

# Mechanisms of Thrombocytopenia in Malignant Tertian Malaria

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## Summary

The mechanism of thrombocytopenia in six patients with falciparum malaria has been studied. All the patients recovered after antimalarial therapy, and cerebral malaria was not a feature. Radioactive-labelled platelets and fibrinogen were injected into the patients during the phase of thrombocytopenia. In all cases recovery of injected platelets was notably subnormal, indicating excessive splenic pooling of platelets. Platelet life span was moderately shortened in all patients, and platelet turnover increased approximately two-fold. Fibrinogen catabolism was moderately increased in all patients, but coagulation tests failed to reveal evidence of disseminated intravascular coagulation. The results suggest that in uncomplicated cases of malaria thrombocytopenia is the result of splenic pooling of platelets aggravated by a moderate decrease in platelet life span. In such cases thrombocytopenia is thus not the result of disseminated intravascular coagulation (D.I.C.), and heparin therapy is not indicated unless there is unequivocal ancillary evidence of D.I.C.

## Introduction

The cause of the thrombocytopenia frequently encountered in *Plasmodium falciparum* malaria infection in man is poorly understood. The possible mechanisms suggested include consumption of platelets as part of disseminated intravascular coagulation (D.I.C.), a well described feature in severe cases of *P. falciparum* malaria (Devakul *et al.*, 1966; Dennis *et al.*, 1967; Borochovit *et al.*, 1970), or excessive removal of normal or immunologically damaged platelets by a hypertrophied reticuloendothelial system (Hill *et al.*, 1964; Sheagren *et al.*, 1970; Shulman *et al.*, 1970; Beale *et al.*, 1972). Both mechanisms involve changes in platelet kinetics, as yet not reported in *P. falciparum* malaria.

In the present study platelet kinetic were measured in six patients with moderately severe *P. falciparum* malaria and thrombocytopenia. At the same time coagulation factors and plasma clearance of <sup>125</sup>I-labelled fibrinogen were measured. The results show excessive pooling of platelets associated with moderate shortening of platelet life span and increased platelet turnover. The rate of clearance from the plasma of injected <sup>125</sup>I fibrinogen was moderately increased in all patients but changes in coagulation factors were minimal. These findings provide evidence that thrombocytopenia in uncomplicated cases of *P. falciparum* malaria is the result of

enlargement of the spleen, which accompanies the infection, as well as of decreased platelet survival.

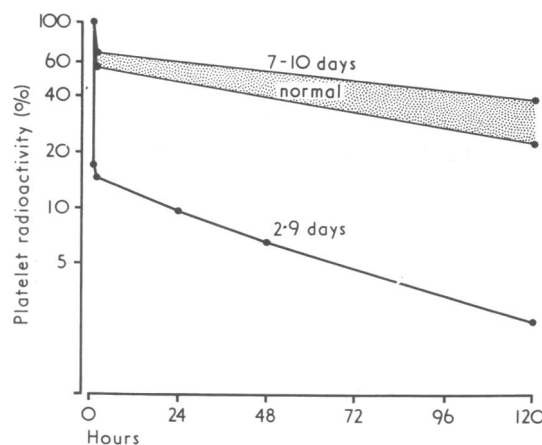
## Material and Methods

### SUBJECTS STUDIED

Six white patients aged 16-68 were investigated. They developed clinical symptoms of malaria shortly after returning from malaria-infested areas. Four of the patients had not taken prophylactic antimalarial tablets, while the remaining two had taken inadequate doses of prescribed tablets. The relevant clinical and haematological data are summarized in table I.

### METHODS

Thrombokinet studies with labelled platelets were carried out as described by Harker and Finch (1969). Allogeneic platelets from ABO and Rh compatible donors were labelled with <sup>51</sup>Cr and a measured volume was infused into the six patients. An aliquot of the infusion was retained for determination of the total platelet radioactivity injected. Blood samples were collected at one minute after the infusion of the platelets and a further four samples during the following hour, the fifth sample being drawn at exactly one hour after infusion. Subsequent samples were drawn at 24, 48, and 72 hours. Radioactivity of the platelet "buttons" prepared from the blood samples and from the suspension infused were determined in a well-type scintillation counter. Radioactivity from <sup>125</sup>I fibrinogen (see below) adsorbed on to the platelets was excluded by counting on a narrow window coinciding with the <sup>51</sup>Cr peak. The platelet recovery was calculated from the platelet radioactivity extrapolated to zero time multiplied by the blood volume (estimated from height and weight) divided by the total platelet radioactivity injected. Platelet survival was estimated from disappearance curves by extrapolating to zero activity. In all six patients an exponential disappearance curve was obtained, and plate-



Case 1. Platelet radioactivity after injection of <sup>51</sup>Cr-labelled allogeneic platelets into patient with *P. falciparum* malaria.

