GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY
AND FALCIPARUM MALARIA

by

Robin D. Fowell and George J. Brewer,
Army Medical Research Unit of the Department of Medicine
the Division of Biological Sciences, University of Chicago,
Chicago, Illinois, United States of America

This report presents the results of studies aimed at evaluating the hypothesis
that glucose-6-phosphate dehydrogenase (G6PD) deficiency, an inherited disorder that
involves the human erythrocyte (Cerson et al., 1956; Tarlov et al., 1962), confers
a biological advantage against falciparum malaria (Motulsky et al., 1959;

METHODS

Experimental design

Investigations of the duration of the prepatent period and of the early clinical
course of mosquito-induced falciparum malaria (McLendon strain) were conducted with
16 American Negro males who volunteered to participate in the studies. Eight
volunteers were G6PD-deficient (activity of erythrocytic G6PD approximately one-
tenth normal). Eight volunteers were not G6PD-deficient. The 16 volunteers
were studied in groups of four. Each group consisted of two G6PD-deficient men
and two men not G6PD-deficient.

1 These studies were supported, in major part, by the Medical Research and
Development Command, Office of the Surgeon General, Department of the Army,
United States of America, under Contract DA-49-007-MD-566 with the University of
Chicago, and, in part, by the Douglas Smith Foundation of the University of Chicago.
Subjects

Volunteers were inmates of the Illinois State Penitentiary, Stateville Branch, Joliet, Illinois. All volunteers were healthy: chest microfilms, physical examinations, levels of haemoglobin in the blood, levels of the haematocrit, reticulocyte counts, total leucocyte counts, differential counts of leucocytes, urinalyses, and phenolsulphonphthalein tests were normal. Red cells of all subjects were morphologically normal. Paper electrophoresis of haemoglobin of all volunteers disclosed that migration was that of haemoglobin A, with no evidence of haemoglobins S or C. The eight G6PD-deficient subjects were between 26 and 38 years old (mean age: 32 years); they weighed between 139 and 217 pounds (67 and 98 kg) (mean weight: 179 pounds = 81 kg). The eight individuals not G6PD-deficient were between 28 and 36 years old (mean age: 33 years); they weighed between 135 and 205 pounds (61 and 93 kg) (mean weight: 172 pounds = 78 kg).

None of the volunteers had a prior history of malaria or of an illness suggesting malaria. Volunteers were selected for study only if they had apparently never resided in or visited a malarious area.

G6PD deficiency was detected initially by the methaemoglobin reduction test of Brewer et al. (1962); findings were confirmed by measurements of activity of G6PD in haemolysates. A modification of the method of assay of G6PD described by Glock & McLean (1953) similar to that of Zinkham & Lenhard (1959) was employed.

Mosquito-induced infections

The four groups of subjects, each consisting of two G6PD-deficient men and two men not G6PD-deficient, were designated groups A, B, C and D. All four members of each group were bitten, at approximately the same time, by the same 10 Anopheles stephensi infected with the McLendon strain of Plasmodium falciparum. The interrupted bite technique described by Alving et al. (1948) was employed.

The density of sporozoites in the salivary glands of each infected mosquito used was graded, after dissection and disruption of the glands, from one plus to four plus. The total number of pluses recorded for the 10 infected mosquitoes that bit all four members of group A was 40; total pluses recorded for groups B, C and D were 39, 34 and 30.
The day of bite was designated day 0, the first day after bite day 1, the second day after bite day 2, and so on. All members of each group were hospitalized on day 6 of each study.

Parasite counts

Thick films of each subject's blood were examined at 12-hour intervals, beginning on day 6 of each study, until overt parasitaemia was detected. Parasite counts were then carried out at four- to six-hour intervals until blood schizontocidal medication was administered. Parasite counts were performed by a previously described modification (Alving et al., 1948; Powell et al., 1964) of the method of Earle & Perez (1932). Parasite counts presented in this report refer to trophozoites, not gametocytes; mature erythrocytic schizonts were rarely seen.

Termination of acute attacks of malaria

Blood schizontocidal medication was administered when the parasite count first exceeded 5000/mm³ or whenever otherwise warranted clinically. Fifteen hundred mg of chloroquine base was administered orally over three days (600 mg the first day, 300 mg six hours later, and 300 mg on each of the next two days) to terminate acute attacks of malaria and to effect radical cure of the infections. The severity of the attacks of malaria in two volunteers (one G6PD-deficient and one not G6PD-deficient), as noted subsequently, necessitated initiation of therapy with chloroquine before the parasite count exceeded 5000/mm³. Administration of chloroquine to one volunteer (Fig. 1, subject 3), a G6PD-deficient individual who was not experiencing a severe attack of malaria, was delayed while studies on the infectivity of gametocytes in the blood of this volunteer were carried out; he received subscurvative doses of 377-C-54 (2:5-bis(cyclohexylaminomethyl) naphthalene-1:6-diol hydrochloride) to control the level of asexual erythrocytic forms of P. falciparum in the blood. Radical cure of the infection in this volunteer was achieved by subsequent administration of chloroquine.

