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Louis H. Miller, Shunichi Usami, Shu Chien

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Research Article

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Alteration in the Rheologic Properties of *Plasmodium knowlesi*–Infected Red Cells. A Possible Mechanism for Capillary Obstruction

LOUIS H. MILLER, SHUNICHI USAMI, and SHU CHIEN

From the Division of Tropical Medicine and the Laboratory of Hemorheology, Department of Physiology, College of Physicians and Surgeons, Columbia University, New York 10032

ABSTRACT Red cells parasitized by *Plasmodium knowlesi* concentrate within the microcirculation of many organs including cerebral capillaries in rhesus monkeys. The possibility that *P. knowlesi* could alter the rheologic properties of red cells so that they are trapped within capillaries was investigated in the present study. The viscosity of *P. knowlesi*–infected red cells suspended in Ringer's solution was increased at all shear rates at hematocrits above 30%. At moderate parasitemia the resistance to flow through 5 μ polycarbonate sieves was increased; at high parasitemia the pores were obstructed. Mature trophozoites caused more obstruction than young trophozoites (rings) at any given level of parasitemia. The reduction of deformability of red cells infected by schizonts of *P. knowlesi* was further demonstrated by their exclusion from rouleaux in a plasma suspension. Therefore, the red cells infected by *P. knowlesi* become less deformable, and this reduction in red cell deformability may explain the obstruction of cerebral capillaries.

INTRODUCTION

Cerebral malaria, renal failure, and centrilobular necrosis of the liver often occur concurrently in patients who are heavily infected by *Plasmodium falciparum*. Similar renal (1) and hepatic (2) lesions are observed in other diseases which produce hemolytic anemia, hypovolemic shock, and splanchnic vasoconstriction. The cerebral pathology, however, is unique to malaria. Many of the cerebral capillaries are filled by parasitized red cells; ring hemorrhages develop around obstructed vessels. It is possible that *P. falciparum* malaria may alter the red cell so that it is unable to pass through the capillary bed in the brain. To test this hypothesis we have studied the rheologic properties of red cells parasitized by *Plasmodium knowlesi*, a primate malaria which produces obstruction of cerebral capillaries and venules (3)[1] and has been shown to cause microcirculatory disturbance by Knisely, Stratman-Thomas, Eliot, and Black (4).

METHODS

Macaca mulatta (rhesus monkeys) that were free of tuberculosis, malaria, and diarrhea were infected intravenously by *P. knowlesi*.[2] The asexual parasite had a synchronized 24 hr cycle (ring to schizont) which began at approximately 6 a.m. At parasitemias above 10%, schizonts were present in the peripheral blood until 11 a.m. At different times in the asexual cycle and at various parasitemias, the following studies were performed.

Parasitemia

The number and stage of parasites per 1000 red cells were counted on Giemsa-stained thin blood films.

Rouleaux formation

Aggregation of suspensions (hematocrit = 1–2%) of normal red cells and red cells infected by schizonts of *P. knowlesi* was observed microscopically in plasma and in Ringer's solution. (The Ringer solution used in this study contained 0.25% albumin and 12 mM Tris, and was adjusted to pH 7.4.) One drop of the red cell suspension was placed on a slide and was covered by an 1 mm cover glass which was elevated at one end by another 1 mm cover glass.

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[1] Miller, L. H., H. N. Fremount, and S. A. Luse. 1971. *Amer. J. Trop. Med. Hyg.* In press.

[2] *P. knowlesi* was kindly supplied by Major Robert Hickman, Walter Reed Army Institute of Research, Washington, D. C.

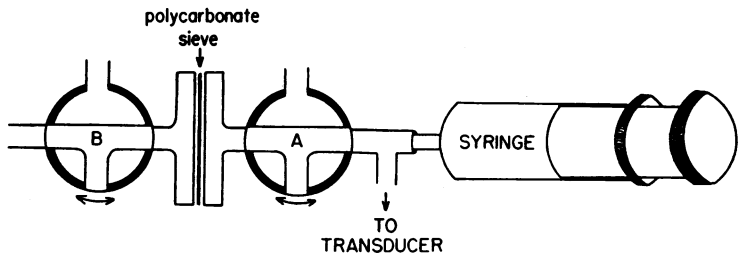

FIGURE 1 Apparatus for measurement of red cell filtration. (A) and (B) are three-way stopcocks on each side of a filter holder which contains a polycarbonate sieve.

