Update/Le point

Glucose-6-phosphate dehydrogenase deficiency*

WHO Working Group[1]

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the commonest enzyme disorder of human beings and a globally important cause of neonatal jaundice, which can lead to kernicterus and death or spastic cerebral palsy. It can also lead to life-threatening haemolytic crises in childhood and at later ages, by interacting with specific drugs and with fava beans in the diet. The complications of G6PD deficiency can largely be prevented by education and information, and neonatal jaundice can be successfully treated by phototherapy, a cheap and simple approach suitable for use in primary health care.

This update describes developments in the methodology for characterizing G6PD deficiency, recent knowledge of the factors that can cause haemolysis, community approaches for prevention of haemolytic crises and neonatal jaundice, and the implications of recent advances at the DNA level.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the commonest disease-producing enzyme disorder of human beings. More than 300 variants of G6PD characterized by standard methods are now known, and the recent isolation of the *Gd* gene promises important fundamental advances in the understanding of enzyme structure and function.

Since comprehensive reviews already exist (*1-3*), this article deals with recent developments in the methodology for characterizing G6PD deficiency, its epidemiology and the factors that can cause haemolysis, re-evaluation of its relevance for public health, community approaches for prevention of haemolytic crises and neonatal jaundice, and the implications of recent advances at the DNA level.

Role of the enzyme

Glucose-6-phosphate dehydrogenase is a "housekeeping" enzyme, vital for the life of every cell. Complete absence of the enzyme is unknown in the human species. Within the restricted metabolism of the red cell, G6PD occupies a particularly important place. It catalyses the first step in the hexose monophosphate pathway, converting glucose-6-phosphate to 6-phosphogluconolactone and reducing the cofactor nicotinamide-adenine dinucleotide phosphate (NADP) to NADPH. The second enzymic step in the pathway is also associated with the reduction of NADP to NADPH.

In the red cell this pathway is the only source of NADPH, which is necessary to protect the cell and its haemoglobin from oxidation in view of their role in oxygen transport. The $-SH$ groups of several enzymes and of the β-chain of haemoglobin are particularly vulnerable to oxidation, with potentially serious consequences. Protection against oxidation is mediated by glutathione which is actively synthesized and is present in high concentration in red cells, almost entirely in the reduced form (GSH) (Fig. 1). The latter can restore oxidized $-SH$ groups, and reacts with peroxides via glutathione peroxidase, becoming itself oxidized (to GSSG) in the process. NADPH is required for regeneration of GSH by the enzyme glutathione reductase; this is considered to be the most important function of NADPH in the red cell.

Though G6PD deficiency affects every cell in the body, its primary effects are haematological because the red cell has no alternative source of NADPH. Other more complex types of cells are protected by additional enzyme systems (such as the less specific

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* This article is based on the updated version (March 1989) of the report of a WHO Working Group on Glucose-6-Phosphate Dehydrogenase Deficiency, which met in Geneva on 3-4 September 1985. Requests for single copies of the full report (document WHO/HDP/WG/G6PD/85.9) or reprints of this article should be sent to Hereditary Diseases Programme, Division of Noncommunicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland. A French translation of this article will appear in a later issue of the *Bulletin*.

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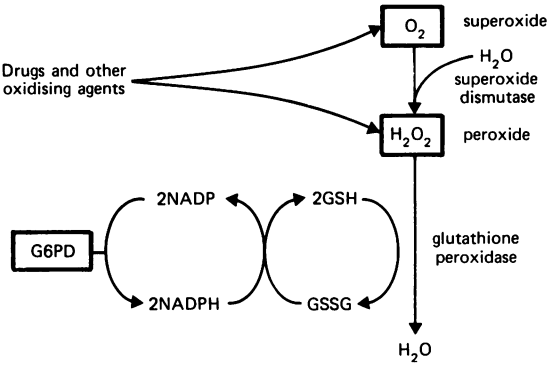
Fig. 1. **G6PD generates the NADPH which protects the red cell against peroxides and superoxides generated by oxidative stresses.**



hexose-6-phosphate dehydrogenase) that can generate NADPH in the absence of adequate G6PD activity.

In normal intact red cells NADP is mostly in the reduced form NADPH, and G6PD operates at only about 2% of its theoretical maximum rate. This is because, under normal circumstances, (1) the quantities of glucose-6-phosphate and NADP are well below saturating levels, (2) NADPH and ATP inhibit the enzyme, and (3) most of the NADP present is not free but bound to catalase. Oxidative stress leading to increased oxidation of NADPH simultaneously releases enzyme inhibition and increases the level of NADP, so that G6PD activity increases proportionately. Consequently, the normal red cell responds to oxidation by increasing its reducing capacity, and its large reserves allow it to deal with very significant levels of oxidative stress. This is why a major reduction in G6PD activity has little clinical effect under ordinary circumstances, but may become dramatically apparent in the presence of oxidative stress.

