GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN MALTA

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Summary: In a survey carried out to establish the incidence of G-6-PD deficiency in Malta, a total of 1514 samples were tested by the brilliant cresyl blue screening method. 2.7% of the male and 1.9% of the female population were found to be enzyme deficient. Of the 295 samples tested by the quantitative assay, 22 were found to be enzyme deficient, and half of these showed an enzyme activity of less than 10% of the normal, and in the other half the activity ranged between 10 and 25%.

Glucose-6-phosphate dehydrogenase deficiency is a hereditary enzyme defect that is linked with the X-chromosome and becomes manifest in heterozygous males and homozygous females with a varying degree of expression. Two main variants have been recognised: that found in Negroes and that found in Caucasians. Affected individuals in the last group are specially prone to favism and neonatal haemolysis.

In view of the high incidence of G-6PD deficiency in Mediterranean countries, such as Italy (W.H.O. Report 1966), Greece (Stammatoyamnopoulos, G., *et al., 1966*) and North Africa (Vergnes, H., 1965), it was considered useful to establish the frequency of this congenital deficiency in the Maltese population. Malta has a population of only 318,000 and consequently, consanguineous marriages are relatively common, though precise figures are not available. It was therefore expected that the gene frequency in the local population would be high. G-6-PD deficiency is reputed to confer a selective advantage to heterozygotes with regard to malaria in countries where it is endemic (Motulsky, A.G., 1964). In this connection, it is relevant to point out that malaria was endemic in Malta until about the middle of the last century (Cassar, P., 1964).

A definitive survey of the Maltese Islands was carried out in order to detect deficiency of G-6-PD in erythrocytes using a screening method. A quantitative assay was used to confirm the positive results obtained by the screening test. Several cases presenting with the clinical picture of a haemolytic disorder, and their near relatives, were also investigated by the quantitative method.

The results of these two series of observations are recorded in this presentation.

Methods

1. The brilliant cresyl blue dye test of Motulsky was used in the screening procedure, as recommended by the W.H.O. Scientific Group Report (W.H.O. Report, 1967).

This test is based on the principle that NADP is reduced to NADPH by G-6-PD in the presence of Glucose-6-phosphate, and added brilliant cresyl blue is decolourised. With normal blood, decolourisation occurs within 40 to 60 minutes. Decolourisation times falling between 65 and 90 minutes were interpreted as indicating mild deficiency, and decolourisation times longer than 90 minutes as indicating a severe degree of enzyme deficiency.

All the samples of capillary blood were tested within 1 to 2 hours.

2. Quantitative assay of G-6-PD in erythrocytes depends on the increase in absorbance of U.V. light at 340 mμ, by NADPH which is generated during incubation of a buffered red cell haemolysate with substrate containing G-6-P, Mg++ and NADP. The results were related to the values obtained in normal controls, and were expressed as a percentage of the mean normal enzyme activity.

Results

In the survey, 1514 blood samples were tested; of these 1145 were males and 369 were females, and were obtained from the following three groups of subjects:

(a) normal healthy children attending various schools,

(b) hospital patients suffering from various medical or surgical conditions, and

(c) patients attending the diabetes out-patient clinic of the hospital.

Table I shows that 31 male subjects had a decolourisation time longer than 65 minutes — an incidence of 2.7%. There was no difference in the incidence of enzyme deficiency between these three groups. Of the 369 female subjects tested, 7 had a decolourisation time longer than the normal — an incidence of 1.9%, but in none was it longer than 90 minutes.

Because of the heterogeneity of the Maltese population, it was considered advisable to analyse the various groups on a socio-geographical basis to find out whether there existed any local variation in the incidence of G-6-PD deficiency. As shown in *Table II*, rural areas with a high

TABLE I

Incidence of G-6-PD Deficiency

	No. Examined	*No. Affected (BCB Decolourisation Time)*					
Males		*65 – 90 mins.*		*Over 90 min.*		*Total Incidence*	
		No.	*%*	*No.*	*%*	*No.*	*%*
Healthy	842	11	1.3	15	1.8	26	3.1
Hospital patients	186	—	—	3	1.6	3	1.6
Diabetics	117	—	—	2	1.7	2	1.7
	1145	11	1.0	20	1.7	31	2.7
Females							
Healthy	86	—	—	—	—	—	—
Hospital Patients	58	1	1.7	—	—	1	1.7
Diabetics	225	6	2.7	—	—	6	2.7
	369	7	1.9	—	—	7	1.9

TABLE II

Incidence of G-6-PD according to region

Locality	*Type*	*Total examined*	*Sex*	*Affected*	*Incidence*
Floriana	Urban	125	M	3	2.4%
Mellieha	Rural	204	M	3	1.5%
Nadur, Gozo	Rural	105	M	6	3.6%
Zebbug, Gozo	Rural	56	M	0	0%
		68	F	0	0%
Private School	Mixed	175	M	3	1.7%

rate of intermarriage do not show an increased incidence of G-6-PD deficiency when compared with an urban population or a mixed population of school-children.

