0 to 10% for different tribal and geographic groups. The entity of deficient red-cell glucose-6-phosphate dehydrogenase should be considered in the differential diagnosis of cases of acute haemolytic anaemia.

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