The activity of G6PD, like that of most other red cell enzymes, diminishes as the cell ages; in G6PD-normal red cells, lack of enzyme never endangers cell survival. However, in G6PD-deficient cases the older red cells are even more deficient than the younger ones, and G6PD eventually becomes a limiting factor. This is probably the main cause of the associated mild haemolysis and reduced red cell life-span.

The normal G6PD enzyme is genetically polymorphic. The most common form worldwide is the B form, and many pathological variants are presumably derived from this form. However, in Africa up to 40% of the population carry an electrophoretically different variant, the A form.

The gene for G6PD is located on the X-chromosome, and G6PD deficiency is inherited in a sex-linked fashion, being fully expressed in hemizygous males and homozygous females, but in only a proportion of female heterozygotes. In general, in adult females only one X-chromosome in each cell is active, the other being inactive (visible as the so-called Barr body). The pattern of X-chromosome inactivation is essentially random, so that most female heterozygotes for G6PD deficiency carry a double population of red cells, some with and some without the enzyme deficiency, with an average of about 50% of each. However, in individuals there may be substantial deviations from the mean. Because of this mosaicism in heterozygous females, G6PD has become quite popular in analysing tumour or other abnormal cell populations, to find out whether they are homogeneous or heterogeneous. When the population is homogeneous it is also presumed to be monoclonal.

Disadvantages of G6PD deficiency

G6PD deficiency certainly carries a disadvantage in terms of the potentially lethal or crippling complications it can cause in hemizygous males and some females. Considering the contribution of neonatal jaundice to mortality and chronic morbidity in developing countries, the disadvantage must be substantial,[a] but to assess this accurately is difficult because of inadequate statistical data. The high frequency of G6PD deficiency in so many populations, however, points to a selective advantage that must outweigh or balance the disadvantages. It has been demonstrated both clinically and *in vitro* that, like the haemoglobinopathies, G6PD deficiency confers powerful protection against *Plasmodium falciparum* malaria, which accounts for its high frequency in nearly all parts of the world where malaria is, or has been, common.

It is interesting that the selective advantage appears to favour the heterozygous female more than the hemizygous male. This may be because the existence of two red cell populations ensures the exposure of the parasites alternately to a G6PD-rich and a G6PD-depleted environment, so that adaptation of the parasite does not occur on a long-term basis, and the female is protected (*4*).

A very large number of variants of G6PD are known. However, as with the abnormal haemoglobins, many are rare, or restricted to particular locations, and have been identified either because they cause a chronic haemolytic anaemia, or because testing is so simple that many large surveys have been conducted. Most of the rare variants do not have any public health significance, but, like the abnormal haemoglobins, simply confirm that, at some time, every mutation that can occur will occur. This article is concerned mainly

[a] **World Health Organization.** *Informal Consultation on Serious Childhood Diseases: Priority issues and possible action at family, community and health centre levels.* Working paper for the Meeting in Geneva, 9–11 July 1984.

with those variants that are common, and cause enough pathology to constitute a public health problem.

In principle, an enzyme deficiency may be caused by absent synthesis, decreased synthesis, accelerated breakdown, decreased catalytic activity, or a combination of these causes. Neither absent synthesis nor decreased synthesis of G6PD has yet been demonstrated, which suggests that the responsible mutations are most commonly to be found in the coding, rather than in the non-coding sections of the gene. Accelerated breakdown with or without decreased activity is probably the commonest cause of G6PD deficiency, and decreased activity alone also occurs. These quantitive changes may also be associated with important qualitative changes in the functional properties of the enzyme.

Over 300 allelic variants are now known, divided into five classes according to the level of enzyme activity in the red cells, and the clinical manifestations (Table 1). Class I includes severely deficient variants that are associated with a chronic non-spherocytic haemolytic anaemia (CNSHA). Class II variants have less than 10% of residual enzyme activity but without CNSHA, and include the common Mediterranean and common severe oriental variants. Class III variants are moderately deficient (10–60% residual enzyme activity), and include the common African (A) form. Class IV variants have normal enzyme activity, and in class V the enzyme activity is increased. In practice, clinical manifestations are confined to variants associated with enzyme deficiency, and the common pathological variants are all in classes II and III. From the public health point of view, the importance of a variant depends on its clinical implications and its prevalence: for the purpose of this report, a variant is considered common, or polymorphic, if it occurs with a frequency of 1% or more among males in a particular population.

Diagnosis and screening

A WHO Scientific Group in 1967 (*1*) recommended that the following enzyme characteristics should be used for identification of G6PD variants: (*a*) red cell G6PD activity; (*b*) electrophoretic migration; (*c*) Michaelis constants (K_m) for G6P; (*d*) relative rate of utilization of 2-deoxy G6P (2dG6P); and (*e*) thermal stability. The general adoption of these recommendations has greatly improved the quality of the information available on G6PD deficiency. Comments and recommendations derived from further experience are given in the more recent WHO report, on which the present article is based (see footnote on p. 601).

