HAEMOLYTIC ANAEMIA FOLLOWING MALARIAL THERAPY*

BY

A. L. HILTON AND G. OWEN†

Doncaster Royal Infirmary

In a man with syphilitic optic atrophy treated with malaria in conjunction with penicillin, a severe intravascular haemolysis unexpectedly complicated the course of fever therapy. The Coombs's test was positive and free oxyhaemoglobin, methaemalbumin, and a saline anti-D were present in the serum, although there was no past history of a blood transfusion. The patient's blood group was then examined and was found to be Group A Rhesus-negative while the donor was Group A Rhesus-positive.

Through the co-operation of Mr. P. G. Shute of the Malaria Reference Laboratory we were able to contact all the physicians who had also used the same donor's blood to inoculate their patients, and we learnt that among these eight further patients there had been cases of severe anaemia and two deaths. The Rhesus-negative subjects in the series had suffered a severe anaemia probably haemolytic in origin.

Butts (1950) studied patients who had recovered from blackwater fever in Cuba and Guatemala. Although some doubt was expressed whether all the cases were true examples of blackwater fever, 40 per cent. of the group were Rhesus-negative. This paper presents some evidence to support a possible association between Rhesus-negative patients and the occurrence of severe haemolysis during induced malaria.

Case Report

A robust man aged 43 was admitted to hospital on December 7, 1956. His wife had been treated for primary syphilis in 1945, but he denied knowledge of any early symptoms of the disease. There was 2½ years' history of

increasing loss of vision to 6/60 and a deterioration of colour vision in the right eye, with blindness in the left eye for 9 months. There was no history of malaria.

Examination.—There was bilateral optic atrophy, but no other abnormal physical signs. The electrocardiogram was normal. The Wassermann reaction was positive in the blood and cerebrospinal fluid; the latter also contained 20 cells per c.mm., protein 50 mg. per 100 ml., and Lange curve 3322100000.

Treatment.—5 ml. heparinized blood from the Malaria Reference Laboratory containing *Plasmodium vivax* was injected intramuscularly below the right scapula. 4 days later the patient's temperature was 100°F. and after 7 days the pyrexia became intermittent. An attempt was made to modify the frequency of the attacks of fever by giving thiobismol 0.2 g. intramuscularly (Shute, 1952). Crystalline and procaine penicillin, chloral hydrate, ferrous sulphate, and compound codeine tablets containing phenacetin were also given before the haemolytic episode. Scanty malaria ring forms were present and the haemoglobin was 100 per cent. on the 13th day. On the 23rd day, after seven rigors, the patient felt very ill and insisted that the treatment should be stopped. He was jaundiced, the spleen was palpable three fingers below the costal margin, and the urine was red-brown. The malarial therapy was stopped and the infection was terminated with paludrine 0.1 g. thrice daily.

Further Investigations.—White blood count 9,600 per c.mm., reticulocytes 32 per cent., packed cell volume 22 per cent., mean corpuscular haemoglobin concentration 33 per cent. The haemoglobin fell to 49 per cent. Blood films showed marked anisocytosis, macrocytosis, and erythrophagocytosis with an occasional normoblast and myelocyte. No malarial parasites were seen. Serum haemoglobin 129 mg. per 100 ml., Schumm's test for methaemalbumin positive, thymol turbidity 12 units, zinc sulphate 16 units, electrophoresis showed an increase in gamma globulin. Cryoglobulins were present but no acid haemolysin was detected. Cold, auto- and pan-agglutinins active in saline and albumin were present at 4°C. The Donath-Landsteiner reaction was negative,

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† Now at the City General Hospital, Sheffield.

Progress.—The patient progressed satisfactorily. A chest x ray was normal, and 3 weeks later the haemoglobin had risen to 70 per cent., reticulocytes 6 per cent., electrophoretic pattern normal, no cryoglobulins detected. There was a cold agglutinin titre of 1 in 5, active at 37°C., and Coombs's test was negative.

Virus studies of the blood of the patient and donor gave negative results.

The colour vision improved and the visual acuity was temporarily restored to 6/24 before returning to 6/60.

Further Investigations.—On April 25, 1963, the patient denied any deterioration in vision. The glucose-6-phosphate dehydrogenase was 570 units/ml. blood, and no antinuclear factor or auto-immune antibodies were detected in the serum.

Follow-up of Other Recipients

The Table shows the diagnosis, blood group, haematological investigations, and final outcome, where known, of the nine patients who received the same donor's blood. Of the seven surviving patients, three of the Rhesus-negative type became severely anaemic and two of these three developed an anti-D antibody. It would have been of great interest to know the blood group of Case 4, in which the haemoglobin fell to 66 per cent. before death. *P. vivax* was identified in this patient.