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TABLE I—Clinical and Haematological Features in Six Patients on Admission to Hospital with *P. falciparum* Malaria

Case No.	Age	Sex	Prophylaxis	Main Symptoms	Duration of Symptoms (Days)	Clinical Findings	Haemoglobin (g/100)	Leucocytes (per $\mu$ l)	Platelets (per $\mu$ l)	Parasite Counts (per $\mu$ l)
1	25	M.	None	Night sweats and rigors	5	Spleen palpable 1 cm below costal margin	13.9	3,900	55,000	Scanty
2	68	M.	None	Weakness and fever	7	Nil of note	12.8	3,900	35,000	25,000
3	23	F.	Inadequate	Slight headache and fever	7	Nil of note	12.0	14,900	32,000	200,000
4	16	M.	None	Headache, fever, and sweating	9	Slight abdominal tenderness	13.0	5,300	90,000	Scanty
5	43	M.	None	Weakness, confusion, and sweating	11	Jaundice, dark stools, spleen 4 fingerbreadths	13.9	10,300	55,000	950,000
6	40	F.	Inadequate	Weakness and fever	48	Pallor	7.8	2,100	40,000	120,000

let life span was calculated from the half time divided by the natural logarithm of 2. Platelet turnover was calculated from the peripheral platelet count divided by the platelet life span in days, and corrected for recovery (Harker and Finch, 1969).

In all six patients 100  $\mu$ Ci  $^{125}$ I-labelled fibrinogen (Radiochemical Centre, Amersham) was injected intravenously one hour after the platelet infusion and samples of venous blood drawn at 20 minutes and four hours, and then daily for three to five days. All patients were given either potassium iodide or Lugol's iodine to block the  $^{125}$ I uptake by the thyroid gland.  $^{125}$ I radioactivity was measured in 10-ml samples of plasma. Contaminating  $^{51}$ Cr radioactivity released into the plasma from the labelled platelets was excluded by selection of appropriate counting conditions. The plasma radioactivity was plotted against time on semi-log paper, and the half life calculated according to Regoeczi (1971). During the course of the malaria study aliquots of the same batches of  $^{125}$ I fibrinogen were injected into four other patients for the investigation of deep vein thrombosis. Two patients were receiving heparin treatment. O'Brien *et al.* (1972) reported that the rate of clearance of injected  $^{125}$ I fibrinogen from the plasma of subjects with venous thrombosis is not significantly different from that of normal subjects.

Blood for coagulation studies was collected into 3.8% trisodium citrate in the proportion of 9:1. The samples were taken shortly before infusion of the  $^{125}$ I-labelled fibrinogen. For the detection of fibrinogen-fibrin reacting antigen (F.R. antigen, previously termed F.D.P.) clotted blood was collected in tubes containing 1  $\mu$ g Trasylol to prevent in-vitro fibrinolysis. Standard techniques for coagulation studies were used (Denson, 1966; Ellis and Stransky, 1961) and F.R. antigen was detected using Wellcome haemagglutination inhibition test kits.

## Results

### PLATELET KINETICS

In all six patients the pattern of platelet radioactivity after the infusion of  $^{51}$ Cr-labelled platelets was essentially similar (graph). After the initial rise there was a rapid fall in platelet radioactivity maximal at one hour. Platelet recovery in

the six patients was greatly reduced, ranging from 7 to 19% (mean 12.2%) (table II). In five healthy subjects studied with the identical technique platelet recovery ranged from 55 to 69% (mean 61.6%).