At the public health level, very simple screening methods for G6PD deficiency regardless of type are needed because, in principle, it is desirable to detect:

— hemizygous males (and homozygous females) in order to advise them to take simple precautions to avoid an acute haemolytic crisis;
— newborn hemizygous males (and homozygous females) to ensure that neonatal jaundice is detected and treated early;
— heterozygous females in order to give specific advice about the care of their male newborn infants.

Diagnosis of G6PD deficiency in adults and neonates by quantitative estimation of the enzyme level in red cells is very easy. The method described in the 1967 report detects all hemizygous males, all homozygous females, and more than 80% of female heterozygotes, but requires basic biochemical laboratory equipment that is not available in many areas

Table 1: Summary of G6PD variants[a]

Class[b]	No. of polymorphic	Electrophoretic mobility			% with electrophoretic change	Total
		Fast	Normal	Slow		
I	1 (1)[c]	20	30	32	63	82
II	49 (45)	29	39	41	64	109
III	22 (30)	25	13	36	82	74
IV	14 (32)	15	2	26	95	43
V	—	2	—		100	2
Total	86 (28)	91	84	135	73	310

[a] Source: reference *2*

[b] Class I —associated with chronic non-spherocytic haemolytic anaemia (CNSHA).
Class II —severely deficient: less than 10% residual activity.
Class III—moderately deficient: 10–60% residual activity.
Class IV—normal activity: 60–150%.
Class V —increased activity.

[c] Figures in parentheses are percentages.

where G6PD deficiency is most common (*1*). That report describes six simple methods that are perfectly adequate for a semi-quantitative assessment of screening for normal and deficient males (and homozygous females), but do not reliably detect heterozygous females. A seventh screening test, the ultraviolet spot test, has also been described.[b] These screening tests are quick, easy, very inexpensive, and suitable for use on large population samples. When possible, neonatal screening using one of these methods should be routinely performed on babies in populations with a high prevalence of G6PD deficiency.

Geographical distribution and frequency

The frequency of G6PD deficiency is usually expressed as the proportion of a sample of males that is found to be hemizygous. This figure is usually considered to be equal to the gene frequency; the prevalences of G6PD-deficient females and female heterozygotes are derived by calculation. The results, which are probably a good approximation of the true figures, should be viewed with some reservations, since so little is still known about the mechanism and relative effectiveness of the selective advantage conferred by the gene in males and females. A second difficulty in estimating the frequency of G6PD deficiency on a large scale is the fact that it can vary markedly, even over a small area. Nevertheless, largely based on the data compiled by Livingstone (*5*), the approximate frequencies of G6PD deficiency in the different countries of the world are summarized in Fig. 2 and Table 2.

G6PD deficiency is a significant public health problem. About 7.5% of the world population carry one or two genes for G6PD deficiency, the proportion ranging from a maximum of 35% in parts of Africa, to 0.1% in Japan and parts of Europe. About 2.9% of the world population are genetically G6PD-deficient. It is important to note that though X-linked disorders are usually thought to affect males only, in this case, because of the high frequency of the gene, and the high incidence of consanguineous marriages, homozygous females contribute about 10% of those genetically G6PD-deficient. In addition, perhaps 10% of heterozygous females are also effectively G6PD-deficient due to unequal inactivation of their X-chromosomes, so about 3.4% of the world population are at risk for complications of G6PD deficiency. With about 130 million births annually, about 4.5 million G6PD-deficient children particularly vulnerable to neonatal jaundice and acute haemolytic crises are born every year. Furthermore, owing to global migrations, the complications of G6PD-deficiency may now occur in at least certain population groups in most countries and regions of the world.

Clinical aspects

G6PD deficiency is a globally important cause of neonatal jaundice, which can lead to kernicterus and death or spastic cerebral palsy. It can also lead to life-threatening haemolytic crises in childhood and at later ages by interacting with specific drugs, and with fava beans in the diet. The frequency and severity of these complications is heavily influenced by extrinsic and cultural factors and by other genetic tendencies.

In normal circumstances, heterozygous females show no haematological abnormality. However, a very mild chronic haemolysis is present in hemizygous males with both the *Gd* A(–) and the Mediterranean variants (see below). The mean red cell life-span is reduced to about 100 days, and the mean Hb level is about 1g/dl less than that in unaffected males (*6*). This is no doubt one of the basic causes of jaundice in the neonatal period.

Neonatal jaundice

The association between G6PD deficiency and neonatal jaundice has been reported independently in many countries, and is now unquestioned (*3*). However, the nature of the association is far from clear, since by no means all G6PD-deficient babies develop jaundice. The extent of the risk seems to vary considerably between populations, and in the same population in different environments, and even at different times in the same population. Clearly, it is affected by many genetic, exogenous and cultural factors.