Viscometry

The viscosity of *P. knowlesi*-infected red cells in 5 rhesus monkeys was compared with the control data obtained from 10 uninfected rhesus monkeys. The red cells were washed twice in Ringer's solution, and the buffy coat was removed. Viscosity was measured in a coaxial cylinder viscometer at 37°C and at several shear rates (52, 5.2, 0.52, 0.104, and 0.052 sec^{-1}) (5). Apparent viscosity (referred to as viscosity in the present paper) is the ratio of shear stress to shear rate. Hematocrit was corrected for fluid trapping by a factor of 0.93 determined with the use of albumin-^{131}I.

Red cell filtration

The constant-pressure method of red cell filtration through polycarbonate sieves (6) was modified to study filtration pressure under constant flow rate.

Apparatus for measurement of red cell filtration. Polycarbonate sieves (Nucleopore: General Electric Company Schenectady, N. Y.), which had a mean pore diameter of 5 μ and a pore density of approximately 3×10^4 pores/cm^2, were cut from one roll to give circular filters 13 mm in diameter. A sieve was placed in a Millipore filter holder which was connected to a 50 ml glass syringe and a pressure transducer by a T-tube as shown in Fig. 1. The syringe was driven by a Harvard infusion pump (model 600–900), and the flow rate was calibrated for each syringe used. The pressure-time curve was recorded on a Sanborn 150 recorder.

Preparation of the red cell suspension. The Ringer solution was filtered through a 4 μ polycarbonate sieve before use in preparation of red cell suspensions. Heparinized blood was washed one time with Ringer's solution. The buffy coat was removed, and the red cells were resuspended in Ringer's solution to a concentration of 5×10^4 red cell/mm^3.

Measurement of pressure-time curve during filtration. The polycarbonate sieve was washed in distilled water, fitted into the filter holder, and flushed with 100 ml of Ringer's solution. The Ringer solution was pumped through the sieve at approximately 2 ml/min, and a pressure-time curve recorded. After recording the pressure-time curve for Ringer's solution, the filter holder was detached from stopcock A with the stopcock B closed (one-fourth turn clockwise from position shown in Fig. 1) so that the Ringer solution could not flow out of the filter holder. The filter holder was opened, and Ringer's solution was removed from above the sieve by blotting with tissue and was replaced by the red cell suspension. The 50 ml glass syringe containing the red cell suspension was attached to the T-tube, and the T-tube and stopcock A were filled by the suspension. The filter-holder was attached to the stopcock A which was set (one-fourth turn clockwise from position shown in Fig. 1) to avoid a rise in pressure during this procedure. Stopcocks A and B were then returned to the positions shown in Fig. 1 to allow the free flow of the red cell suspension through the filter. The syringe was pumped at a rate of approximately 2 ml/min, and a pressure-time curve was recorded. The filtration of red cells was performed within 1 hr of drawing the blood and at a temperature between 22° and 24°C. The pH of the red cell suspension at the end of the experiment was between 7.3 and 7.4.

Calculations. A straight line was fitted by eye to the slope of the pressure-time curve for the red cell suspension (Fig. 2). The change in pressure per second was calculated from the slope, and was converted to change in pressure per milliliter of suspension filtered, since the red cell solution was pumped through the sieve at a constant rate.

The pressure-time curve was back extrapolated to obtain the zero-time pressure (P_0), and the zero-time resistance (R_0) was calculated as P_0 divided by the pump flow rate. The resistance for Ringer solution (R_R) was similarly

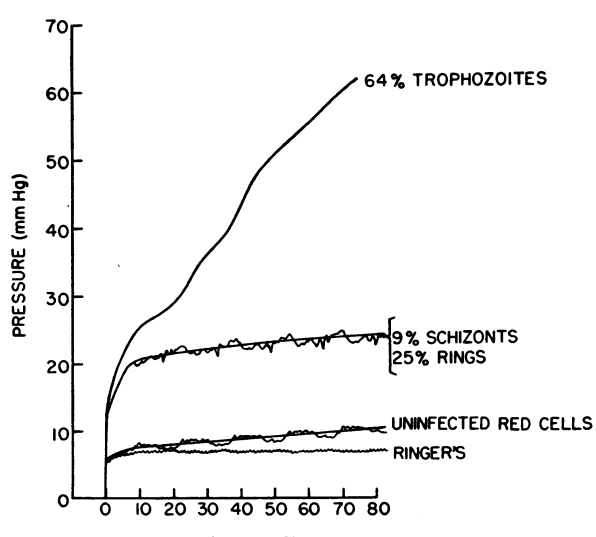

FIGURE 2 Pressure-time curves for Ringer's solution and suspensions of uninfected and *P. knowlesi*-infected red cells through 5 μ polycarbonate sieves.

calculated, and R_0/R_R gave the relative resistance for the red cell suspension.