The studies were carried out under extremely close medical supervision. No adverse complications occurred.

Statistical evaluation

Differences between corresponding mean values (e.g., the difference, at a given time, between the mean parasite count in G6PD-deficient subjects and that in subjects not G6PD-deficient) were evaluated for statistical significance by the "t-test" for small samples as described by Bancroft (1962).
RESULTS

All eight G6PD-deficient subjects and seven of the eight subjects not G6PD-deficient developed patent falciparum infections (Fig. 1). Subject 9 (Fig. 1, group C) failed to develop clinically-apparent malaria during a follow-up period of 60 days. Overt parasitaemia was first detected on day 9 in all members of group A, on day 10 in all members of group B, on days 10 or 11 in the three members of group C who developed patent infections, and on day 12 in all members of group D. The mean length of the prepatent period in G6PD-deficient subjects was 10.4 days. The mean length of the prepatent period in subjects not G6PD-deficient (excluding subject 9) was also 10.4 days.

All 15 subjects who developed patent infections sustained acute clinical attacks of falciparum malaria. Rates of increase in levels of overt parasitaemia, fever and other symptoms, and times of initiation of blood schizontocidal therapy varied. No grossly apparent pattern emerged suggesting a marked difference in these respects in the G6PD-deficient individuals compared to those not G6PD-deficient. Subject 12, a G6PD-deficient man, sustained the most abrupt acute clinical attack observed during the course of the study; treatment with chloroquine was instituted when the parasite count was 1180/μm³. Subject 10, not G6PD-deficient, experienced repeated spiking fever, accompanied by other symptoms of falciparum malaria, when levels of parasitaemia were relatively low; therapy with chloroquine was initiated when the parasite count was 1400/μm³. The other 13 subjects who developed patent infections received blood schizontocidal therapy instituted immediately after the parasite count first exceeded 5000/μm³ (Fig. 1).

Mean levels of parasitaemia in G6PD-deficient subjects and in those not G6PD-deficient (excluding subject 9) were calculated for each 12-hour interval during the 108-hour period following the onset of overt parasitaemia (Fig. 2). Mean values calculated for the interval between hours 108 and 120 are separated from the remainder of the data because the administration of chloroquine to five G6PD-deficient subjects and to five subjects not G6PD-deficient appreciably altered the findings during the latter interval. None of the differences between corresponding mean values proved statistically significant (P > 0.05).
An average of 5.7 days elapsed between the onset of overt parasitaemia and the initiation of administration of blood schizontocidal therapy to subjects not G6PD-deficient. The corresponding value calculated for the studies with the G6PD-deficient subjects was 6.9 days. The difference proved not statistically significant ($0.4 < P < 0.5$).

The early course of parasitaemia and fever in subject 3 (Fig. 1), a G6PD-deficient volunteer, was similar to that of other subjects, but subsequently he had few symptoms of malaria other than transient fever. These other symptoms were mild. The count of asexual erythrocytic forms of P. falciparum in the blood of this subject did not exceed $5000/\text{mm}^3$ until day 24 (Fig. 1), at which time detectable gametocytæmia was present. Subcurative doses of 377-C-54 were administered to control levels of asexual erythrocytic forms of P. falciparum and uninfected A. stephensi were then allowed to bite this G6PD-deficient volunteer. Oocysts developed in the stomachs of these mosquitoes and large numbers of sporozoites were detected subsequently in the salivary glands. Some of these mosquitoes were allowed to bite other non-immune volunteers. The latter volunteers developed falciparum malaria after prepotent periods of 10 to 12 days.

**DISCUSSION**

The investigations presented in this report represent relatively short-term studies conducted with a small number of healthy American Negro males infected with a single strain of P. falciparum in a non-endemic area. Results of such studies do not necessarily provide accurate insight into what may occur over many generations as large numbers of individuals - particularly young children - in endemic areas sustain repeated falciparum infections, as well as a host of other maladies, that are allowed to pursue their natural course.