Known relevant variables are as follows:

— the nature of the variant concerned, and the level of G6PD activity in the liver;
— the genetic background (for instance, there is good evidence of genetically-determined slower maturation of liver enzymes in Asian than in European babies, while genetic variation in other red-cell enzymes would be expected to have an additive effect);
— exogenous factors such as the maturity of the infant (neonatal jaundice is more severe and more harmful in premature infants) and the method of feeding (neonatal jaundice is more common in breast-fed babies);
— exposure of the newborn to environmental agents, such as clothes that have been stored in contact with naphthalene (moth) balls, or herbal medicines, or dressing the umbilical cord with antiseptic "mentholated" powders;
— the frequency of infectious causes of neonatal jaundice in newborns, such as umbilical sepsis (G6PD

[b] *Report of a WHO Working Group on Glucose-6-phosphate Dehydrogenase Deficiency (G6PG), Geneva, 3–4 September 1985* (unpublished document WHO/HDP/WG/G6PD/85.9), Annex 2, p. 37.

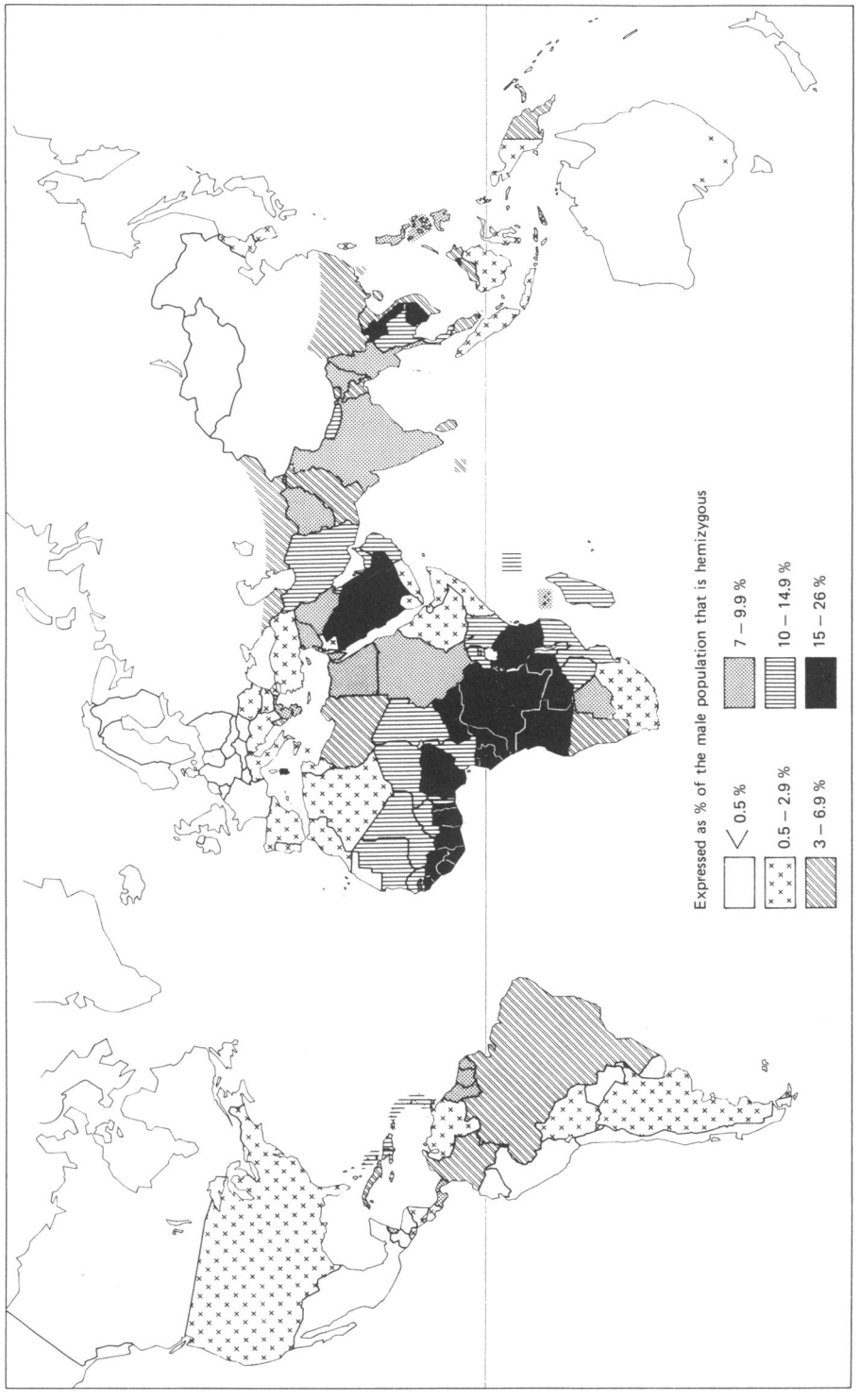


Fig. 2. **World distribution of G6PD deficiency.**

Table 2: **Global frequency of G6PD deficiency (assuming that birth incidence is approximately equal to adult prevalence)**