RESULTS

Normal rhesus monkeys. The range for viscosity of red cell suspensions in Ringer's solution at different hematocrits and at shear rates 52 sec^{-1} and 0.052 sec^{-1} for 10 normal rhesus monkeys is shown in Fig. 3. In filtration studies on 13 uninfected monkeys, the rate of pressure rise was 0.93 ±0.45 mm Hg/ml (mean ±SD), and the relative resistance was 1.09 ±0.14.

Microscopic observations on schizonts in plasma and in Ringer's solution. In light microscopy, the red cells which contained *P. knowlesi* malaria pigment were seen to be spur shaped (Fig. 4). Since on Giemsa-stained smear all pigment-containing parasites were mature trophozoites or schizonts, these mature forms must be those present in the spur cells. When suspended in plasma the uninfected red cells formed typical rouleaux, but schizonts of *P. knowlesi* were excluded from the rouleaux and existed as monodispersed particles (Fig. 4). Both infected and uninfected red cells were monodispersed in the Ringer solution.

Effect of P. knowlesi on viscosity of red cells in Ringer's solution. At a hematocrit of 44% the viscosity of the suspension containing *P. knowlesi*–infected red cells was significantly greater than normal red cells at all shear rates (Fig. 5). The per cent increase in viscosity of samples with infected cells over normal red cells was similar at all shear rates. At both 52 and 0.052 sec^{-1}, the increase in viscosity due to the presence of infected red cells was detectable only when the hematocrit was above 30% (Fig. 3).

Effect of P. knowlesi on the flow of red cells through 5 μ polycarbonate sieves. The asexual maturation and the degree of parasitemia influenced the flow of red cells through the sieves (Figs. 2, 6A, and 6B). It should be noted that the samples referred to as rings (young trophozoites) and schizonts contained less than 10% schizonts with one exception (18% schizonts and 28% rings). Trophozoites (approximately 9–14 hr old) at parasitemia below 25% and rings and schizonts at parasitemia below 50% usually produced a normal rate of pressure rise but caused an increase in the relative resistance. At higher parasitemia the rate of pressure rise was increased. The more mature trophozoites increased the rate of pressure rise more than the younger trophozoites for any given degree of parasitemia.

DISCUSSION

The data in the present study indicate that *P. knowlesi* alters the normal rheologic properties of the red cell. At hematocrits above 30% the viscosity of infected red cells in Ringer's solution was increased at all shear rates.

