GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN MALTA — A PRELIMINARY STUDY

MAURICE N. CAUCHI

M.D. (MALTA), M.Sc. (LOND.), PH.D. (LOND.), D.P.H.

Department of Pathology
Royal University of Malta

It has been suggested that glucose-6-phosphate dehydrogenase deficiency (G6PD) affects more than 100 million people of all races throughout the world (Carson and Frischer, 1966), and that as many as 30% of cases of chronic non-spherocytic haemolytic anaemia are associated with a form of G6PD deficiency (Wintrobe, 1967). The special relevance of this problem to Malta can readily be appreciated when it is remembered that G6PD deficiency is particularly common in countries bordering the Mediterranean. In some surveys up to 32% of Greeks and 35% of Italians tested were G6PD deficient (WHO, 1967).

G6PD is an enzyme present in red cells and catalyzes the reaction: G6PD —› 6PG (fig. 1). During this process NADP (nicotinamide adenine dinucleotide phosphate) is reduced to NADPH which is essential for keeping glutathione (GSSG) in the reduced state (GSH). This is called the Pentose Phosphate pathway, and although only about 20% of glucose-6-phosphate is metabolised via this mechanism, all NADPH required by the cell is produced in this way.

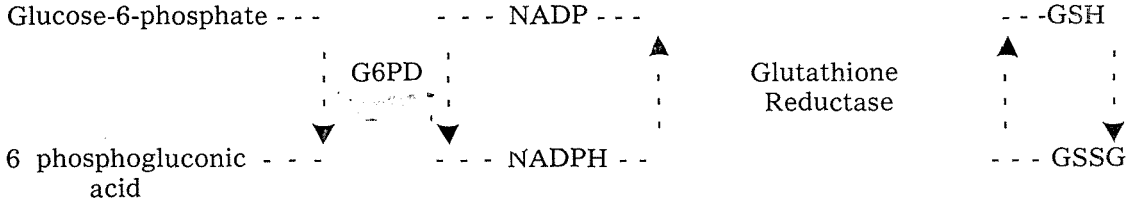

Fig. 1. Role of G6PD in the production of NADPH

The consequences of G6PD deficiency have been known for a long time. Haemolysis following ingestion of certain beans has been called favism, Baghdad Spring Fever, etc. The introduction of 8-aminoquinoline in 1926 as an antimalaria drug has been associated with severe and even fatal haemolysis. The list of drugs known to affect deficient individuals has grown considerably in recent years, and includes sulphapyridine, sulphadiazine, primaquine, trinitrotoluene, quindine, naphthaline, nitrite, and chloramphenicol (WHO, 1967).

The inheritance of G6PD deficiency follows a well described pattern. The locus responsible for this enzyme is situated on the X-chromosome, so that this condition is transmitted as a sex-linked character affecting males (hemizygotes) — female carrying one affected chromosome (heterozygotes) show varying degrees of enzyme deficiency.

Methods of Study

Various screening procedures have been described to detect G6PD deficiency, including the Methaemoglobin reduction test (Breuer *et al.*, 1962), the M.T.T. linked spot test (Fairbanks and Beutler, 1962), the Methylene Blue absorption test (Oski and Growney, 1965, Sass *et al.*, 1966), the fluorescent test (Beutler, 1966), and the ascorbic acid cyanide test (Rakitzis, 1964, Jacob and Jandl, 1966). The screening me-

thod used here is that described by Motulsky and Campbell-Kraus, 1961). This test is based on the principle that the dye Brilliant Cresyl Blue (BCB) is reduced to a colourless state by the action of NADPH produced through the action of G6PD.

For this test 0.02 ml of blood is added to 1 ml distilled water. To this are added 0.01 ml Sodium G6P (825 mg/100ml), 0.1 ml NADP (50 mg/10 ml), 0.25 ml BCB (32 mg/100 ml) and 0.2 ml TRIS buffer (pH 8.5): 8.96 gm/97 ml + 3 ml HC1. The mixture is covered by paraffin oil, and incubated at 37°C. The time taken for decolourisation to take place is an index of G6PD activity in the red cells. Normally specimens decolorise by 65 mins.