Region	% of all births (male + female)				
	Male hemizygote	Female homozygote	Female heterozygote	Total with 1 or 2 genes for G6PD deficiency	G6PD-deficient (estimated total)[a]
Africa	5.6	0.9	9.4	15.9	7.4
Americas	1.4	0.09	2.4	3.9	1.7
Asia	2.3	0.2	4.2	6.7	2.9
Europe	0.34	0.02	0.67	1.0	0.4
Oceania	0.9	0.06	1.8	2.8	1.1
Total	2.6	0.3	4.6	7.5	3.4

[a] Figure obtained by adding % of male hemizygotes, % of female homozygotes, and 10% of female heterozygotes.

deficiency leads to a higher serum bilirubin rise in these circumstances);
— the consumption of potentially haemolytic agents, such as fava beans, drugs or herbal remedies by pregnant heterozygotes (haemolytic factors may be transmitted to the fetus in the mother's blood, and to the newborn in her milk).

However, even when all such variables are taken into account, there are still unknown factors affecting the frequency and severity of neonatal jaundice associated with G6PD deficiency. For instance, an interesting discrepancy has been noted between the high frequency of the condition among African babies and a lower frequency among Black American babies, though recent work suggests that perhaps the discrepancy is smaller than had been thought. It is also possible that the level of vitamin E may be relevant, and the determination of its level in the plasma of newborns could be of considerable interest.

Favism

This term is used to describe the occurrence of an acute haemolytic reaction (see below) in a G6PD-deficient individual following the ingestion of broad beans (*Vicia faba*). It occurs most frequently in children below 5 years of age, is relatively uncommon in adults (*7*), and can be fatal. It used to be common in Mediterranean areas where fava beans are often eaten, but has not been described in Africans. It is therefore thought to be associated with the common Mediterranean B(–) form, rather than with the common African A(–) form of G6PD.

Acute haemolytic anaemia

G6PD-deficient individuals are at risk for iatrogenic disease due to drug administration. In fact, G6PD deficiency was discovered through an investigation of the haemolytic anaemia induced by the antimalarial drug primaquine in some Black American soldiers during the Second World War (*8*). Since then, several other drugs have been found to have a similar effect, due to oxidizing activity, which depletes the reduced glutathione in red cells and converts NADPH to NADP. The mechanism of red cell destruction during the haemolytic crisis is still poorly understood, but it is clear that oxidative damage leads to denaturation and precipitation of haemoglobin to form Heinz bodies, which cause the red cells to become trapped in the spleen, where they are destroyed.

Acute haemolysis begins within one or two days of the administration of the drug. The reaction may vary from transient mild anaemia to rapidly progressing anaemia with back and abdominal pain, jaundice and haemoglobinuria, and transient splenomegaly. Heinz bodies are found in the red cells in the peripheral blood.

One of the most curious features of the acute haemolytic reaction is that it is erratic, in the sense that the same agent may cause haemolysis in one G6PD-deficient person but not in another, and in the same person at one time but not another. Differences between individuals may be determined by different G6PD variants and other genetic differences within the red cell, or in some other organ such as the liver. Genetic factors affecting the rate of absorption and metabolism of the drugs may also be involved, e.g., the so-called fast-acetylator/slow-acetylator polymorphism, which influences the rate at which certain drugs are inactivated in the liver. However, genetic factors cannot be invoked to explain intra-patient variability. The differences must be due to something in the environment, involving more than one triggering factor. For instance, when a patient is pyrexial in association with infection it is usually assumed that the infection causes haemolysis; but it is also possible that fever in itself causes haemolysis, though this has not been definitely demonstrated.

In order to prevent the above complications, it is important both to screen newborns to identify affected males, and to clearly identify drugs that can cause an acute haemolytic reaction. For the sake of the affected individuals, it is as important to avoid incriminating useful drugs that are innocent, as to identify those that are responsible. The list proposed by Beutler (*2*) is recommended, with some modifications (Table 3).

Tests for drugs that might lead to acute haemolytic anaemia. The danger of precipitating an acute haemolytic reaction in the significant, G6PD-deficient section of the world's population is obviously an important consideration in the development and testing of new drugs, especially antimalarials and other agents that might be used extensively in the tropics. Recommendations are needed for methods of testing drugs for their ability to cause haemolysis.

Two *in vitro* tests have been extensively used. One is based on measuring the fall in gluthathione under drug challenge in a system consisting of G6PD-deficient red cells, an NADPH-generating system, and a preparation of rat liver microsomes (*9*). This method has the advantage that measurement of glutathione stability is very simple, but on the other hand, though the preparation of rat liver microsomes metabolizes drugs, there is no guarantee that the metabolic products are indeed the same as those circulating in the plasma of a person who has ingested that drug. The second test depends on measuring the hexose-monophosphate pathway activity in G6PD-normal red cells in the presence, and in the absence of a drug (*10*). Instead of

Table 3: **Drugs to be avoided in G6PD deficiency**

- Drugs given below in **bold** print should be avoided by people with all forms of G6PD deficiency.
- Drugs in normal print should be avoided, in addition, by G6PD-deficient persons of Mediterranean, Middle Eastern, and Asian origin.
- Items in normal print and within square brackets apply only to people with the African A(−) variant.