Discussion

There have been various estimates of the degree of haemolysis which may occur during the course of

induced malaria. Driver, Gammel, and Karnosh (1926) inoculated 79 patients with *P. vivax*, mostly intravenously, and described an average destruction of about 25 per cent. of the patient's red blood cells. The R.B.C. were reduced to 1,250,000 per c.mm. and the haemoglobin to 30 per cent. in one subject after the fourth chill. The collapse was remedied only by intravenous quinine and a blood transfusion. Read, Kaplan, Becker, and Boyd (1946) noted nine cases of jaundice thought to be haemolytic in origin in 211 patients inoculated with *P. vivax* and in 89 infected with *P. malariae*. Anaemia was considered to be relatively benign and constant and so was not regularly recorded, but the relationship between the number of rigors and anaemia can be gathered from a small series analysed because of the development of oedema. A *P. vivax* infection was induced in thirteen patients in whom the haemoglobin averaged 65 per cent. after an average of fifteen rigors. It is unlikely that any patient in our series experienced this number of rigors and it would appear reasonable to suppose that unparasitized red blood cells were involved in the haemolytic process.

There are few published examples of severe haemolysis occurring during the course of induced malaria. Rudolf (1925) reports a case of haemoglobinuria which took place 71 days after the last dose of quinine and 88 days after the last paroxysm of an induced *P. vivax* infection. He also referred to a patient given *P. vivax* who developed haemoglobinuria immediately after the administration of

TABLE
SUMMARY OF THE OUTCOME OF MALARIAL THERAPY IN NINE PATIENTS

Case No.	Diagnosis	Blood Group	Number of Rigors or Periods of Fever	Haematological Findings	Final Results
1	Optic Atrophy	A Rh - Negative	Seven rigors, then actively terminated	Haemolytic anaemia (case described)	Satisfactory
2	G.P.I.	A Rh - Positive	Five rigors	Presumed no haemolysis	Satisfactory
3	G.P.I.	—	Six rigors, then actively terminated	Presumed no haemolysis	Satisfactory
4	G.P.I.	—	Three weeks, then termination attempted	Haemolysis. Hb fell to 66 per cent.	<i>Post mortem</i> "effects of malaria and bronchopneumonia".
5	G.P.I.	A Rh - Positive	Three weeks, then actively terminated	Presumed no haemolysis	Satisfactory
6	G.P.I.	O Rh - Negative	—	Anti-D developed Hb fell to 64 per cent. Serum Bilirubin 1.75 mg. per 100 ml.	Satisfactory
7	—	B No Rh grouping	Four rigors	—	<i>Post mortem</i> "myocardial failure due to malaria therapy"
8	—	O Rh - Positive	—	Presumed no haemolysis	Satisfactory
9	G.P.I.	A Rh - Negative	Three rigors, then actively terminated	Haemolytic anaemia Hb fell to 30 per cent.	Satisfactory

quinine. Kitchen and Sadler (1945) described haemoglobinuria after quinine had been given for a persistent, induced *P. falciparum* infection in a patient who had also been treated with *P. vivax* and *P. malariae*. Severe haemolysis associated with the production of methaemalbumin is, however, usually reported as a rare complication of naturally-acquired malaria. In fact, methaemalbumin was first described in a case of blackwater fever by Fairley and Bromfield (1934). It is now thought that oxyhaemoglobin liberated from the damaged cells combines with haptoglobin. The excess free oxyhaemoglobin is unstable and breaks down to release haem groups, which are oxidized and combine with albumin to form methaemalbumin (Allison and Rees, 1957). Consequently advantage was taken of the copious literature on the aetiology of blackwater fever to seek an explanation for the haemolytic episodes in therapeutic malaria and to see whether there were any points of similarity.

Blackwater fever commonly occurs during a recurrence of a *Plasmodium falciparum* infection and after the administration of an antimalarial drug, usually quinine. These prerequisites do not seem to be essential, as Findlay (1949) has described cases among military personnel in West Africa in which the haemoglobinuria took place before quinine was given and during a first attack of malaria. Foy and Kondi (1937) found *P. vivax* in 33 per cent. of their cases in Macedonia although they did not rule out the possibility of an earlier infection with *P. falciparum*.

Foy and Kondi (1936) carried out cross-infection experiments inoculating the inmates of a mental institution with blood from blackwater fever with negative results. They were therefore unable to incriminate either a specific organism or a haemolytic strain of parasite. It was also thought unlikely that our donor's blood contained a virulent organism apart from *P. vivax* and this was supported by the absence of a readily identifiable pattern in the development of the complication.

