MALARIA AND STRESS IN RELATION TO HAEMOGLOBINS S AND C*

BY

G. R. THOMPSON,† M.D., M.R.C.P., D.T.M.&H.

Late Captain, R.A.M.C.; Queen Alexandra Military Hospital, Millbank, London

In a previous communication (Thompson, 1962) evidence was presented in favour of the existence of partial protection against *Plasmodium falciparum* malaria among Ghanaians with either the sickle-cell or haemoglobin C trait. Comparison of mean parasite densities among young children in Accra showed significantly lighter infections among heterozygous carriers of haemoglobin S or C than among those possessing normal haemoglobin alone. The protection conferred by haemoglobin S was apparently greater than that due to haemoglobin C, but the difference demonstrated was not significant. The statistical treatment of these results was criticized by J. P. Garlick (personal communication, 1962) on the grounds that the t test was an invalid method of comparing untransformed parasite densities with large standard deviations and positively skewed distributions. Reappraisal of previous results has been necessary on this account, and has resulted in a more accurate assessment of the relationship between malaria and haemoglobins S and C respectively.

A much smaller advantage for heterozygous carriers of haemoglobin C was forecast by Allison (1961), who thought that it might be difficult to obtain unequivocal evidence of the existence of a protective effect against malaria in the field. Equilibrium between the haemoglobin S and C genes in Ghana could be envisaged if it was demonstrated that haemoglobin S heterozygotes possessed a greater advantage over the normal than did haemoglobin C heterozygotes, since this would help to compensate for the known lower survival rate of haemoglobin S homozygotes as compared with their haemoglobin C counterparts. However, the accepted efficiency of haemoglobin S in limiting malarial mortality is not entirely without its disadvantages. There now exist several reports illustrating the morbidity and mortality attributed to the sickle-cell trait in negroes exposed to stress not only at high altitudes (Rotter *et al.*, 1956) but also at sea-level (Edington and Lehmann, 1955; Ende *et al.*, 1955; Ober *et al.*, 1960; McCormick, 1961). Some further examples are reported in this paper.

Methods

Haemoglobin electrophoresis and sickling tests were carried out using standard procedures as previously described (Thompson, 1962). Foetal haemoglobin was estimated by the alkali denaturation method of Singer *et al.* (1951).

All case reports relate to Ghanaian patients observed at the Military Hospital, Accra, during 1960–1.

Further Analysis of Relation Between Malaria and Haemoglobin Type

Comparison of mean parasite densities had previously shown apparently significant differences between the three main haemoglobin groups of Accra police children (Thompson, 1962). However, logarithmic transformation of the original parasite densities has enabled a more acceptable statistical comparison to be made between their means. Results, shown in Table I, reveal that the previous difference between the AA and AC groups is no longer significant, although the difference between the AA and AS groups retains its high level of significance. These findings strongly suggest a superiority on the part of haemoglobin S in limiting parasitaemia, although on direct comparison the difference between the means of the AS and AC groups remains insignificant.

TABLE I.—*Comparison of Mean Log_{10} Densities of P. falciparum in Ghanaian Children Aged 1–6 Years*

Hb Type	Mean Log_{10} Density	t	P
AA	3·20	3·360	<0·001
AS	2·78		
AA	3·20	1·023	>0·3
AC	3·05		
AS	2·78	1·533	>0·1
AC	3·05		

It now becomes necessary to determine whether the protective effect previously postulated for haemoglobin C can be supported on any other grounds. Table II shows the frequency distribution of increasing intensities of *P. falciparum* infections among children grouped according to haemoglobin type, and includes homozygous carriers of haemoglobins S and C, although sickle-cell haemoglobin C heterozygotes have been omitted to avoid confusion. Thus group Hb-A = AA, Hb-S = AS + SS, and Hb-C = AC + CC. The differences between the three haemoglobin groups are shown in Table II, and it can be seen that carriers of haemoglobin C apparently occupy an intermediate position between those with haemoglobin S on the one hand and normal haemoglobin on the other. Comparison of the number of children with parasite densities above an arbitrary level (Raper, 1955) shows the incidence of densities exceeding log 3.75 (5,630/c.mm.) to be lower in both groups S and C than in group A. These differences are significant at the 5% level ($\chi_2 = 6.172$ for 2 d.f.) and tend to suggest that haemoglobin C may have a similar action to haemoglobin S in limiting high levels of parasitaemia during infections with *P. falciparum*.