TABLE II—Results of Platelet Kinetic Studies with  $^{51}$ Cr-labelled Allogeneic Platelets in Six Patients with *P. falciparum* Malaria

Case No.	Platelet (count/ $\mu$ l)	Platelet Recovery (%)	Platelet Life Span (Days)	Platelet Turnover (per $\mu$ l/day)
1	55,000	14	2.9	122,000
2	35,000	9	2.9	121,000
3	32,000	15	2.0	96,000
4	90,000	7	3.3	345,000
5	55,000	19	2.6	102,000
6	40,000	9	3.7	97,000
Normal values	140-400,000	>55	7-10	25-50,000

After the rapid fall in the first hour after infusion platelet radioactivity decreased at a much slower rate during the subsequent 72 hours. Platelet life span calculated from this part of the curve was shortened in all patients, ranging from 2.0 to 3.7 days (mean 2.9) (table II), compared with 7-10 days in normal subjects studied with the identical technique (Lurie *et al.*, 1969). Platelet turnover was increased in all patients, ranging from 96,000 to 345,000/ $\mu$ l/day (mean 147,000/ $\mu$ l/day) (table II). In normal subjects platelet turnover averaged 37,000/ $\mu$ l/day (range 25,000-50,000).

### $^{125}$ I-LABELLED FIBRINOGEN HALF LIFE AND COAGULATION TESTS

In the six malaria patients  $^{125}$ I fibrinogen half life ranged from 22 to 48 hours (mean 35.5 hours) (table III). In the four control subjects the half life was 64, 67, 84, and 96 hours respectively (mean 77.8). Other than a slight increase in the concentration of F.R. antigen, the results of the coagulation tests in cases 1, 2, 3, 5, and 6 were within normal limits. In case 4 the plasma fibrinogen concentration (169 mg/100 ml) was slightly reduced and the factor VIII level (61%) was towards the lower limit of normal. The  $^{125}$ I fibrinogen half life (33 hours) in this patient was not significantly different from the mean (35.5 hours) for the group of six patients.

TABLE III—Results of Coagulation Tests and  $^{125}$ I Fibrinogen Half-life in Six Patients with *P. falciparum* Malaria

Case No.	Prothrombin Time (Sec)	Kaolin-cephalin Clotting Time (Sec)	Fibrinogen (mg/100 ml)	F.R. Antigen ( $\mu$ g/ml)	Factor V (%)	Factor VIII (%)	$^{125}$ I Fibrinogen (Hours)
1	11.3	50.9	334	20	110	110	30
2	11.0	34.0	400	20	100	116	22
3	13.8	59.9	226	0	150	126	32
4	11.5	57.8	169	5	88	61	33
5	9.1	45.5	256	20	130	156	48
6	11.1	44.3	242	4	125	100	48
Normal values	10.3-12.9	40-60	200-400	2-10	50-150	50-150	64-96

## Discussion

The introduction of techniques to study platelet kinetics has enabled the mechanisms of thrombocytopenia in disease states to be better defined (Aas and Gardner, 1958). After injection of labelled platelets into normal subjects the percentage of the platelets recovered varies inversely with the size of the splenic pool and is a reliable indication of its size.

In the present study platelet recovery values after the injection of labelled platelets were greatly reduced, and in three subjects were less than 10%. These recoveries are comparable to those reported in subjects with congestive splenomegaly (Aster, 1966; Harker and Finch, 1969), and platelet pooling in the spleen appears to be a major factor in the aetiology of the thrombocytopenia in malaria. Splenomegaly with congestion of the red cords is a characteristic feature of malaria and the increased pooling may simply be a reflection of this. However, in thrombocytopenic states due solely to excessive splenic pooling platelet life span is usually normal or near normal (Aster, 1966; Harker and Finch, 1969). This was not the case in the present study where platelet life span was shortened. Decrease in platelet life span could result from the removal of normal platelets by an abnormally avid reticuloendothelial system, or the removal of damaged platelets by a normal reticuloendothelial system, or a combination of both. Hyperplasia of reticuloendothelial elements with evidence of increased phagocytic function is characteristic of the histology of the spleen and liver in malaria. Sheagren *et al.* (1970) assessed reticuloendothelial system phagocytic function in malaria by measuring the rate of clearance of <sup>125</sup>I-labelled human serum albumin. Phagocytic activity was markedly enhanced during the acute illness, returning to normal after treatment and complete recovery. The authors thought that this increased reticuloendothelial system activity may result in phagocytosis of normal platelets thus contributing to the thrombocytopenia in the disease.