Our studies were conducted under carefully controlled conditions. Immunity to malaria was excluded as a possible complicating factor. The experimental approach we employed, although far from ideal, is a more direct one than certain approaches others have used previously to test the hypothesis that G6PD deficiency confers a biological advantage against falciparum malaria.
We detected no prolongation of the prepatent period of mosquito-induced falciparum malaria in G6PD-deficient American Negro men compared to that in American Negro men not G6PD-deficient. Studies carried out after the onset of patency failed to disclose significant differences between corresponding mean levels of parasitaemia in G6PD-deficient subjects compared to those in subjects not G6PD-deficient. The mean time elapsing between the onset of patency and the initiation of administration of blood schizontocidal medication in studies with G6PD-deficient subjects was not significantly different from that which was obtained in studies with subjects not G6PD-deficient. These data do not support the hypothesis that G6PD deficiency confers a biological advantage against falciparum malaria.

Our findings indicate that there was no retardation of exoerythrocytic development or of different patterns of proliferation of asexual erythrocytic forms of *P. falciparum* in the G6PD-deficient subjects. It has been suggested that growth of falciparum parasites in G6PD-deficient red cells may be suboptimal, (Motulsky, 1964). Theoretical reasons cited to explain possible suboptimal growth of falciparum parasites in G6PD-deficient red cells include the results of studies carried out with *P. lophurae* and with *P. knowlesi*. Our investigations with *P. falciparum* indicate that, under the particular experimental conditions employed, the environment for the growth of asexual erythrocytic forms of *P. falciparum* provided by G6PD-deficient red cells was not significantly less favourable than that provided by red cells not G6PD-deficient. The development of viable, infective gametocytes in the red cells of one G6PD-deficient individual was indicated by passage of the strain from that individual through mosquitoes to other volunteers.

Our findings do not render invalid the hypothesis that G6PD deficiency confers a biological advantage against falciparum malaria. Our studies carried out when overt parasitaemia was present, were limited to times when levels of falciparum parasites in the blood were relatively low. Parasite counts in eight volunteers (four G6PD-deficient and four not G6PD-deficient) exceeded 10,000/mm³ during the course of these studies (just after initiation of therapy with...
chloroquine). Parasite counts in one man not G6PD-deficient and in two G6PD-deficient men transiently exceeded 20,000/mm$^3$. Only one volunteer, a G6PD-deficient subject, developed parasitaemia exceeding 30,000/mm$^3$ during the studies. The data presented, therefore, 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 (Motulsky, 1964). Considerable additional evidence concerning this hypothesis is needed.

ACKNOWLEDGEMENTS

These studies were made possible through the co-operation of the inmates and administrative staff of the Illinois State Penitentiary, Stateville Branch, Joliet, Illinois. The authors wish to acknowledge particularly the help of the late Dr Alf S. Alving of the University of Chicago. Drs Geoffrey M. Jeffery and William E. Collins made possible initiation of our studies by kindly sending us blood infected with the McLemore strain of P. falciparum.
RESUME

Ce rapport expose les résultats d'études entreprises pour juger de l'hypothèse selon laquelle la carence en glucose-6-phosphate deshydrogénase (G-6-P-D), anomalie congénitale du système enzymatique qui affecte l'érythrocyte chez l'homme, confère un avantage biologique contre le paludisme à *P. falciparum*.

Les recherches concernant la durée de la période de prépatence et l'évolution clinique précoce du paludisme ont été menées sur 16 volontaires non immuns, choisis parmi des Noirs américains de sexe masculin en bonne santé qui ont été infectés par une souche unique (McLendon) de *Plasmodium falciparum*, dans une zone où le paludisme n'était pas endémique. Huit de ces volontaires présentaient une carence en G-6-P-D, contrairement aux huit autres.

Les résultats de ces études ne donnent pas nécessairement une image exacte de ce qui peut se produire sur plusieurs générations car, dans les zones d'endémicité, un grand nombre d'individus, surtout parmi les jeunes enfants, contractent des infections répétées à *P. falciparum* dont le cours naturel n'est pas entravé. Les études en cause ont été faites dans des conditions rigoureusement contrôlées. L'immunité à l'égard du paludisme a été éliminée en tant que facteur de complication.

On n'a constaté aucune prolongation de la période de prépatence du paludisme à *P. falciparum* transmis par les moustiques chez les volontaires présentant une carence en G-6-P-D par rapport aux autres volontaires. Il n'y a pas eu de différence significative entre les sujets carencés en G-6-P-D et les autres sujets du point de vue des degrés moyens de parasitémie enregistrés au début de la période de patence.

Le laps de temps qui s'est écoulé en moyenne entre le début de la période de patence et la première administration de schizontocide sanguin n'a pas varié de façon significative d'un groupe à l'autre.

