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Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency, Thalassaemia, and Abnormal Haemoglobins in the Philippines*†

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During 1960 blood specimens were obtained from 403 persons living in Manila (Series A), who were students in medical schools, student nurses, and in-patients in a children's home. Of these, 295 were males and 108 females (all student nurses) and they originated in all parts of the Philippines. In addition 112 blood specimens were obtained from Philippino male cannery workers living in Alaska§ (Series B). Venous samples of blood were collected into acid dextrose citrate solution and sent by air to Seattle in refrigerated containers immediately after drawing. They arrived within 24–48 hours in excellent condition.

The primary interest of this study was to determine the prevalence of G6PD deficiency, thalassaemia, and abnormal haemoglobins in the Philippines, which until recently had been an area of heavy malarial infestation. In addition blood and serum group studies are described in a companion paper.

G6PD Deficiency

Brilliant cresyl blue decolorization tests (Motulsky and Campbell-Kraut, 1961) were performed on the 295 males of Series A. Of these, 12 were unequivocally deficient in G6PD (4·1%). In Series B the prevalence of G6PD deficiency was higher, 15 or 13·4% of the 112 lacking the enzyme.

The birth places of all members of both series were recorded (Fig. 1). The birth places of the 12 G6PD deficient members of Series A who originated from all over the Philippines were distributed rather uniformly over the islands. In Series B, on the other hand, 89 of the 112 examined, including all 15 G6PD deficient individuals, came from 4 coastal provinces of North West Luzon (Ilocos Norte, Ilocos Sur, La Union and Pangasinan). In the main Series A, 25 persons, including one that was G6PD deficient, came from these four provinces. At first sight the disparity in G6PD deficiency frequency between these two sub-groups is rather striking (15 out of 89 as opposed to 1 of 25) but the numbers are small and the χ^2 test gives no indication of a statistically significant heterogeneity.

This evidence, however, is suggestive though by no means conclusive that a focus of high incidence of enzyme deficiency may exist in the coastal area of N.W. Luzon (14%). For the remainder of the country a prevalence of 3·8% was obtained, which agrees well with that reported by Buenaventura (1961).

Brilliant cresyl blue decolorization times of all those enzyme-deficient individuals were very long, more than 6 hours in all cases and more than 24 in most. This is in marked contrast to findings in enzyme-deficient Negroes, who have decolorization times of as little as 2 hours, but similar to findings in enzyme-deficient Greeks and Italians.

This difference was confirmed by quantitative assays of enzyme activity, by a modification of the test of Glock and McLean (1953) (Zinkham, Lenhard, and Childs, 1958) in G6PD deficient and normal subjects both Negro and Philippino (Table I).

The enzyme level in normal Philippinos is not significantly lower than in normal Negroes. The slight decrease is probably due to ageing of the specimens which had to be sent from Alaska to

