the other genotypes, but the details have not yet been worked out.

Although the genotypes associated with severe suxamethonium sensitivity are relatively rare (about 1 in 2,000 of the general population), at least one of the genes concerned (E_1^a) is relatively common and is present in about 1 in 25 people. Such heterozygous individuals have a demonstrable peculiarity in their serum cholinesterase, but are in other respects perfectly healthy. The peculiarity must therefore be regarded as a not uncommon normal variation. One may well ask why it occurs with such a frequency and what selection pressures have maintained it in the population. As yet we have no real clue about this. It is, however, worth mentioning that it is not the only inherited variation in serum cholinesterase which is relatively common. It has recently been found that up to 10% of individuals have an extra electrophoretic component of the enzyme which is genetically determined (Harris, Hopkinson, Robson & Whittaker 1963, Harris, Robson, Glen-Bott & Thornton 1963). Its significance is as yet uncertain, and I have not discussed it here because it appears at present to have nothing to do with suxamethonium sensitivity.

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Glucose 6-phosphate Dehydrogenase Deficiency

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is a good example of the interaction between a gene and its environment. In the absence of any prejudicial factor the majority of individuals with this enzyme deficiency live unaffected lives, but in the presence of a number of different chemical agents acute red cell destruction will occur. A few patients with almost total deficiency of the enzyme have a severe hæmolytic anæmia from birth. Although deficiency of glucose 6-phosphate dehydrogenase may occur in other tissues besides the red cell it does not appear that these tissues suffer any damage.

The function of this enzyme in the red cell appears to be principally to maintain an efficient protective mechanism against the oxidation of hæmoglobin. Hæmoglobin is an unstable molecule and on its own undergoes slow oxidation to methæmoglobin; the presence of many different compounds hastens this process and will actually decompose methæmoglobin with the formation of verdo- and choleglobins. A key factor in the red cell which protects hæmoglobin against oxidation is the compound glutathione which in the reduced state provides 2 mEq of potential hydrogen ion. The continuous renewal of reduced from oxidized glutathione is accomplished by a metabolic system known as the pentose phosphate pathway and in it G6PD is the initial activator (Fig 1).

Normally in the red cell 90% of glucose is converted anaerobically to lactic acid and only 10% is oxidized to pentose phosphates: the limiting factor here is the availability of TPN. However, the formation of oxidized glutathione or of methæmoglobin leads to their reaction with TPNH through the appropriate reducing enzyme and to the formation of TPN; the presence of excessive TPN now permits additional oxidation of glucose to pentose, and in this way glutathione and methæmoglobin are reduced (de Loecker & Prankerd 1961). In practice this system of reducing methæmoglobin seems only to operate under adverse environmental conditions, the more usual reduction being accomplished in conjunction with DPN and glycolysis.

In the presence of methylene blue the metabolism of glucose through the pentose phosphate pathway can also be considerably increased and the importance of G6PD in controlling this metabolic route can be shown by comparing glucose oxidation in the red cell in normal and enzyme deficient subjects (Table 1).

In vitro experiments have demonstrated the protective action of reduced glutathione on hæmoglobin. In mixtures containing these compounds the addition of ascorbic acid leads first to the oxidation of glutathione and only subsequently to the oxidation of hæmoglobin and the formation of choleglobin (Jandl *et al.* 1960).

In subjects whose red cells are deficient in G6PD similar oxidative end-products of hæmo-

Table 1
Metabolic data in normal and G6PD-deficient red cells with and without the addition of methylene blue