These screening procedures have the great advantage of being quick, and simple to perform, so that large numbers of tests can be done at the same time. For more detailed investigation of those cases that show enzyme deficiency by the screening test, a more accurate but more laborious quantitative test is done. Basically the test depends on the increase in absorbance at 340 mu following the production of NADPH.

The relationship between the BCB tests (in minutes) and the quantitative tests (in units/100 ml packed cells) is not a linear one as seen in the following experiment:

For the BCB (qualitative) test, samples of blood were taken and diluted so as to contain 20, 15, 10, 5 and 0 u litres/ml of distilled water. The time taken for decolourisation of the BCB after incuba-

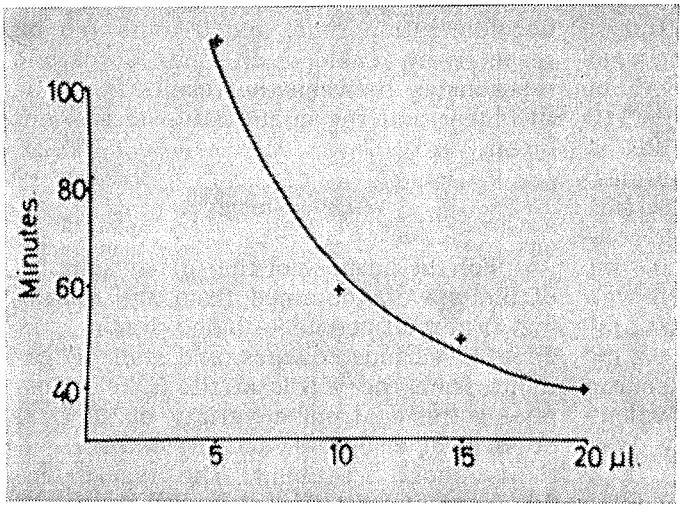

Fig. 2: The effect of dilution on decolourisation time in the BCB test.

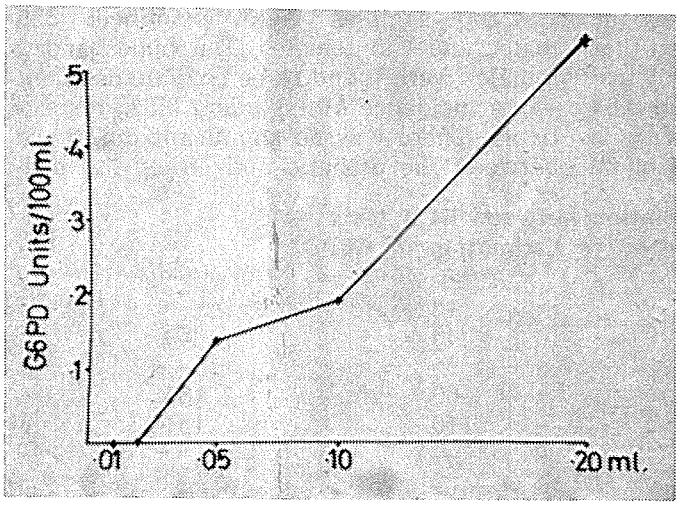

Fig. 3: The effect of dilution on the activity of samples as measured by the quantitative test (units/100ml packed cells).

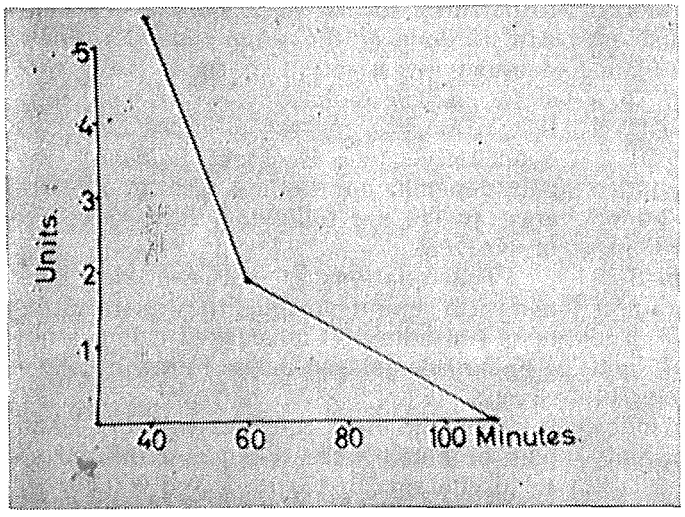

Fig. 4: Correlation between the result of the BCB test (minutes) and the quantitative test (units/100 ml. packed cells).

tion is shown in fig. 2. It is seen that the rate of decolourisation is a function of the volume of blood examined: when 20 ul of blood were used decolourisation occurred in 40 minutes, whereas when only 5 ul were used, decolourisation occurred in 110 minutes; i.e. a low activity of G6PD is associated with long decolourisation times.

