GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY, SICKLING AND MALARIA IN AFRICAN CHILDREN IN SOUTH-WESTERN NIGERIA

by

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INTRODUCTION

Results of field studies on the hypothesis that malaria favours the enzyme-deficient gene have been conflicting. Thus Allison & Clyde (1961), Harris & Gilles (1961) and others, have put forward evidence in favour of the theory, while Kidson & Gorman (1962) and Kruatrachue et al. (1962) have challenged the concept. In hospital studies dealing only with severely ill children the findings have been equally conflicting. Thus Gilles & Taylor (1961) showed that the incidence of cerebral and other complicated forms of malaria was significantly lower in enzyme-deficient subjects than in those with normal enzyme activity, while Porter et al. (1964) showed that three of eight children with severe malaria were enzyme deficient; (G-6-PD phenotype A).

In this paper the relationship is recorded between (1) erythrocyte glucose-6-phosphate dehydrogenase deficiency (G-6-PD) and malaria, (2) haemoglobin S and malaria, and (3) haemoglobin C and malaria; in 100 children under four years of age, clinically diagnosed as having severe falciparum malaria and in all of whom the diagnosis was confirmed by parasite counts of 100 000 per mm.3 or over.

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1 This study received financial assistance from the World Health Organization.
All the children studied were Nigerian, of both sexes, aged six months to four years. They had all been admitted to the emergency room of the children's department of University College Hospital, Ibadan, with the presumptive diagnosis of severe falciparum malaria, i.e., high fever (over 103°F, 39.4°C) with convulsions or coma. In several instances this presumptive diagnosis was not confirmed by blood film examination and the symptoms were due to other conditions, e.g., meningitis. Only those children in whom in addition to the above clinical presentation, parasite counts of 100,000 per mm$^3$ or over were found, were accepted for this study. It took three years to collect the series of 100 children who satisfied these criteria.

METHODS

For the diagnosis of haemoglobin S and C paper electrophoresis was applied using standard techniques. Blood was collected in acid-citrate-dextrose-inosine solution and assayed for erythrocytes G-6-PD activity by the qualitative and quantitative tests of Motulsky and Campbell-Kraut (described in Gilles & Taylor, 1961, and Capps et al., 1963). The Fairbanks & Beutler (1962) spot test for the detection of enzyme deficiency was also employed on the last 40 cases, and good agreement with the Motulsky and Campbell-Kraut qualitative test was obtained. On other subjects, however, outside this series, the latter test was found to be more useful in selecting suspected female heterozygotes with intermediate enzyme levels for confirmation by the Motulsky and Campbell-Kraut quantitative assay. Thick blood films were stained with Field's stain, and the number of parasites/400 w.b.c. counted; it was assumed that the white cell count in these young children was 10,000 per mm$^3$.

RESULTS

G-6-PD deficiency and malaria. Table 1 shows the presence of G-6-PD deficiency in children suffering from severe malaria and in control children attending the clinic for other purposes. It can be seen that the incidence of G-6-PD deficiency in children suffering from severe malaria is significantly lower than in the controls.
When statistical analysis is made taking the two sexes separately, male children suffering from severe malaria have a significantly lower incidence of G-6-PD deficiency at the 5% level than the control group, while the difference in the female children is not statistically significant (Table 2).

**Levels of erythrocyte G-6-PD activity.** The quantitative levels of G-6-PD activity in the 94 children with severe falciparum malaria but normal enzyme activity ranged from 175-1085 units/ml/100 rbc's. The levels for the six deficient children were as follows:

1. Male 139 units/ml/100 rbc's  
2. Male 132 " " " "  
3. Male 91 " " " "  
4. Male 75 " " " "  
5. Male 34 " " " "  
6. Female 134 " " " "

The quantitative test also gave a deficient result in all of these six children.

Child 6 is most probably a female heterozygote with an intermediate level of enzyme, while it is thought that cases 1 and 2, who are male hemizygotes with full expression of the deficiency, had somewhat elevated enzyme levels as a result of a detected reticulocytosis.

**Enzyme levels before and after treatment.** Fletcher & Maegraith (1962) have shown that *Plasmodium knowlesi* infection in rhesus monkeys increases erythrocyte glucose-6-phosphate dehydrogenase. A similar effect by *P. falciparum* might therefore give rise to false positive results, i.e., a deficient person appearing normal.

In order to check this possibility enzyme levels were estimated just before curative treatment began and again usually 48 hours later when very few, if any, parasites were detectable in thick blood films. The second blood sample sometimes had a lower enzyme activity than the initial one but it was never so low as to alter the pretreatment assessment of normal activity. It is suspected that overnight storage at 4°C of blood from children undergoing chloroquine treatment may result in a decrease in enzyme activity. Alternatively, these decreases in activity may reflect the removal from the circulation of parasitized erythrocytes with increased enzyme contents. Both these possibilities are being studied at
the present time. Whenever it was possible to collect a third sample a week after commencement of treatment the enzyme activity was often found to be elevated above the initial pretreatment level with evidence of a concurrent reticulocytosis.