	O_2 µl uptake 3 h/2 ml cells	Glucose utilization (µM/ml cells/h)	Lactate production (µM/ml cells/h)	% Glucose converted to CO_2
Normal control (6)	20·6±2·75	2·05±0·1	4·4±0·3	8·5±1·2
G6PD deficiency	7	1·65	4·7	4·1
	7	1·70	4·2	3·9
	8	1·75	4·0	4·1
(Blood with methylene blue (0·005 M)				
Normal control (6)	428±9·0	3·35±0·2	4·0±0·3	70±4·3
G6PD deficiency	150	2·5	3·5	8·9
	155	2·9	3·1	11·6
	147	2·5	3·1	11·6

globin may be seen *in vivo* and can be demonstrated in a stainable form as Heinz bodies. The presence of Heinz bodies is associated with shortening of red cell survival to a few days, but it is not clear through what mechanism cell survival is shortened, although we found (Prankerd 1958) that *in vitro* incubation of G6PD-deficient red cells with phenylhydrazine in the presence of oxygen led to an increased loss of lipids on washing compared with cells incubated under nitrogen. It is quite possible that oxidative destruction of lipids occurs at the same time as hæmoglobin. In a very elegant experiment Beutler *et al.* (1954) have demonstrated that the likelihood of a particular cell with G6PD deficiency being destroyed by an agent is directly related to its age.

Other chemical changes are also found in G6PD-deficient cells apart from the lack of enzyme. Particularly relevant are the low levels of GSH and its instability, and the low content of catalase, which also contributes to the inefficient protection of these cells from oxidation, catalase being responsible for the destruction of hydrogen peroxide (Tarlor *et al.* 1962).

The clinical disorder associated with G6PD deficiency was first observed in Negroes who developed an acute hæmolytic anæmia when treated with the antimalarial drug primaquine and

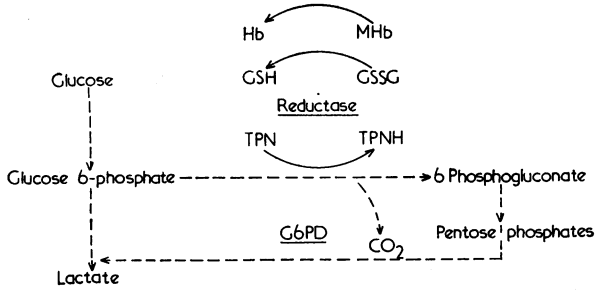

Fig 1 *Reactions in the pentose pathway in the red cell providing reduced glutathione*

a series of investigations at the University of Chicago (Beutler 1959) showed that the hæmolysis was related to an intrinsic red cell defect associated with the presence of Heinz bodies, that the cells were deficient in reduced glutathione and finally that this deficiency was probably caused by diminished activity of G6PD. The clinical pattern of events in a susceptible subject is hæmolysis beginning within five days of starting the drug, associated with reticulocytosis, often hæmoglobinuria and usually anæmia. However, regardless of whether the offending drug is continued or not, hæmolysis is of limited duration, and for a period of some weeks after the hæmolytic process the subject is insensitive to the drug if it is readministered. The self-limitation of red cell destruction is due to the fact that only cells over a certain age are sensitive to lysis.

In America susceptible individuals constitute about 10% of the Negro population and investigation into their families shows that the defect is inherited and appears to be transmitted by an incompletely dominant gene carried on the X chromosome. In other countries the incidence of this enzyme deficiency varies (Table 2).

A wide range of chemical agents are known to produce hæmolysis in affected individuals, and these show little chemical relationship, but all react actively with hæmoglobin (Table 3).

Any of these agents will produce hæmolysis in normal individuals if given in sufficient quantity, but in the affected a relatively small dose is hæmolytic. This is well illustrated by phenylhydrazine which in a dose of 100 mg daily produces hæmolysis in normal individuals but a dose of 50 mg daily will do so in G6PD-deficient subjects. Individuals with this enzyme deficiency

Table 2
G6PD deficiency in various races, detected partly by enzyme estimation and partly by glutathione stability (figures taken from published literature)

	Percentage of population with G6PD deficiency
Caucasians, northern	0·1
Caucasians, southern	
Northern and Southern Italians	2–3
Sardinians	3–30
Greeks	2–5
Negroes	
USA	10
Western Africans, Nigerians	10
Central Africans, Congolese	15–20
Eastern Africans, Tanganyikans	15–30
Southern Africans, Bantu	2
Jews	
European	0·2
Iraqi	25
Turkish	5
Kurdish	60
Asiatics	
Chinese	2
Japanese	0
Filipinos	12