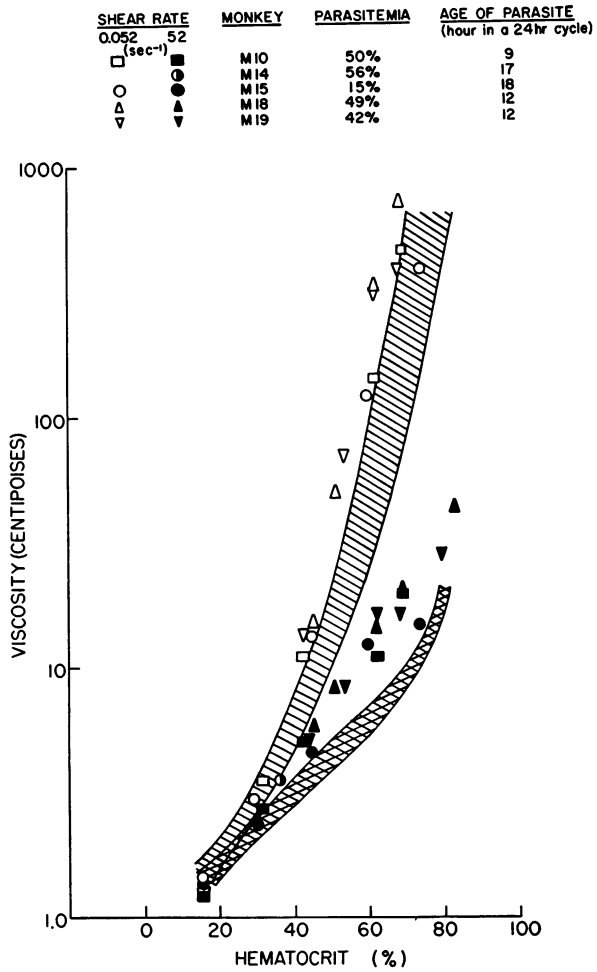

FIGURE 3 Relationship between viscosity and hematocrit at shear rates 0.052 (upper curves) and 52 (lower curve) sec^{-1} for rhesus red cells in Ringer's suspension. The shaded areas are the range for 10 normal monkeys.

The increased viscosity in a nonaggregating medium reflects a decrease in cell deformability.

The alteration in red cell deformability by *P. knowlesi* was also demonstrated by sieving of red cells through 5 μ pores. The plugging of pores, as indicated by an increased slope of the pressure-time curve, was most marked at high parasitemia. At any given parasitemia the stages of the parasite also affected red cell filtration (Fig. 6). An increased relative resistance associated with a normal rate of pressure rise was observed in red cells infected by trophozoites of moderate parasitemia (< 25%) and a mixture of rings and schizonts of high parasitemia (25–50%). This may reflect a difficulty of the infected red cell traversing the pores without actual obstruction. There are approximately 4×10^4 pores in the 13 mm diameter sieve. With a flow rate of 2 ml/min and a red cell suspension of 5×10^4 cells/mm^3, approxi-

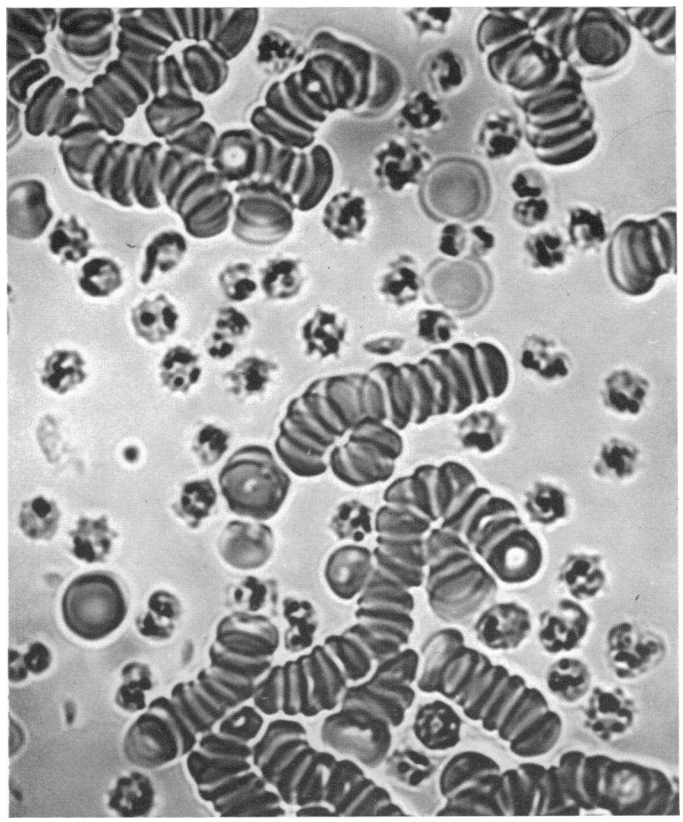

FIGURE 4 The spur-shaped red cells which contain pigmented schizonts of *P. knowlesi* are excluded from rouleaux and monodispersed in plasma. × 1100.

mately 40 red cells pass through each pore per second. At 10% parasitemia approximately four infected red cells pass through each pore per second. The lack of an increase in the rate of pressure rise indicates that at this rate of passage of the infected red cell, there was no demonstrable obstruction. At 50% parasitemia approximately 20 infected red cells would have to pass through each pore per second, and the interaction of infected red cells may then result in obstruction of pores.