Haemoglobin S and malaria. Table 3 shows the incidence of haemoglobin S in children suffering from severe malaria and in control children. It can be seen that the incidence of AS in children suffering from severe malaria is significantly lower than in the controls.

Haemoglobin C and malaria. Table 4 shows the incidence of haemoglobin C in children suffering from severe malaria and in control children. There is no difference between the two groups.

Relation between G-6-PD and haemoglobin genotypes. The six G-6-PD deficient children were all haemoglobin AA; the four AS children had normal G-6-PD activity as did the six AC children. In this series of children there was clearly no association between the various genetic traits investigated.

DISCUSSION

G-6-PD and "malaria hypothesis". Proof of the malaria protection hypothesis has been sought in various ways: (1) gene frequency distribution studies in populations living in areas of different malarial endemicity; (2) malaria parasite density surveys in G-6-PD normal and deficient children; (3) induced falciparum malaria in human volunteers; (4) G-6-PD deficiency among patients with severe clinical falciparum malaria. To date in almost every instance the results have been conflicting.

In a recent publication Powell & Brewer (1965) failed to disclose significant differences between corresponding mean levels of parasitaemia in G-6-PD deficient subjects compared to those in subjects not G-6-PD deficient. The authors admit however that their studies were limited to times when levels of falciparum parasites in the blood were relatively low, and that the data they present do not shed light upon the critically important issue concerning what obtains when there are very high levels of parasitaemia attended by substantial risks of mortality. It is this very issue that we have attempted to tackle in our study.
Identification of G-6-PD subjects in post mortem blood is complicated by the broad range of enzyme values observed in red cells obtained at autopsy (Zinkham, 1961), and moreover the tests have to be performed soon after death. This means that the nearest one can get to this ideal is to study children who are suffering from potentially lethal infections.

Field (1949) in Malaya, found that case mortality rates of malaria only rose significantly when the peripheral blood before treatment showed at least 100 000 parasites per mm$^3$ (39 deaths in 177 patients, i.e., 22%). Although no comparable figures are available for Africa, it is the experience of one of us (H. M. Gilles) that the criteria we have laid down for the selection of children for this present study are indicative of severe malaria infection in African children which would result - if untreated - in a mortality rate not dissimilar to that recorded above. It is accepted, however, that differences of opinion are bound to occur, since physicians are not endowed with prophetic powers. It must be borne in mind moreover that clear cut differences between G-6-PD normal and deficient children could sometimes be partly obscured by the presence in surveys of female heterozygotes to whom the protective advantages afforded by possession of full expression of the abnormal gene may not be available because of their intermediate enzyme levels. The point can be made that similar considerations may apply at limited times through reticulocytosis in male hemizygotes in whom a higher than normal population of young erythrocytes with their higher enzyme contents might support parasite growth. Nevertheless the evidence we have presented strongly supports the view that G-6-PD deficiency offers a selective advantage to the carrier against potentially lethal malaria infection. The incidence of G-6-PD deficiency in the control children of this study was similar to that reported in previous surveys from Nigeria (Gilles & Taylor, 1961; Harris & Gilles, 1961; Capps et al., 1963).

Malaria and haemoglobin S. Evidence has accumulated over the past years in favour of the hypothesis that the sickle cell trait protects the bearer against the lethal effects of P. falciparum malaria. The most convincing evidence was produced by Edington & Watson-Williams (1965) who showed that in 42 children dying of cerebral malaria, haemoglobin S was never present. Our study confirms the rarity of haemoglobin S in children suffering from severe falciparum malaria.
Malaria and haemoglobin C. The evidence that haemoglobin C protects against the lethal effects of malaria has never been convincing (Edington & Laing, 1957). Thompson (1962) working in Accra, claimed that possession of the C trait protected against malaria. His results are open to criticism on at least two grounds, (1) children of policemen are a selected and privileged group, (2) malaria control measures in Accra over the past years have diminished the intensity of transmission. Edington & Watson-Williams (1955) could find no evidence of protection in their post mortem material, nor does this study support the hypothesis that haemoglobin C protects against malaria. It was suggested by Livingstone (1959) that the distribution of haemoglobin C in West Africa showed some relationship to that of *P. malariae* and that protection against this parasite may possibly occur. Our studies in nephrotic children do not substantiate this view. Thus the distribution of the haemoglobin C trait in 156 children suffering from the nephrotic syndrome associated with *P. malariae* was similar to that for the general population (Hendrickse & Gilles, 1963). It could of course be argued that the susceptibility of nephrotic children to *P. malariae* more than counteracts any protective effect that the HbC might provide.