TABLE II.—*Frequency Distribution of Log Parasite Densities of P. falciparum Among 240 Children with Positive Blood Films*

Hb Group	Total	Log_{10} Parasite Density		
		1·25–2·50	2·501–3·75	3·751–5·00
Hb-A ..	176	35 (19%)	99 (57%)	42 (24%)
Hb-S ..	34	15 (44%)	16 (47%)	3 (9%)
Hb-C ..	30	7 (23%)	20 (67%)	3 (10%)

None of the children with either abnormal haemoglobin had infections whose parasite densities exceeded 25,000/c.mm., although 10 children with normal haemoglobin had them in the 25,000–100,000/c.mm. range. In a series of 50 fatal cases of falciparum malaria among Asiatics in Malaya only 6% had parasite densities below 25,000/c.mm. (Field, 1949). Although the data for Field's treated adults are not comparable with those of young untreated children in Ghana, nevertheless his findings do imply that limitation of the intensity of *P. falciparum* infections would considerably reduce the malarial mortality rate among children at risk.

*This work formed part of a thesis submitted for the degree of M.D. in the University of London.

†Present address: Department of Medicine, Hammersmith Hospital and Postgraduate Medical School, London.

Case Reports Illustrating Pathological Responses to Stress of Subjects with Sickle-Cell Trait

Splenic Infarction

The clinical features of splenic infarction in association with sickling have been described in detail by Cooley *et al.* (1954), although it was later shown that one of their cases had sickle-cell haemoglobin C disease (Smith and Conley, 1955). Rotter *et al.* (1956) observed three cases of splenic infarction (due to air travel) in American negroes proved to have the sickle-cell trait. The syndrome was observed on several occasions in Accra, and five examples are reported below.

Case 1.—An adult policeman was returning from the Congo in an unpressurized transport plane flying at about 10,000 ft. (3,050 m.) when he developed severe left-sided abdominal pain. On admission to hospital in Accra examination revealed marked guarding in the left hypochondrium associated with an enlarged and extremely tender spleen. Chest *x*-ray films initially showed elevation of the left diaphragm together with splenic enlargement, and subsequently a left pleural effusion. Expectant treatment was adopted and abdominal pain and fever gradually settled within a month. Two weeks after his discharge he was readmitted with severe haemoptysis. Radiologically there was patchy consolidation with some cavitation in the basal segments of the left lower lobe. It seemed possible that these pulmonary manifestations could have resulted from extension upwards of a secondarily infected splenic infarct. The response to antibiotic therapy was good, but three months later left basal pneumonia recurred and was complicated by the development of a right subphrenic abscess. This was drained and the patient eventually made a good recovery. Haemoglobin type AS; Hb 9.6 g./100 ml.; foetal haemoglobin <2%.

Case 2.—An adult soldier was returning from the Congo in identical circumstances as in Case 1 when he developed similar symptoms and signs and was diagnosed as a case of acute splenic infarction. His condition settled uneventfully after a prophylactic course of penicillin. Haemoglobin type AS; Hb 13.1 g./100 ml. Haemoglobin S formed 40% of the mixture of haemoglobins A and S. Foetal haemoglobin <2%.

Case 3.—A soldier's wife was admitted to hospital as a new case of pulmonary tuberculosis. Chest *x*-ray examination showed acute bilateral cavitating disease and the sputum contained tubercle bacilli. On the fifth day after admission. during which her temperature rose to 103° F. (39.4° C.), pain developed suddenly in the left side of the abdomen, associated with an acutely tender spleen. Examination of blood films excluded malaria. Treatment with antituberculous drugs soon brought her fever under control, and the presumed splenic infarct resolved without complications. Haemoglobin type AS; Hb 7.2 g./100 ml.; foetal haemoglobin not estimated.

Case 4.—A male civilian aged 20 was admitted to hospital with a traumatic fracture of the pelvis complicated by a partial rupture of the urethra. Subsequently he ran a persistent low-grade fever accompanied by some pyuria. Towards the end of the fifth week his temperature rose to 102° F. (38.9° C.) and he developed pain in the left side of the chest. On examination the spleen was enlarged and tender, and a splenic rub was audible. Chest *x*-ray examination showed marked elevation of the left diaphragm. Fever and leucocytosis persisted. and six weeks after the onset of splenic pain pyrexia became hectic and the patient coughed up large quantities of caseous material. Tomography showed a cavity in the region of the left diaphragm, but it was not possible to determine whether this was above or below the latter. It was felt that the patient had sustained a splenic infarct which had developed into an abscess and then drained spontaneously via the left lung in a similar manner to Case 1. Good resolution followed intensive antibiotic therapy. Haemoglobin type AS. Haemoglobin S formed 40% of the mixture of haemoglobins A and S. Foetal haemoglobin <2%.

Case 5.—An adult airman was admitted to hospital with typhoid fever. During the second week of admission he developed sudden pain in the left hypochondrium. On examination the spleen was found to have enlarged further than noted previously. It was acutely tender, and a superimposed friction rub later became audible. Chloramphenicol therapy was maintained and a good recovery eventually followed. Haemoglobin type AS; foetal haemoglobin not estimated.