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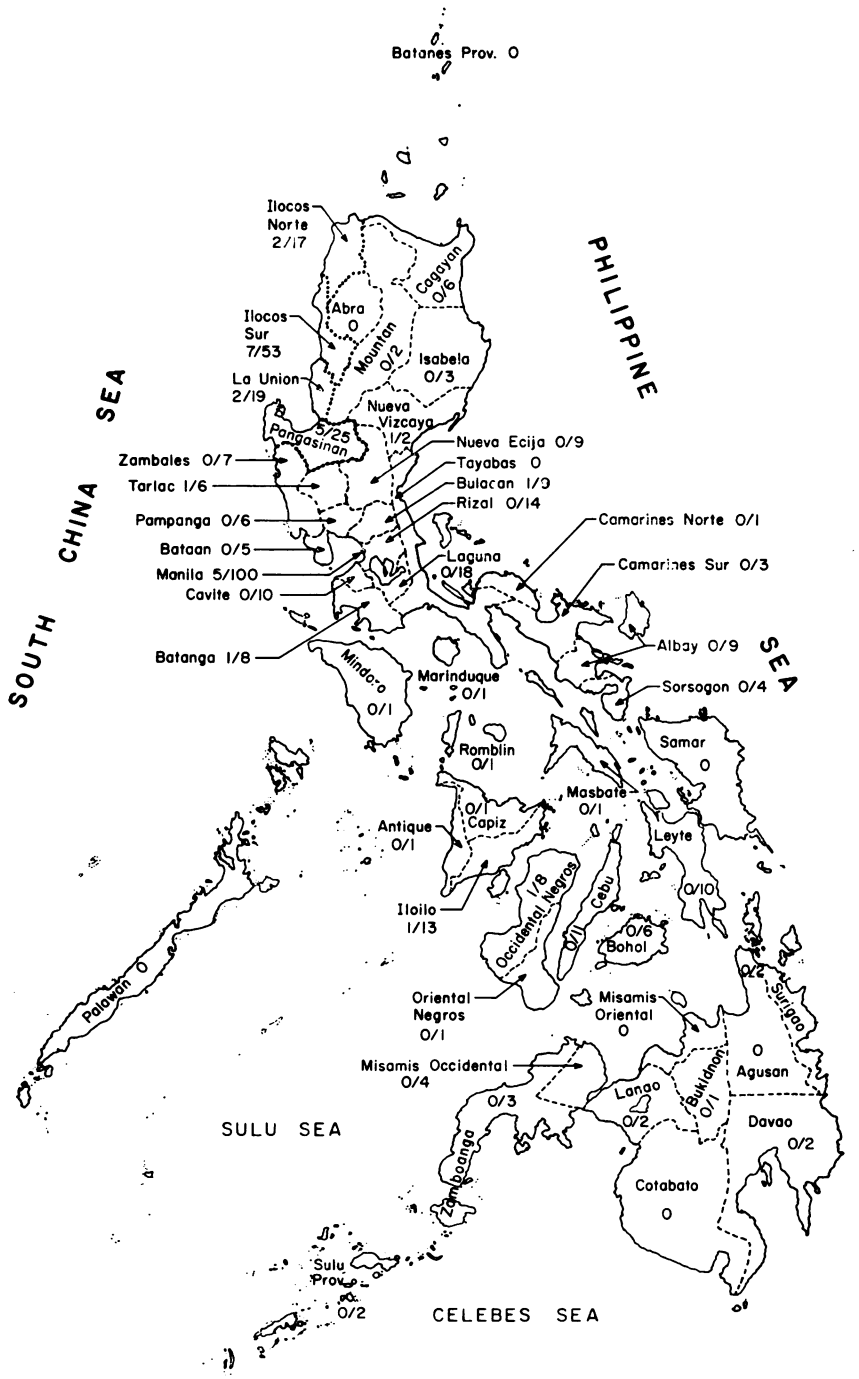

FIG. 1. Map of the Philippine Islands indicating the distribution of enzyme deficiency. The number of enzyme-deficient males among the total number of males tested from each province is indicated. Note the higher frequency in the N.W. provinces (16/114).

TABLE I

G6PD LEVELS IN NORMAL AND DEFICIENT NEGROES AND PHILIPPINOS

	Number	Mean Enzyme Level*	Difference
Negro male normals	11	318 ± 23	p > 0·10
Philippino male normals	10	262 ± 32	
Negro male deficient	16	23 ± 16	p < 0·001
Philippino male deficient	15	3·5 ± 1·2	

† Expressed as Δ OD/min./100 ml. packed R.B.C's.

TABLE II

FINDINGS IN SEVEN CHILDREN WITH SEVERE SPLENOMEGALIC ANAEMIA AND IN SOME OF THEIR PARENTS

	Sex	Age	Blood Film*	Osmotic Fragility*	HbF level %	HbA_2 level
Patients						
A	F	4 mth.	A	—	21·0	—
B	M	3 yr.	A	—	2·3	Raised
C	M	2 yr.	A	—	2·7	Raised
D	M	8 mth.	A	—	55·0	Normal
E	F	12 yr.	A	—	5·8	Raised
F	M	7 mth.	A	—	0·8	—
G	F	10 yr.	N	—	1·4	Normal
Parents						
A	M	44 yr.	A	A	4·0	Raised
A	F	34 yr.	A	A	2·0	—
B	M	24 yr.	A	A	1·1	Normal
C	M	24 yr.	N	N	1·0	Normal
D	F	27 yr.	A	—	1·0	Raised
D	M	30 yr.	A	A	2·8	Raised
F	F	25 yr.	N	N	1·0	Normal

*A= abnormal; N= normal.