The decreased life span of the platelets observed in the present study could be antibody mediated. There is a rise in IgA, IgG, and IgM immunoglobulins primarily during the early period after infection (Tobie *et al.*, 1966; Collins *et al.*, 1971). Beale *et al.* (1972) suggested that in malaria immunologically altered platelets are removed at an excessive rate from the blood by the reticuloendothelial system. The results of the present study suggest that this role is a relatively minor one, as the platelet kinetics in the malaria patient do not resemble closely those reported in acute immune thrombocytopenia where platelet recovery is usually normal, platelet life span is reduced to a few hours or less, and platelet turnover is increased from 4 to 9 times (Aster and Jandl, 1964; Harker and Finch, 1969). By contrast, the malaria patients showed markedly reduced platelet recoveries and only moderate shortening of life span.

No evidence of decreased platelet production in malaria has been found in the present study. Platelet turnover was increased some threefold in five patients and tenfold in one. This moderate increase of platelet turnover in five patients is compatible with splenic pooling (Harker and Finch, 1969). The results of these studies with <sup>51</sup>Cr-labelled platelets thus show at least two factors in the mechanism of the thrombocytopenia in malaria, excessive splenic pooling of platelets and decreased platelet life span. The former mechanism is the more important, for the recovery values were extremely low while the decrease in platelet life span was moderate and not as severe as is usually found in patients with acute immune thrombocytopenia.

Thrombocytopenia in severe malaria may be the result of D.I.C. (Dennis *et al.*, 1967; Devakul *et al.*, 1966; Borochovitz *et al.*, 1970), but D.I.C. is not a feature of uncomplicated malaria (Shulman *et al.*, 1970; Devakul *et al.*, 1966; Jaroovesama, 1972; Reid and Nkruma, 1972). In the present study coagulation tests failed to produce evidence of

D.I.C. in five of the patients studied. In the sixth patient plasma fibrinogen was moderately decreased, but levels of F.R. antigen were only slightly raised. All patients in the present study showed abnormally rapid removal of injected <sup>125</sup>I fibrinogen from the plasma. While the clearance rates were not as rapid as is usually found in D.I.C. they were nonetheless more rapid than those of the control subjects. This was not associated with changes in coagulation tests in five patients. In the one patient with moderate reduction in plasma fibrinogen the rate of catabolism of <sup>125</sup>I fibrinogen was not more rapid than the group as a whole. Thus no supportive evidence has been found that the rapid removal of <sup>125</sup>I fibrinogen was associated with D.I.C. Of particular importance is the finding of little or no rise in F.R. antigen levels. Fibrinogen is catabolized directly, without preliminary conversion to fibrin, so that fibrinogen catabolism can be changed without involving coagulation (Regoeczi, 1966). The site of fibrinogen catabolism is probably the cells of reticuloendothelial system, for experimentally induced hyperendotheliosis of the reticuloendothelial system is accompanied by an increased rate of fibrinogen catabolism in rabbits (Regoeczi, 1970). It is possible that in uncomplicated malaria rapid disappearance from the plasma of injected <sup>125</sup>I fibrinogen reflects clearance into the overactive reticuloendothelial system; a mechanism similar to that found with <sup>125</sup>I-labelled microaggregated human serum albumin (Sheagren *et al.*, 1970). However, when the clearance of injected <sup>125</sup>I fibrinogen from the plasma is very rapid in malaria patients this may be on the basis of D.I.C.

Evidence for D.I.C. in some patients with malaria is irrefutable. Results of the present study, however, indicate that this is not the only mechanism which may produce thrombocytopenia in malaria. The clinical implications of these findings relate particularly to the use of heparin in malaria. In the presence of unequivocal D.I.C. the use of heparin is justified in malaria. The results of the present study, however, indicate that if heparin is to be used evidence of D.I.C. other than thrombocytopenia and increased fibrinogen catabolism must be found, particularly in uncomplicated cases.

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## Simple Method for Detection of Infection of Peritoneum during Dialysis

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### Summary

The lysozyme (muramidase) content of peritoneal fluid samples has been found to be an early indicator of the onset of infection in the course of peritoneal dialysis. A level of 10.0  $\mu\text{g/ml}$  indicates peritoneal infection and one of 7.5  $\mu\text{g/ml}$  is highly suspicious.

### Introduction

Early detection of peritoneal infection in the course of dialysis is clearly important. It is well recognized, however, that a positive culture from peritoneal fluid effluent does not invariably indicate significant infection of the peritoneum because contamination of the outflow fluid is common (Frank *et al.*, 1948; Odel *et al.*, 1948). Such positive cultures do in any case take one or two days before reports are obtained. The presence of polymorphs is taken by some as an index of infection but there is also no doubt that chemical irritation of the peritoneum with glucose enhances the diapedesis of leucocytes (Boen, 1961). Others rely on naked eye estimation of the turbidity of the fluid due to precipitation of increased protein in cold fluid but in the course of a dialysis protein loss into the peritoneum increases, and the amount of protein lost is greater in patients with glomerulonephritis than pyelonephritis (Bonomini *et al.*, 1967).