Fatal Sickling Crisis

Fatal crises in persons with the sickle-cell trait were reported from Ghana by Edington and Lehmann (1955) and Edington (1957), although the latter stressed the need to exclude sickle-cell thalassaemia by family studies and foetal haemoglobin estimations. Ober *et al.* (1960) reported a fatal episode of sickling in an American negro with no increase of foetal haemoglobin; and McCormick (1961), in a series of 120 necropsies on proved cases with the sickle-cell trait, concluded that sickling caused or contributed to death in 12.5%.

Case 6.—An adult male civilian in an alcoholic stupor was admitted to hospital for temporary observation. Soon after admission he vomited and rapidly developed profound and irreversible circulatory failure which was accompanied by epileptiform convulsions and which culminated in death 30 minutes later. Post-mortem examination revealed inhalation of a small quantity of vomit and the presence of numerous sickle-cells in the congested spleen, although it was not possible to differentiate between ante- and post-mortem sickling from the pathological findings alone. However, the clinical course was identical to fatal sickling crises observed personally in pregnant women with sickle-cell haemoglobin C disease, in which situation this complication is well recognized (Edington, 1957), and it is felt that intravascular sickling played a major part in determining the fatal outcome of this case. Haemoglobin type AS. Foetal haemoglobin <2%.

Discussion

It has become increasingly evident that under certain circumstances persons with the sickle-cell trait can undergo sickling crises or develop organic infarcts. Known precipitating factors include exposure to high altitudes (Sullivan, 1950) and infections, particularly those involving the respiratory tract (McCormick, 1961).

Findlay *et al.* (1947) were unable to demonstrate *in vivo* sickling at altitudes below 15,000 ft. (4,570 m.) in three West Africans with the trait. Rotter *et al.* (1956) suggested that special features associated with the splenic circulation might explain the occurrence of local sickling among subjects exposed to the relatively minor degree of hypoxia occurring at 10,000–15,000 ft. (3,050–4,570 m.), and emphasized the importance of the amount of haemoglobin S present in the blood of persons with the sickle-cell trait. In each of their three cases of splenic infarction and in the fatal case of Ober *et al.* (1960) haemoglobin S formed over 40% of the total haemoglobin present. In both cases of splenic infarction in the present series in which this estimation was carried out the proportion of haemoglobin S was 40%. Neel *et al.* (1951) demonstrated the existence of two modes of haemoglobin S concentration in sicklers with the trait, with values around 34–36% and 40–42%, and it seems that members of the latter group are predominantly at risk.

The role of infection in the present series is noteworthy. Ham and Castle (1940) demonstrated measurable rises in the plasma proteins of persons with acute infections, with consequent increases in blood viscosity of up to 70%. Associated pyrexia causes increased oxygen uptake by tissues, and the potential combination of local stasis, acidosis, and anoxaemia may initiate sickling in susceptible organs like the spleen. Other factors predisposing to

anoxaemia are anaemia, present in Cases 1 and 3, and alcoholic intoxication. McCormick (1961) noted the association between sickling crises and alcohol and it may have been an important factor in the death in Case 6, although inhalation of vomit probably caused the extra hypoxia that is presumed to have precipitated a terminal sickling crisis.

One further feature common to all subjects developing splenic infarcts in the present series was their lack of mobility prior to the onset of symptoms. All had been sitting, either in an aeroplane or propped up in a hospital bed, for some time previously, and it may be that this tended to favour stasis of the splenic circulation, thus potentiating locally any anoxaemia consequent on hypoxia or infection. Once established, splenic infarcts appear to be highly susceptible to secondary infection, and prophylactic antibiotics are advocated to prevent abscess formation and its attendant complications (Conn, 1954).

In contrast to the sickle-cell trait clinical manifestations of the haemoglobin C trait are few. Two cases of idiopathic haematuria were reported by Myerson *et al.* (1959) and Smith and Krevans (1959), but otherwise the condition appears to be essentially benign.

In conclusion it seems that heterozygous carriers of haemoglobin S are better protected against the lethal effects of malaria than are haemoglobin C heterozygotes, but that possession of haemoglobin S in concentrations greater than 40% can give rise to undesirable side-effects. Conversely, the protective ability suggested for haemoglobin C is apparently unaccompanied by any comparable disadvantages, but, being of a lower order, it is less easily demonstrated convincingly in a small sample.

Summary

Previously quoted figures comparing malarial parasite densities in Accra children have been re-analysed. Results for carriers of haemoglobin C, although less conclusive than originally claimed, do suggest some degree of protection against *P. falciparum* infections. The superior efficiency of haemoglobin S in this respect is confirmed.

Case reports illustrating the occurrence of splenic infarcts and a fatal crisis in persons with the sickle-cell trait are presented. The roles of infection and circulatory stasis, and of the proportion of haemoglobin S present in the blood, are discussed in relation to the development of sickling *in vivo* in such cases.