Seattle, whereas the Negroes were resident in Seattle. On the other hand, enzyme levels among the deficient Philippinos are very low, whereas in Negroes enzyme deficiency is only partial. It is of interest that Kidson and Gorman (1962) found a similar complete type of deficiency in various Melanesian populations of the Pacific in New Guinea and New Britain.

Abnormal Haemoglobins

Haemolysates in Series A and B were examined by paper electrophoresis. No component other than Hb A was found. In addition, no major abnormal haemoglobin was found in the blood samples from seven children with severe haemolytic anaemia or in their close relatives (see Table II). Some of these patients had been clinically suspected of having haemoglobin E-thalassaemia disease.

Thalassaemia

The diagnosis of thalassaemia requires considerably more exacting techniques than the detection of the other abnormalities discussed. In the present survey, the osmotic fragility test was employed as a screening procedure. Series A only was tested. It was found that the percentage of lysis in 0·45% NaCl solution of cells stored in ACD and examined several days after collection gave a good approximation to a normal distribution (Fig. 2). Initially 20 samples showing lysis of less than 60% and lying outside the main distribution were submitted to further study. The percentage of A_2 haemoglobin was first estimated by cyanogum electrophoresis (Raymond and Weintraub, 1959). This was followed by starch grain electrophoresis (Gerald and Diamond, 1958) in all cases where high values were suggested by visual inspection of cyanogum gels. Furthermore, an alkaline denaturation method (Singer, Chernoff, and Singer, 1951a, b) was used to estimate foetal haemoglobin (Hb F) levels in all these samples and in 100 additional specimens with normal osmotic fragilities from Series A. This test was also performed on all the members of Series B. In all cases where an increase of HbA_2 was suspected blood films stained with Wright's stain were examined.

In 7 cases a raised HbA_2 level was suggested on cyanogum screening and in 5 of them starch grain electrophoresis confirmed this impression. In

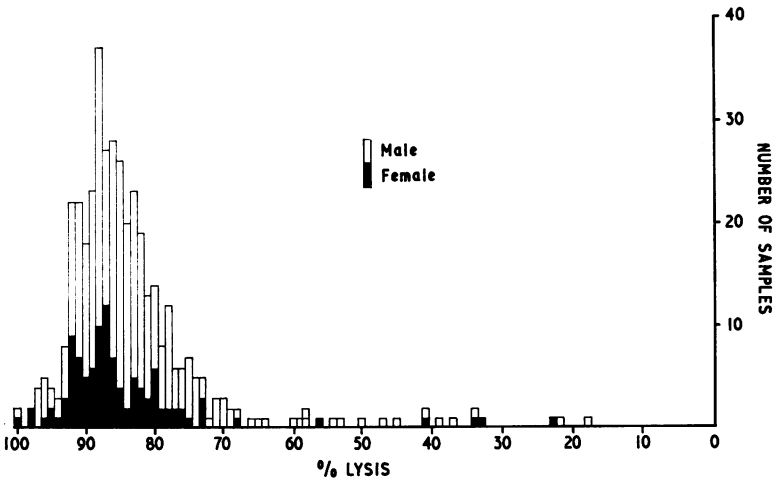

FIG. 2. Osmotic fragility at 0·45% NaCl. Series A only tested. Detailed studies were performed on samples with lysis of 60% or less (see text). At least five subjects with abnormal fragility had β-thalassaemia.

4 of these 5 samples the HbF level was also raised (> 2%). HbF was not raised in the remaining 15 subjects with lysis of less than 60%.

These findings indicate (Table III) a minimum

TABLE III

FINDINGS IN 5 CASES WITH RAISED OSMOTIC RESISTANCE AND RAISED HbA_2 LEVELS

Sex	Age	Osmotic Fragility % Lysis in 0·45 NaCl	HbF Level (%)*	HbA_2 Level (%)†
M	23	41	1·6	3·5
F	19	24	3·1	5·3
M	21	18	2·6	3·4
M	19	23	4·9	6·1
M	22	55	2·5	5·3

Note: In all five cases blood films showed morphological abnormalities typical of thalassaemia trait.

* HbF levels of above 2% are abnormal.
† HbA_2 levels of above 3·2% are abnormal under these conditions of testing.

prevalence of β thalassaemia trait of 5/403 (Series A only) or a little over 1% in the Philippines. That this is a reasonable estimate is confirmed by the fact that normal levels of HbA_2 were found in 14 further subjects with moderately raised osmotic resistance (lysis between 60 and 75%). Increased levels of HbF were not found in over 200 tests in Series A and B apart from these trait carriers.