This report concerns the simple expedient of estimating the lysozyme (muramidase) content of coincident serum and peritoneal fluid samples in order to gauge the onset of peritoneal infection.

### Methods

A 5.0-ml sample of clotted blood was taken morning and evening during the course of three-day peritoneal dialysis (P.D.) on 50 patients, and also peritoneal effluent fluid every sixth cycle. Samples were kept at 4°C before estimation of the lysozyme content by the technique of Harrison *et al.* (1968) using *Micrococcus lysodeikticus* (Sigma Ltd.) as substrate and lysozyme (Sigma, grade 1) at standard dilutions of 10, 5, 4, 3, 2, 1, and 0  $\mu\text{g/ml}$ . Serum and P.D. fluid lysozyme levels were read from the standard curve. Blank readings were made to correct for turbidity of both serum and P.D. fluid. Peritoneal dialysis itself was performed by standard technique using two-litre cycles of acetate fluid containing 200 units heparin added to the first cycle only. Record was made of the turbidity of peritoneal fluid

samples, of any admixture with blood, of the subsequent bacterial culture results, and of the clinical course of the patient and whether antibiotics were added to the dialysis fluid. A simple clinical score was made by which one point was scored for clinical evidence of peritoneal infection, one for a positive bacterial culture, and another for turbid dialysate fluid.

Finally, the inhibitory capacity of extra heparin on the lysozyme assay was estimated by adding known amounts of heparin to peritoneal fluid of known lysozyme content.

### Results

Serum lysozyme levels varied widely between patients with a mean level of 7.6  $\mu\text{g/ml}$  (S.D. 5.3, range 1.0-14.7). However, the mean lysozyme content of the peritoneal fluid samples was 4.2  $\mu\text{g/ml}$  (S.D. 1.7). This should be compared with the mean peritoneal level of 2.8  $\mu\text{g/ml}$  (S.D. 1.4) for six patients who were studied during their first peritoneal dialysis and with a mean level of 8.8  $\mu\text{g/ml}$  (S.D. 3.3) for patients with infection of the peritoneum. Patients developing infection during the course of dialysis invariably showed a rise of peritoneal fluid lysozyme to more than 7.5  $\mu\text{g/ml}$  and usually to above 10.0. Generally the mean P.D. fluid/serum lysozyme ratio was  $0.47 \pm 0.2$ , hence an occasional patient will be seen in whom a high serum level might determine a high fluid level. The ratio rose during the course of a three-day dialysis by an average of 40%, reflecting the fact that there was some rise of P.D. fluid lysozyme over the course of three days. Often during infection the P.D. fluid lysozyme content exceeded the serum level.

Listed in the table are the results of the 16 patients out of 50 who had a positive clinical score. A score of unity was usually made because of turbid peritoneal fluid but, in retrospect, only in one case was the P.D. fluid lysozyme high enough to indicate that there was infection. One case was given a unity score because of the subsequent finding of bacteria in peritoneal fluid culture, but this was clearly a false positive finding. Patients with a score of two or three units certainly had infection and, as already noted, such patients usually had lysozyme levels of 10  $\mu\text{g/ml}$ . Failure to obtain a positive culture was most certainly due in some cases to the accepted unit policy of treatment with intraperitoneal antibiotics at the first suspicion of infection.

To illustrate the uses of the assay four examples are shown in the figures. A rapid rise of P.D. fluid lysozyme indicative of peritoneal infection and an equally rapid resolution with antibiotic treatment is shown in fig. 1. A patient's graph whose P.D. fluid lysozyme was 9-10  $\mu\text{g/ml}$  but in whom there was only slow response to therapy is shown in fig. 2. Fig. 3 shows the effect on lysozyme levels of infected peritoneal fluid, accompanied by a good response to therapy. The course of a patient with haemorrhagic pancreatitis who showed high but fluctuant levels of P.D. fluid lysozyme together with high serum levels is illustrated in fig. 4. Clearly, if peritoneal dialysis is used for treatment of pancreatitis a monitor of lysozyme levels can indicate progress.