The recent report of a WHO scientific group (1966) stressed the importance of conducting further studies of the malaria hypothesis in children between four months and four years with heavy parasitaemia; our investigation is in keeping with this recommendation.

**SUMMARY**

1. The relationship between erythrocyte glucose-6-phosphate dehydrogenase deficiency, haemoglobin S, haemoglobin C and malaria has been studied in 100 Nigerian children under four years of age clinically diagnosed as having severe falciparum malaria and in all of whom the diagnosis was confirmed by parasite counts of 100,000 per mm$^3$ or over.

2. The results obtained support the hypotheses that G-6-PD deficiency and haemoglobin S offer a selective advantage to the carrier against potentially lethal malaria infection. Haemoglobin C offers no such advantage.
ACKNOWLEDGEMENTS

Our thanks are due to Dr Gupta, Paediatrician, Adeoyo Hospital, Ibadan, for referring some of her patients to us, and to all the house physicians and registrars in the Emergency Treatment Room of the Department of Paediatrics, University College Hospital, Ibadan, without whose co-operation this study would not have been possible.
RESUME

Les rapports entre la carence érythrocytaire en glucose-6-phosphate déshydrogénase (G-6-PD), l’hémoglobine S et l’hémoglobine C, d’une part, et le paludisme, d’autre part, ont été étudiés sur 100 enfants nigériens âgés de moins de 4 ans chez lesquels le diagnostic clinique avait révélé une atteinte grave de paludisme à P. falciparum. Chez tous ces enfants, le diagnostic a été confirmé par la mise en évidence d’une parasitémie de 100 000/mm³ ou davantage.

Les résultats obtenus corroborent l’hypothèse selon laquelle la carence en G-6-PD et la présence d’hémoglobine S confèrent un avantage biologique contre une infection paludéenne dont l’issue peut être fatale. L’hémoglobine C ne confère aucun avantage à cet égard.
REFERENCES


Capps, F. P. A. et al. (1963) Lancet, 2, 379


Fletcher, K. A. & Maegraith, B. G. (1962) Nature (Lond.), 196, 1316


Kruatrachue, M. et al. (1962) Lancet, 2, 1183


Porter, I. H. et al. (1964) Lancet, 1, 895


Thompson, G. R. (1962) Brit. med. J., 1, 682


**TABLE 1. ERYTHROCYTE G-6-PD DEFICIENCY IN CHILDREN SUFFERING FROM SEVERE MALARIA AND IN CONTROL CHILDREN (AGES 6 MONTHS TO 4 YEARS)**

<table>
<thead>
<tr>
<th></th>
<th>Number examined</th>
<th>G-6-PD normal</th>
<th>G-6-PD deficient</th>
<th>% deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>200</td>
<td>166</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>Severe malaria</td>
<td>100</td>
<td>94</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

\[X^2 = 6.926\] 1 df P < 0.01.

The incidence of G-6-PD deficiency in children suffering from severe malaria is significantly lower than in controls.

**TABLE 2. THE INCIDENCE OF G-6-PD DEFICIENCY IN RELATION TO THE SEX OF THE CHILDREN**

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number examined</td>
<td>G-6-PD normal</td>
</tr>
<tr>
<td>Controls</td>
<td>102</td>
<td>80</td>
</tr>
<tr>
<td>Severe malaria</td>
<td>55</td>
<td>50</td>
</tr>
</tbody>
</table>

\(^a\) \[X^2 = 3.9734\] 1 df P < 0.05 > 0.02.

\(^b\) \[X^2 = 3.7629\] 1 df P > 0.05.

**TABLE 3. HEMOGLOBIN S IN CHILDREN SUFFERING FROM SEVERE MALARIA AND IN CONTROL CHILDREN**

<table>
<thead>
<tr>
<th></th>
<th>Number examined</th>
<th>Hb AA</th>
<th>Hb Hs</th>
<th>% Hb Hs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>200</td>
<td>164</td>
<td>36</td>
<td>18%</td>
</tr>
<tr>
<td>Severe malaria</td>
<td>100</td>
<td>96</td>
<td>4</td>
<td>4%</td>
</tr>
</tbody>
</table>

\[X^2 = 10.62\] 1 df P = 0.001.
<table>
<thead>
<tr>
<th></th>
<th>Number examined</th>
<th>Hb AA</th>
<th>Hb AC</th>
<th>% Hb AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>200</td>
<td>186</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Severe malaria</td>
<td>100</td>
<td>94</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

There is clearly no difference in the incidence of haemoglobin C in the two groups of children examined.
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