Table 3
Chemical agents reported to cause destruction of red cells deficient in G6PD

8-Aminoquinolines	Primaquine
	Pamaquine
	Pentaquine
Sulphonamides	Sulphanilamide
	Sulphamethoxypyridazine
	Salicylazosulphapyridine
	Sulphacetamide
Sulphones	Sulfoxone
	Diaminodiphenylsulphone
	Thiazosulphone
Nitrofurans	Nitrofurantoin
	Furazolidone
	Nitrofurazone
Acetylphenylhydrazine	Naphthalene derivatives
Acetanilid	Methylene blue
Acetophenetidin	Ascorbic acid
Antipyrine	Probenecid
Aminopyrine	Quinidine
Acetylsalicylic acid	Trinitrotoluene
Para-aminosalicylic acid	*Fava bean*

appear to show a cross-sensitivity to the drugs listed in Table 3, but in the case of favism the situation is somewhat different (Szeinberg *et al.* 1957). American negroes are apparently insensitive to the fava bean and in S. Caucasians favism is not apparently seen in all individuals who are enzyme deficient. It seems as if an abnormal gene closely linked to the G6PD one may also be concerned and results in the abnormal metabolism of bean products.

The detection of G6PD deficiency is not difficult. The enzyme can easily be assayed directly by measuring the oxidation of TPNH spectrophotometrically in a red cell system containing the requisite substrates (Prankerd 1963). Alternatively the stability of reduced glutathione against oxidation in the presence of acetylphenylhydrazine can be measured (Beutler *et al.* 1957), but this is not a particularly sensitive test. In the absence of suitable equipment and in field studies two simple methods, one involving the reduction of methæmoglobin (Brewer *et al.* 1960) and the other the reduction and decoloration of brilliant cresyl blue (Prankerd 1963), can be used.

Several variants of G6PD deficiency may be seen clinically. In one of the commonest, that producing drug sensitivity in Negroes, only 10–15% of the enzyme is present in the red cells. In S. Caucasians the deficiency is greater and only 0–5% of enzyme is present. These subjects are often sensitive to the fava bean and in many of them a hæmolytic state in the newborn is also a feature. In N. Caucasians a total deficiency of enzyme is occasionally seen and is associated with a severe nonspherocytic hæmolytic disease dating from birth. One such patient had red cells which appeared to be insensitive to primaquine when his cells were transfused to a normal donor taking this drug (Bowdler & Prankerd 1964).

Chemical studies of the enzyme in deficient subjects are still in an early stage. The enzyme seems to be normal in character in Negroes but deficient in quantity. Although I have so far discussed enzyme proteins as if there were only one, electrophoretic studies demonstrate the existence of two isoenzymes in the red cells of normal subjects. In N. Caucasians with hereditary nonspherocytic hæmolytic anæmia one of these proteins appears to be chemically abnormal, but it is very difficult to obtain sufficient enzyme for study (Kirkman & Crowell 1963).

Other differences between G6PD variants also exist; for instance tissue cells other than the red cell, such as leucocytes and liver cells, share the deficiency in S. Caucasians, but not in Negroes. In our patient with nonspherocytic hæmolytic disease leucocyte enzymes were normal in activity.

The prevalence of this harmful genetic abnormality in many communities has led people to wonder how the gene frequency is maintained. Following the analogy of sickle cell disease and its protective effect against malaria the relationship of this defect to malarial infestation has been investigated and it appears that there is also a degree of protection afforded by a deficiency of G6PD. (Allison & Clyde 1961). The mechanism of this could be a result of the instability of reduced glutathione in the red cells because this compound is required for the growth of malarial parasites.

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Acetylation Polymorphisms

The administration of a standardized dose of a drug in exactly the same way to a large number of healthy human beings allows one to construct a frequency distribution histogram of the effect produced by the drug or some feature of its metabolism in the body. The finding of a bimodal or trimodal distribution curve can lead to knowledge of a new genetic polymorphism (Evans 1962).