Antimalarials

Primaquine [people with the African A(−) variant may take it at reduced dosage, 15 mg daily or 45 mg twice weekly under surveillance]

Pamaquine

Chloroquine (may be used under surveillance when required for prophylaxis or treatment of malaria)

Sulphonamides and sulphones

Sulphanilamide
Sulphapyridine
Sulphadimidine
Sulphacetamide (Albucid)
Sulphafurazone (Gantrisin)
Salicylazosulphapyridine (Salazopyrin)
Dapsone[a]
Sulphoxone[a]
Glucosulphone sodium (Promin)
Septrin

Other antibacterial compounds

Nitrofurans—Nitrofurantoin
Furazolidone
Nitrofurazone
[Nalidixic acid]
Chloramphenicol
p-aminosalicylic acid

Analgesics

Acetylsalicylic acid (Aspirin): moderate doses can be used
Acetophenetidin (Phenacetin)
Safe alternative: paracetamol

Anthelminthics

β-naphthol
Stibophan
Niridazole

Miscellaneous

Vitamin K analogues (1 mg of menaphthone can be given to babies)
Naphthalene[a] (moth balls)
Probenecid
Dimercaprol (BAL)
Methylene blue
Arsine[a]
Phenylhydrazine[a]
Acetylphenylhydrazine[a]
Toluidine blue
Mepacrine

[a] These drugs may cause haemolysis in normal individuals if given in large doses. Many other drugs may produce haemolysis in particular individuals.

using the drug itself, plasma from a person who has taken the drug is used. The correlation between clinical haemolysis in G6PD deficiency and both these tests is considered to be rather good, although a few exceptions have been noticed. Both these *in vitro* tests are very promising, and should be perfected with a view to incorporating one or both of them into compulsory drug-testing requirements, before a drug is marketed in regions where G6PD deficiency is common.

The use of *in vivo* tests may also be considered when appropriate. Traditionally, these have been done in two ways: by administering the drug directly to a G6PD-deficient human volunteer, which today would generally be regarded as ethically unacceptable; or by transfusing a small amount of chromium-labelled G6PD-deficient red cells into a normal person, and then challenging that person with the drug. This too is becoming more objectionable, since serious conditions can be transmitted through blood. A third possibility would be an *in vivo* test in a heterozygote. Although there is a potential for haemolysis, if the woman has been shown to have at least 50% normal cells, severe effects are less likely.

Health burden and prevention

It appears that about 7% of the world population carries the gene for G6PD deficiency, the average ranging from 16% in Africa to 1% in Europe. The health burden due to neonatal jaundice associated with G6PD deficiency, however, has not been adequately assessed, partly because of the difficulty of allowing for all the variables involved. Rough estimates can be derived from data in the existing literature.

In Singapore, where the population is largely of southern Chinese origin with an admixture of Malay and Indian, 3.1% of male Chinese babies and 3.5% of male Malay babies are hemizygous. Therefore, in 1953, about 822 male babies out of the total of 53 000 babies born were hemizygotes at risk for kernicterus. In the same year there were 146 deaths due to kernicterus, of which just under half (about 63) were associated with G6PD deficiency (*11*). This represents a mortality of 8% among the infants at risk, and 1.2 per 1000 of all births. If allowance is made for an equal number surviving with spastic cerebral palsy, the morbidity and mortality due to the condition in this place at that time may have involved more than 16% of hemizygous males.

A significant proportion of this severe pathology was traced to Chinese customs, including the use of moth-balls to preserve clothes, and the consumption of herbal remedies by pregnant women, and their administration to the newborn (*11*). A successful educational campaign, combined with heterozygote diagnosis, phototherapy, and exchange-transfusion when required, has since reduced the incidence of kernicterus to a vanishingly low level in Singapore. These figures indicate the importance of G6PD deficiency in the newborn period in the whole of south-east Asia. They also demonstrate the possibility of limiting morbidity and mortality due to neonatal jaundice by very simple methods. The study may be especially relevant for southern China, where the particular combination of the gene and the exacerbating environmental circumstances is probably still to be found. There is similar evidence from Ibadan, Nigeria, where 20% of males are hemizygous, that the serum bilirubin may rise to a potentially lethal level in up to 6% of hemizygous male babies, i.e., in over 1% of all male infants in this population (*12*).