I wish to thank my wife once again for her great assistance in matters technical and typing. Dr. Hermann Lehmann kindly estimated the percentage of haemoglobin S in several blood samples. I am grateful to Dr. J. A. Fraser Roberts, Dr. C. A. B. Smith, and Dr. J. P. Garlick for their helpful criticisms.

References

Allison, A. C. (1961). *Ann. N.Y. Acad. Sci.*, **91**, 710.
Conn, H. O. (1954). *New Engl. J. Med.*, **251**, 417.
Cooley, J. C., Peterson, W. L., Engel, C. E., and Jernigan, J. P. (1954). *J. Amer. med. Ass.*, **154**, 111.
Edington, G. M. (1957). *J. clin. Path.*, **10**, 182.
—— and Lehmann, H. (1955). *Brit. med. J.*, **1**, 1308.
Ende, N., Pizzolato, P., and Ziskind, J. (1955). *Ann. intern. Med.*, **42**, 1065.
Field, J. W. (1949). *Trans. roy. Soc. trop. Med. Hyg.*, **43**, 33.
Findlay, G. M., Boulter, E. A., and MacGibbon, C. B. (1947). *J. roy. Army med. Cps*, **89**, 138.
Ham, T. H., and Castle, W. B. (1940). *Trans. Ass. Amer. Phycns*, **55**, 127.
McCormick, W. F. (1961). *Amer. J. med. Sci.*, **241**, 329.
Myerson, R. M., Harrison, E., and Lohmuller, H. W. (1959). *Amer. J. Med.*, **26**, 543.
Neel, J. V., Wells, I. C., and Itano, H. A. (1951). *J. clin. Invest.*, **30**, 1120.
Ober, W. B., Bruno, M. S., Weinberg, S. B., Jones, F. M., jun., and Weiner, L. (1960). *New Engl. J. Med.*, **263**, 947.
Raper, A. B. (1955). *Brit. med. J.*, **1**, 1186.
Rotter, R., Luttgens, W. F., Peterson, W. L., Stock, A. E., and Motulsky, A. G. (1956). *Ann. intern. Med.*, **44**, 257.
Singer, K., Chernoff, A. I., and Singer, L. (1951). *Blood*, **6**, 413.
Smith, E. W., and Conley, C. L. (1955). *Bull. Johns Hopk. Hosp.*, **96**, 35.
—— and Krevans, J. R. (1959). Ibid., **104**, 17.
Sullivan, B. H., jun. (1950). *Ann. intern. Med.*, **32**, 338.
Thompson, G. R. (1962). *Brit. med. J.*, **1**, 682.

USE OF A PROTHROMBIN METER FOR QUICK'S ONE-STAGE TEST

BY

A. G. JACOBS,* M.B., B.S.
Department of Clinical Pathology, Queen Elizabeth Hospital,

J. A. FREER, A.I.M.L.T.
Birmingham

In spite of the current controversy over the effectiveness of anticoagulant therapy, there is at the moment no sign of a reduction in the number of prothrombin estimations requested to be carried out in routine laboratories. Thus any mechanization that lessens the amount of labour involved in performing these estimations by Quick's method is to be welcomed. The standard technique involving holding the tubes containing the reagents, manipulating them in the water-bath, and working the stop-watches is both tiring and monotonous. Further, in order to obtain consistent results, a degree of skill has to be acquired to judge when clotting has occurred.

Toohey and Cook (1960) described a photoelectrical system for measuring prothrombin times, using the Quick technique, and this machine was later marketed by the Evans Electroselenium Company as a "prothrombin meter" (Fig. 1). Five hundred comparative tests were carried out by Toohey and Cook, and they concluded that the machine produced more accurate and reproducible results than the manual method, but no statistical analysis was made of their results. Before introducing this machine into routine use in this laboratory it was decided to determine prothrombin times on a series of specimens with technicians employing the orthodox method as well as the mechanical method, and then submit the results to statistical analysis.

Material and Methods

Venous blood, collected without stasis, was mixed with 3.8% sodium citrate in the proportion of 9:1. After mixing and centrifuging, the plasma was tested within two hours by a technician who determined the prothrombin time according to Quick's one-stage method (see Dacie, 1956), using 0.1-ml. volumes of citrated plasma, human brain thromboplastin, and M/40 calcium chloride. The thromboplastin used gave times of 11–12 seconds with normal plasma. Each specimen was tested at least twice by the manual method and using the prothrombin meter.

The meter as originally described by Toohey and Cook (1960) determines the time for clot formation of the plasma by shining a beam of light through the specimen under test on to a photosensitive cell. A clock which is incorporated in the machine is started as the reagents are mixed, and a change in the turbidity of the specimen takes place just before clotting occurs, which reduces the amount of

*Present address: Department of Pathology, St. Mary's Hospital, London W.2.