It seems likely that some of the 15 cases with high osmotic resistance but normal HbF and normal HbA_2 levels represent cases of α thalassaemia trait. While no haemoglobin H or Bart's was found on routine paper electrophoresis in Series A and B, this is not an adequate screening technique (Fessas, 1963) for α thalassaemia, nor can much significance be attached to failure to find HbH inclusion bodies by the technique of Gouttas, Fessas, Tsevrenis, and Xefteri (1955). All samples of Series A were examined for such inclusions. However, the delay in time occurring before these preparations could be made would have very much reduced chances of finding rare inclusion bodies. While the inadequacies of the survey precluded detection of α thalassaemia trait carriers, circumstantial evidence that this trait also exists in the Philippines is afforded by the fact that Vella (1959), and Lie-Injo and Ti (1961) found a 1·2–3·2% incidence of γ_4 (Bart's) haemoglobin in the cord blood of ethnically related newborn Malayan infants. This finding strongly suggests the presence of α thalassaemia trait in these infants.

Of the 15 persons who had raised osmotic resistance but did not have β thalassaemia trait, 4 were women and 11 men. This ratio represents very similar proportions of the totals (108 women and 295 men). If iron-deficiency anaemia were responsible for the increased osmotic resistance, a larger proportion of women would be expected. It may be, therefore, that a substantial number of these 15 are in fact carriers of α thalassaemia trait.

Seven children selected because of severe splenomegalic anaemia and their parents (when possible) were studied in an attempt to assess the role of thalassaemia and haemoglobinopathy in the patho-

genesis of severe anaemia in Philippino children. G6PD activity was normal in all samples. No major abnormal haemoglobin components such as HbE were found in any specimens by paper electrophoresis. Table III describes the findings. The diagnosis of thalassaemia major appeared probable in one case only (D). Both parents (DF, DM) showed evidence of thalassaemia minor. In all other cases, an exact diagnosis was uncertain though thalassaemia appeared implicated in patients B, C, and E in view of the raised Hb A_2 levels in these patients. The possibility of interaction of α and β thalassaemia must be considered, though this combination does not lead to overt clinical disease in Greece (Fessas, 1962). On the other hand Bernini, Colucci, de Michele, Piomelli, and Siniscalco (1962), working in Italy, conclude that this combination is sometimes responsible for serious clinical disease. It is also possible that these children (B, C, and E) are β thalassaemia heterozygotes with more severe anaemia than usually found.

Comment

These studies indicate the presence of both β thalassaemia and G6PD deficiency in the Philippines. Similar findings were noted among Taiwanese (Motulsky, Lee, and Fraser, 1965), though the frequency of G6PD deficiency was lower (3%) and that of β thalassaemia somewhat higher (3%) in that island. It is of interest that both these genetically unrelated traits occur in Asiatic populations as they do in Mediterranean populations. Since the evidence for a protective influence of G6PD deficiency against falciparum malaria is fairly good (see Motulsky (1964) for references, and Flatz and Sringam (1963)), the positive correlation between the two traits in both Asiatic and Mediterranean populations suggests that malaria also plays a role in maintenance of the thalassaemia gene. More detailed enzymatic kinetic and electrophoretic studies are required to decide whether the type of severe enzyme deficiency seen in both Mediterranean and Asiatic populations represents a mutation with biochemically identical effects or whether two different mutations affecting the same enzyme are involved (Kirkman, 1963).

Summary

A high frequency (6·6%) of G6PD deficiency was found in 407 Philippino males. G6PD deficiency may be more frequent in the North-Western provinces of the Philippines. G6PD deficiency among Philippinos is severe and is quantitatively similar to the enzyme deficiency found in Mediterranean populations.

In 403 healthy Philippinos of both sexes at least 5 cases of β thalassaemia trait were found. No major abnormal haemoglobin component such as HbE was found in 515 Philippinos screened. A study of 7 children selected for severe splenomegalic anaemia suggested that thalassaemia in either the homozygote or heterozygote state plays an important role in the pathogenesis of clinically significant anaemia in the Philippines.

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