These studies suggest that G6PD deficiency is responsible for up to half the morbidity and mortality associated with neonatal jaundice, and that the global infant mortality due to neonatal jaundice associated with G6PD deficiency is probably in the range of 0.7 to 1.6 per 1000 of all births. An equal number of infants may suffer long-term morbidity from the same cause. However, neonatal jaundice is so influenced by exogenous and cultural factors, such as the extent of breast-feeding or the details of neonatal care, that more complete studies are needed, including a comparison of the incidence of G6PD-related jaundice in rural and urban areas.

Because the relevant information is lacking it is not possible even to make a rough estimate of the health burden of favism or drug-related acute haemolytic anaemia. It is likely that the latter is increasing because of the increasing consumption of drugs in developing countries. However, prevention is simple, and largely depends on awareness of the existence of the problem.

- *Neonatal jaundice* associated with G6PD deficiency can be prevented in many cases by educating the public and health workers to avoid dressing newborn infants in clothes that have been stored in moth-balls, to keep them in a good light, and to avoid traditional medicines. Health workers, particularly midwives and other birth attendants, need to be trained to recognize jaundice in the newborn at an early stage. When it is recognized, it can usually be controlled by exposing the infant to light (*13*). When exchange-transfusion is needed, it is important to test the blood to be transfused to exclude G6PD deficiency; this is also important when transfusion is required for favism, or for acute haemolytic anaemia.

Phototherapy is a very simple way of preventing death or life-long crippling disease in affected babies. The recommended screening methods for detecting hemizygous males and homozygous females are also sufficiently easy and cheap to be widely used. Therefore, whenever possible, neonatal screening should be performed on cord-blood samples in populations where G6PD is common (i.e., where it affects more

than 3–5% of males), in order to monitor these vulnerable infants for jaundice and institute treatment as early as possible.

For developing countries where G6PD deficiency is common and screening facilities are not available, simple recommendations could be developed for early identification of jaundiced infants; exposing the babies to early morning sunlight would be a simple and probably effective means of controlling jaundice. Further field studies are needed to develop optimal recommendations.

- *Favism* can be prevented by neonatal and general population screening combined with education, as in Sardinia (*14*); or simply by advising parents not to feed young children with fava beans, as in Cyprus. The former policy is to be recommended since it focuses on individuals who are definitely at risk. The indiscriminate approach pursued in Cyprus has been successful for one generation, but now that favism has become uncommon, the advice not to give children fava beans begins to sound like an old wive's tale, and younger mothers may deviate from the recommendations (Dr M. Angastiniotis, personal communication, 1984).
- *Acute haemolytic anaemia* can be prevented by detecting male hemizygotes (and female homozygotes), informing them of the risk, and providing them and local health workers with reliable advice about the food and drugs they should avoid.

Preventing the complications of G6PD deficiency depends heavily on education of the population at risk. To be effective, educational aids such as leaflets should be given to people who are tested and found positive. A draft information guide for affected individuals and primary health care workers is included with the report, on which the present article is based (see footnote on p. 7).

Molecular genetic aspects

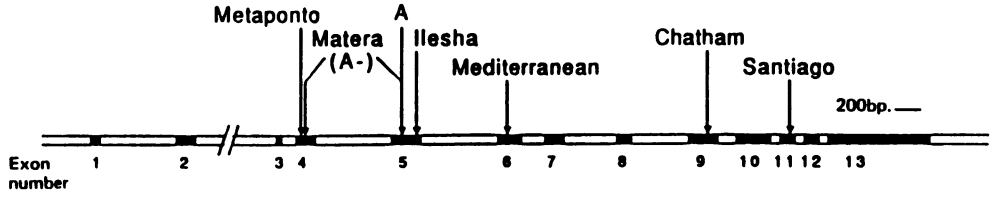
Recent developments have been reviewed (*3*). Some polymorphic and some rare variants have now been cloned and sequenced (Fig. 3), and all but one have been found to differ from normal G6PD (type B) by a single point mutation. The exception is G6PD A(–) in which there are two mutations, one of which is the same as G6PD A, supporting the notion that the former derives from the latter.

Both cDNA and genomic clones specific for the G6PD (*Gd*) gene are now available. Such studies are important to fundamental biological research, since they will permit deduction of structural changes in the enzyme and correlation with known changes in stability and function, and with clinical phenotype. Because so many defined G6PD variants are available, G6PD will probably be the first human enzyme for which the biochemical and clinical consequences of localized changes in the molecule can be accurately pinpointed. The study is likely to follow the haemoglobin model, in which this approach to understanding the pathophysiology associated with changes in an individual protein molecule has been so eminently successful.

It is reasonable to assume that most variants result from point mutations of the mis-sense type. Deletions would cause complete absence of the enzyme, which has never been recorded, presumably because it would be lethal during the early development of G6PD-deficient embryos. DNA analysis does not supersede characterization of the enzyme by studying its behaviour, because it is not yet possible to predict from an amino-acid replacement how, for instance, the stability of the enzyme, the level of its activity, or its affinity for a substrate will be modified.