Our patient was given no antimalarial drug apart from thiobismol, but it must be noted he received phenacetin in the compound codeine tablets. In view of the recorded association between a glucose-6 phosphate dehydrogenase deficiency and a phenacetin induced anaemia (Kellermeyer, Tarlov, Brewer, Carson, and Alving, 1962), this enzyme was estimated and found to be at the lower limit of the normal range.

The influence of blood groups on the course of therapeutic malaria was described by Polayes and Derby (1934). The series consisted of 127 patients; seven developed haemoglobinuria, of whom four received compatible blood. Rhesus-grouping had

not been discovered at that time. The finding of severe anaemia, as we report, in three out of six Rhesus-typed patients would be of real significance if the results were reproduced in a larger series.

The pathogenesis of blackwater fever remains obscure. Butts (1945) drew attention to the increased number of Rhesus-negative persons in Caucasians compared to Negroes and Chinese and correlated this with the incidence of blackwater fever. He suggested that the malaria parasites contained a substance resembling the Rhesus factor which led to auto-immunization and lysis. The lysis of unparasitized red blood cells is due, according to Maegraith, Findlay, and Martin (1943), to a disturbance of the normal balance of the lytic systems of the blood. Gear (1946) had a unifying theory to explain the unsolved problem. He postulated that the parasites or antimalarial drug or both might render the red blood cells autoantigenic. The resultant autoantigen might stimulate the reticulo-endothelial system to produce a haemolysin. The contraction of the spleen by quinine, chill, exertion, or violent emotion could liberate more haemolysins.

Finally the question may be asked whether patients infected with syphilis for many years can develop auto-antibodies which may aggravate any haemolytic tendency during induced malaria. Our patient had a positive Coombs's test which supported the concept of an auto-immune haemolytic process, whilst complement-fixation tests for auto-immune antibodies against specific tissues including spinal cord and the antinuclear factor were all negative.

Summary

A series of nine patients who had received intramuscular injections of blood from the same donor for induction of malaria is reviewed. A severe intravascular haemolysis occurring during the malarial therapy is described. The Rhesus-negative subjects were found to develop an anaemia probably haemolytic in origin. The possible significance of this is briefly discussed.

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REFERENCES

- Allison, A. C., and Rees, W. ap (1957). *Brit. med. J.*, **2**, 1137.
- Butts, D. C. A. (1945). *Amer. J. trop. Med.*, **25**, 417.
- (1950). *Ibid.*, **30**, 663.
- Driver, J. R., Gammel, J. A., and Karnosh, L. J. (1926). *J. Amer. med. Ass.*, **87**, 1821.
- Fairley, N. H., and Bromfield, R. J. (1934). *Trans. roy. Soc. trop. Med. Hyg.*, **28**, 307.
- Findlay, G. M., (1949). *Ann. trop. Med. Parasit.*, **43**, 140.
- Foy, H., and Kondi, A. (1936). *Ibid.*, **30**, 423.
- (1937). *Trans. roy. Soc. trop. Med. Hyg.*, **31**, 123.
- Gear, J. (1946). *Trans. roy. Soc. trop. Med. Hyg.*, **39**, 301.
- Kellermeyer, R. W., Tarlov, A. R., Brewer, G. J., Carson, P. E., and Alving, A. S. (1962). *J. Amer. med. Ass.*, **180**, 388.
- Kitchen, S.F., and Sadler, G. G. (1945). *Amer. J. trop. Med.*, **25**, 379.
- Maegraith, B. G., Findlay, G. M., and Martin, N. H. (1943). *Lancet*, **1**, 573.
- Polayes, S. H., and Derby, I. M. (1934). *J. Amer. med. Ass.*, **102**, 1126.
- Read, H. S., Kaplan, L. I., Becker, F. T., and Boyd, M. F., (1946). *Ann. intern. Med.*, **24**, 444.
- Rudolf, G. de M. (1925). *Brit. med. J.*, **1**, 964.
- Shute, P. G. (1952). *Lancet*, **2**, 333.

Anémie hémolytique après paludothérapie

RÉSUMÉ

On décrit 9 malades qui, après avoir reçu une injection intramusculaire du sang d'un seul donneur pour provoquer une fièvre paludéenne, subirent une hémolyse grave intravasculaire.

Les sujets Rhésus négatifs développèrent une anémie qu'on croyait d'origine hémolytique.

On discute la signification de ce phénomène.