Table III shows the results of the quantitative assays carried out. Of the 295 samples tested by this method, 22 subjects were found to be enzyme deficient, i.e. the activity of the sample was more than two standard deviations below the mean normal level. It was also observed that 11 of these, i.e. 50%, had an enzyme activity of less than 10% of the normal, and the remaining 50% showed activities varying between 10 and 25% of the normal mean.

TABLE III
Quantitative G-6-PD estimations

	Male	*Female*	*Total*
No. Examined	233	62	295
No. Affected	19	3	22
G-6-PD Activity *(% of normal)*			
	Male	*Female*	*Total*
0 – 10%	11	—	11
– 15%	1	1	2
– 20%	5	1	6
– 25%	2	1	3

The family studies shown in *Table IV*, indicated that undetectable levels of G-6-PD in the patients examined were accompanied by a low degree of activity in one or other of the parents, as well as in one or more siblings. In one family (Family Mi) a markedly low value in the patient was associated with normal values in both parents.

The large majority of cases presenting with an acute haemolytic episode were admitted to hospital after having eaten the bean *Vicia faba*.

Discussion

The overall incidence of 2.7% of G-6-PD deficiency in the Maltese population revealed by this study, compares with that of neighbouring countries. In Italy, the incidence varies from less than 1% to as high as 35% in the Po district (W.H.O. Report, 1966). In Algeria, the incidence is 3 to 4% (Vergnes, H., 1965). An incidence of 46% has been found in some regions in Greece as revealed by some surveys (Allisan, A.C., *et al.*, 1964), and an incidence of 3.1% in Cyprus (Plato, C. G., *et al.*, 1964).

As has been shown, there is no correlation between a high rate of intermarriage of G-6-PD deficiency in the Maltese population. This seems to suggest that a considerable loss of genes occurs in the homozygous state. It is well established G-6-PD deficiency is one important cause of jaundice in the newborn (Weatherall, D. J., 1960), and fatal kernicterus might be postulated as one way by which limitation of gene multiplication might occur in localities with a high rate of intermarriages. Further studies are required to establish whether foetal wastage is greater in those areas where intermarriages are common.

Reference has already been made to the relationship between G-6-PD deficiency and malaria. However the evidence so far

TABLE IV
Enzyme activity in families of patients with G-6-PD deficiency

Family	*G-6-PD activity (as % of normal) in:*			
	Propositus	*Father*	*Mother*	*Siblings*
Za	0	33.6	77	28.7 94.5
Du	0	88	68	0, 6.5
Cac	0	109	86	
Bo	0	32	115	
Da	0	57.2	22	53, 43.0
Ga	0	—	39.6	
Cam	0	32.6	7.2	
Mi	13.1	139	255	

is still inconclusive. It is worth noting that malaria was endemic in Malta up to the 19th century, and the Anopheles mosquitoe was described in Malta in 1904 (Zammit, T. and Caruana Scicluna, G., 1905). Though isolated breeding grounds for these mosquitoes still exist to the present day, and sporadic cases of malaria have been diagnosed as late as 1940, malaria does not present an epidemiological problem in Malta.

The pattern of inheritance of the gene responsible for G-6-PD is well established. As the locus responsible for this enzyme is situated on the X chromosome, this condition is transmitted as a sex-linked character, and affects males predominantly, while females carrying one affected chromosome (heterozygotes) show a varying degree of enzyme deficiency. This is also apparent in our family studies where reduced activities were found in 5 of the 8 mothers of patients with G-6-PD deficiency. This pattern of inheritance also shows why the incidence of G-6-PD deficiency found in the survey was higher in males than in females (2.7% for males compared with 1.9% for females) and heterozygous females are not so readily detected. This mode of inheritance also explains why the clinical effects in females are so much milder. None of the females included in the survey had a decolourisation time longer than 90 minutes, whereas the decolourisation time was prolonged to over 90 minutes in 65% of the male population showing abnormal decolourisation times.

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