At present, the simplest way to analyse DNA is by digestion with restriction enzymes, usually followed by Southern blotting and investigation with gene-specific probes. In theory, a DNA digest prepared with a particular restriction enzyme might identify a point mutation directly by revealing a new restriction site absent in control DNA, or by demonstrating the absence of a restriction site known to be present in control DNA. However, in general, for this approach to be successful, one needs to use a large number of restriction enzymes or to have a large measure of good luck. Techniques capable of detecting point mutations

Fig. 3. **The human G6PD gene (size, 17.5 kilobases; total exons, 13; coding exons, 12; messenger RNA, 2269 nucleotides; protein, 515 amino acids; relative molecular mass (M_r), 62 487; one molecule of NADP tightly bound per subunit).**



Exons shown in black, introns shown in white (intron 2 is much longer, which is indicated by a break). Vertical arrows indicate positions of point mutation found in some variants.

not dependent on restriction enzymes are being developed, but are not yet in routine use.

Another, by now classical use of restriction enzymes is for detecting restriction-fragment-length polymorphisms (RFLPs). So far, only one RFLP has been reported at the *Gd* locus. Assuming that others are discovered, it is possible that one or more will prove to be in linkage disequilibrium with a polymorphic G6PD variant. Then any such RFLP will help to assess whether a particular individual has that particular variant. This will be particularly useful in populations in which several variants are present, each with relatively high prevalence, but will not be immediately helpful for new or rare variants. This application of the knowledge of RFLPs will become less important as more point mutations are identified, and DNA amplification by the polymerase chain reaction is more widely used. However, in view of the fact that *Gd* is closely linked with the X-linked form of mental retardation associated with a fragile site, a *Gd*-specific RFLP could be used for heterozygote detection and prenatal diagnosis in this relatively common condition. No probe is yet available for the fragile site.

The most definitive way to determine a structural change in a gene is to construct a genomic DNA library from a subject with the presumed mutation. At present, a good library can be made with 200 μg of DNA, which may be obtained from about 20 ml of blood. Techniques for constructing libraries are becoming increasingly efficient, and the choice of vectors is becoming wider, so that in practice progressively smaller amounts of DNA will be required to obtain a fully representative library (i.e., one that contains sequences representing the entire genome of the propositus).

Once a library is established, it is screened with existing probes for the normal *Gd* gene. Provided that the library is sufficiently representative it will always be possible to isolate clones containing the subject's *Gd* sequence. However, the *Gd* gene is so large (at least 60 kilobases) that with present techniques it could not possibly be contained in a single clone, and this situation is not likely to change in the near future.

Once *Gd* clones from the subject are isolated, the coding portions can be identified from knowledge of the normal gene structure, and can be subcloned and sequenced. The same can be done with non-coding DNA regions within and flanking the gene. Thus in principle, any *Gd* mutation can be fully identified. It must be anticipated, however, that perhaps hidden silent mutations will exist in some or all populations. Therefore the finding of a single base-change in DNA from a subject with G6PD deficiency cannot be deemed automatically to be the basis for enzyme deficiency.

A knowledge of the complete amino-acid sequence of normal G6PD, deduced from its DNA sequence, can now help structural studies at the protein level. Tryptic peptides can be correctly assigned to particular positions along the sequence, and an amino-acid replacement within a peptide can therefore be immediately identified, with its serial number in the complete sequence. On the other hand, inclusion of DNA studies will increase the work required for characterization of variants.

Recommendations

(*a*) Population screening of all newborn babies should be carried out in areas with a prevalence of G6PD deficiency of 3–5% or more in males. The programme should be associated with an education campaign for mothers and health workers.

(*b*) Blood for transfusion of G6PD recipients who have jaundice or haemolysis should be tested and used only if found to be G6PD-normal.

(*c*) *In vitro* tests able to predict risk of haemolysis in G6PD-deficient subjects should be perfected and applied to all new drugs to be introduced in areas where G6PD deficiency is prevalent.

(*d*) Further research needs to be done on factors which, in addition to G6PD deficiency, are determinants for neonatal jaundice and favism.

(*e*) The spectrum of clinical manifestations associated with individual G6PD-deficient variants should be defined further. This can be done only in the respective endemic areas, especially with regard to individual drugs.

(*f*) The updated procedures for characterization described in the full report (see footnote on p. 601) should be used. It is especially important that full clinical, genetic and haematological data be provided for each new variant, together with the biochemical characterization.

(*g*) Now that the primary structure of G6PD is known, studies of its 3-dimensional structure would be possible, and are very important for an understanding of its enzymatic function.

(*h*) The cloning of the G6PD gene makes it possible and important to identify specific structural changes in subjects with both common and rare variants, and especially those associated with G6PD deficiency. To this purpose, it is recommended that DNA be prepared from subjects with known and new variants and that, if possible, either fibroblasts or lymphoblasts be obtained and stored in a central facility.

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