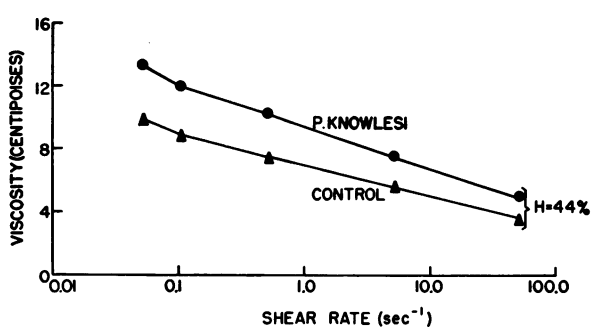

FIGURE 5 The viscosity of *P. knowlesi*-infected red cells in four monkeys was increased over the control values for four uninfected monkeys at hematocrit = 44%. ($P < 0.05$ at all shear rates.)

The inability of *P. knowlesi*-infected red cells to engage in rouleaux is additional evidence for abnormality in deformation, since red cells must deform to make available closely parallel surfaces for bridging by fibrinogen and globulins (7).

A decrease in cell deformability may result from a less flexible membrane and/or an increase in internal viscosity. The normal red cell has a high ratio of surface to volume as reflected by its biconcave shape (8). The spur-shaped distortion of *P. knowlesi* infected red cells (9) (Fig. 4) may decrease the ratio of surface to volume and thus cause a loss of red cell flexibility. Similarly distorted red cells have been observed in ABO agglutination (10), microangiopathic hemolytic anemia (11), hereditary beta lipoprotein deficiency (12), pyruvate kinase deficiency (13), and alcoholic cirrhosis (14). Red cell distortion caused by sickle cell anemia (5, 15), hereditary spherocytosis (16), and spur cells (17) have abnormal rheologic properties as measured by viscometry and sieving similar to the observations in the present study. In addition to the effect of malaria on the red

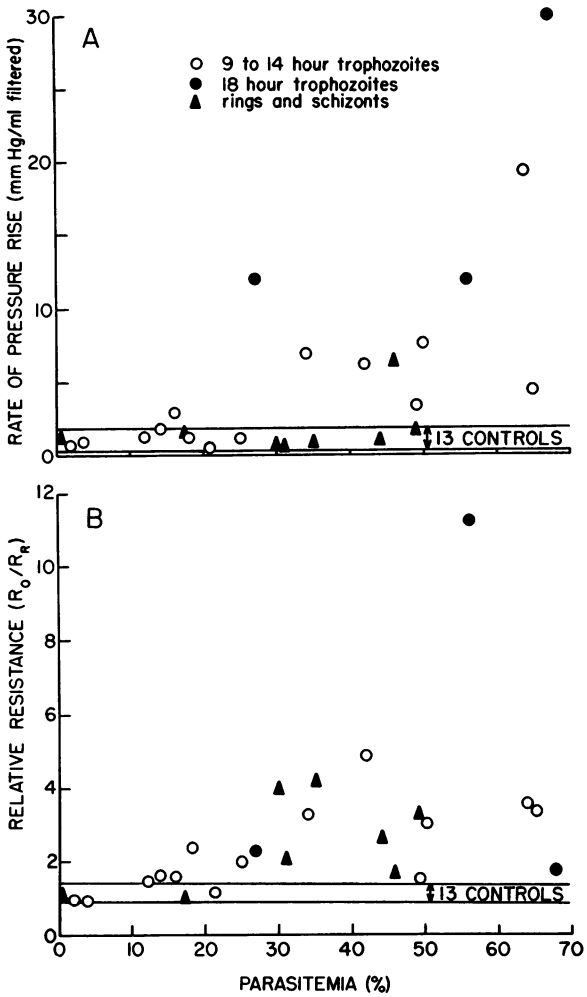

FIGURE 6 The rate of pressure rise (A) and relative resistance (B) during the filtration of *P. knowlesi*-infected red cells through 5 μ polycarbonate sieves at various degrees of parasitemia and different stages of asexual development.

cell membrane, the parasite itself within the red cell may increase the internal viscosity of the cell. Since most other malarias do not distort the red cell membrane, it may be possible to separate the effect of the parasite from that of the altered membrane by similar studies in other malarias.

The present studies on *P. knowlesi* malaria suggest that a reduction of deformability of infected red cells is the mechanism for capillary obstruction. The possible role of reduced deformability of *P. falciparium*-infected red cells in the pathogenesis of cerebral malaria in man deserves investigation.

ACKNOWLEDGMENTS

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