Ces résultats ne confirment pas l'hypothèse d'un avantage biologique conféré par la carence en G-6-P-D contre le paludisme à *P. falciparum*.
Il semble qu’il n’y ait pas eu de retard dans le déroulement de la phase exo-
erythrocytaire ni de différence dans la prolifération des formes érythrocytaires asexuées
de P. falciparum. Dans les conditions particulières de l’expérience, les hématies carac-
térisées par une carence en G-6-P-D ne semblaient pas être un milieu beaucoup moins
favorable à la croissance des formes érythrocytaires asexuées que les hématies normales.
Le développement de gamétocytes viables et infectants dans les hématies d’un des sujets
carencés en G-6-P-D a été mis en évidence par le fait que la souche présente chez ce
sujet a été transmise à d’autres volontaires par l’intermédiaire de moustiques.

Ces constatations n’influent pas nécessairement l’hypothèse de l’avantage biolo-
gique conféré par la carence en G-6-P-D contre le paludisme à P. falciparum, car les
études en question, faites au moment où la parasitémie s’était déclarée, ont été limitées
à des périodes où la densité des P. falciparum dans le sang était relativement faible.
Un seul volontaire a présenté une parasitémie dépassant 30 000/mm³.

Il faudra réunir beaucoup d’autres données pour juger de l’hypothèse considérée
ici, notamment en ce qui concerne l’important problème de l’évolution qui se produit
lorsque la parasitémie atteint des degrés élevés.
REFERENCES

Alving, A. S. et al. (1948) Procedures used at Stateville Penitentiary for the
testing of potential antimalarial agents, J. clin. Invest., 27, 2


test for primaquine-type sensitivity of erythrocytes, J. Amer. med. Ass.,
180, 386

Carson, P. E. et al. (1956) Enzymatic deficiency in primaquine-sensitive
erythrocytes, Science, 124, 484

Earle, W. C. & Perez, M. (1932) Enumeration of parasites in the blood of malarial
patients, J. Lab. clin. Med., 17, 1124

Glock, G. E. & McLean, P. (1953) Further studies on the properties and assay of
glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase of
rat liver, Biochem. J., 55, 400

Med. Hyg., 13, 147

Motulsky, A. G. et al. (1959) Biochemical genetics of glucose-6-phosphate

Powell, R. D. et al. (1964) Studies on a strain of chloroquine-resistant Plasmodium
falciparum from Thailand, Bull. Wld Hlth Org., 30, 29

Tarlov, A. R. et al. (1962) Primaquine sensitivity - glucose-6-phosphate dehydrogenase
deficiency. An inborn error of metabolism of medical and biological
significance, Arch. intern. Med., 109, 209

from patients with congenital nonspherocytic hemolytic anemia, J. Pediatr.,
55, 319
Curves of temperature (T) (°C.) and (P) parasitemia (levels of asexual erythrocytic forms per mm$^3$.) in volunteers bitten by mosquitoes infected with the McLeodan strain of P. falciparum. Abscissa indicate days 9 through 24 (after bite by infected mosquitoes) of each study. Arrows designate times of initiation of blood schizontocidal therapy (subject 3 received 377-C-54, all other subjects received chloroquine).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NORMAL</th>
<th>G6PD-DEFICIENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><img src="image" alt="Graphs" /></td>
<td><img src="image" alt="Graphs" /></td>
</tr>
<tr>
<td>B</td>
<td><img src="image" alt="Graphs" /></td>
<td><img src="image" alt="Graphs" /></td>
</tr>
<tr>
<td>C</td>
<td><img src="image" alt="Graphs" /></td>
<td><img src="image" alt="Graphs" /></td>
</tr>
<tr>
<td>D</td>
<td><img src="image" alt="Graphs" /></td>
<td><img src="image" alt="Graphs" /></td>
</tr>
</tbody>
</table>

The compound designated as 377-C-54 is a hydroxynaphthalene derivative (1,6-dihydroxy-2,5-bis (cyclohexylaminomethyl) naphthalene) with an activity against avian and simian malaria infection and some activity against human plasmodia. (Editor's remarks.)
Fig. 2
Mean levels of parasitemia (ordinate) (levels of asexual erythrocytic forms of P. falciparum per cu. mm.) in G6PD-deficient volunteers compared to those in volunteers not G6PD-deficient (excluding subject 9). Mean levels of parasitaemia were calculated for each 12-hour interval (abscissa) beginning with the onset of patency.
The purpose of the WHO/Mal series of documents is threefold:

(a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;

(b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;

(c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

The issue of a paper in this series does not therefore constitute formal publication and a paper so issued may, with the agreement of the author and WHO, be published in a WHO periodical or elsewhere.

Authors alone are responsible for views expressed in signed articles. The mention of manufacturing companies or of their proprietary products does not imply that they are recommended or endorsed by the World Health Organization.