For the quantitative test, samples (from the same blood as used above) containing 0.2, 0.1, 0.05, 0.02, and 0.01 ml were tested and the quantity of G6PD was estimated quantitatively (units/100 ml packed cells). It is seen (fig. 3) that 0.2 ml blood — the quantity normally taken for the test — contained an activity equivalent to 0.54 units, whereas no activity was measurable in 0.02 ml blood.

If one plots the results obtained by the BCB test against those obtained by the quantitative method (fig. 4), it is seen that 100% activity is equivalent to a decolourisation time of 40 min (BCB test) and 0.54 units (quantitative test); 50% activity is equivalent to a decolourisation time of 60 minutes and 0.183 units respectively; while 5% activity is equivalent to 110 min. decolourisation time, and 0 units/100 ml respectively (Table 1). In other words, the relationship between the results from the BCB test and the quantitative test is not linear.

G6PD Survey

For the purpose of the survey samples of blood were obtained from the following: i) normal people — blood donors, students at various colleges, and staff; ii) patients from the wards at St. Luke's Hospital suffering from a variety of surgical or medical disorders; and iii) diabetic patients under treatment. The results are tabulated in table 2.

800 samples were examined: 555 males and 245 females. Ten males and 2 females were found to be enzyme deficient — an incidence of 1.8% and 0.8% respectively. There was no significant difference between the diabetic and either the nor-

Table 1. Relation between BCB tests and the Quantitative Estimation of G6PD

Sample	*Activity as % of original dilution*	*BBC Test minutes*	*Quantitative Test units/100 ml packed cells*
1	100%	40	.54
2	75%	50	—
3	50%	60	.183
4	25%	110	.131
5	10%	—	0
6	5%	—	0

Table 2. Incidence of G6PD Deficiency

	MALES			*FEMALES*		
	No. Examined	*Affected*	%	*No. Examined*	*Affected*	%
Normal	269	5	1.86	27	—	—
Patients	187	3	1.61	46	—	—
Diabetics	99	9	2.2	172	2	1.17
Total	555	10	1.8	245	2	0.81

mal or the ordinary patients groups.

The lower incidence of G6PD deficiency in females can be explained by the relative lack of sensitivity of the BCB test in heterozygotes.

Work is going on at the moment in order to establish whether the incidence of G6PD deficiency varies from one part of the Island to the other.

Acknowledgements

I would like to thank Prof. G. P. Xuereb and all members of the Pathology Department for their constant help and encouragement. I also thank those consultants at St. Luke's Hospital, as well as the rectors of various colleges whose cooperation made this work possible. Mr. F. Fenech is thanked for his technical assistance.

References

BEUTLER, E. (1966) Blood, *28*, 553.

BREWER, G.J., *et al.* (1962) J. Amer. med. Ass. *180*, 386.

CARSON, P.E. and FRISCHER, H. (1966). Am. J. Med. *41*, 744.

FAIRBANKS, V.F. and BEUTLER, E. (1962) Blood, *20*, 591.

JACOB, H.S. and JANDL, J.H. (1966). New. Eng. J. Med., *274*, 1162.

MOTULSKY, A.K. and CAMPBELL-KRAUT, J.M. (1961) in Blumberg B.S. "Proceedings of the conference on Genegetic Polymorphisms and geographic variations in disease" New York, Grune and Stratton.

OSKI, F.A. and GROWNEY, P.M. (1965) J. Pediat., *66*, 90.

RAKITZIS, E.J (1964) Cancer, *2*, 1182.

SASS, M.D. *et al.* (1966) J. Lab. Clin. Med. *68*, 156.

WINTROBE, M.M. Clinical Hematology VI ed. Henry Kimptom London 1967 p. 657.