

Hemolytic Activity in Human Malaria Infections

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OBJECTIVE: To investigate the hemolytic factors associated with human malarial infections with particular emphasis on host complement activity in Chloroquine resistant and non-resistant malaria and immunological phenomena producing host cell lysis.

DESCRIPTION: Previous studies at SEATO Medical Research Laboratory (1968–69), using *P. Inui* and *P. coatneyi* in monkeys showed marked decrease in erythrocyte survival time in the course of chronic infections with scanty parasitemia, or even in the absence of parasites. Studies also showed that inappropriate erythrocyte destruction was mediated by some humoral factor associated with chronic infection. Equally striking is the marked decrease of complement activity in the infected monkeys using the spectrophotometric method for complement assay as described by Hook and Muschel (1964) and Fogel et al at WRAIR (1966).

A study of complement activity levels of acute malaria patients (1970–1971) found this activity to decrease during infection and rise rapidly to normal levels after successful chemotherapy. Preliminary studies of patients infected with both *P. falciparum* and *P. vivax* indicate that there is a pronounced depletion of C' activity in human malaria infections in Thailand.

PROGRESS: All patients studied in this report were selected from Ayuthaya Province, about 120 kilometers north of Bangkok. Four females and ten males aged between 18–41 years and infected with *P. falciparum* were followed from the day of treatment through the complete course and for as long as 144–150 days. There were another 18 patients who were hospitalized for a short period of 1 to 9 days, and could not be followed further. One female and 7 males of the 18–41 years age group were infected with *P. vivax* and were also followed for as long as 150 days. A cumulative total of forty normal Thais aged between 18–27 years were used as controls, with only two being from the Bangkok area.

All patients were treated with intramuscular Chloroquine, 150 mg. base, or intramuscular quinine, 300–600 mg, and then continued with chloroquine orally for a total of 1500 mg base. Primaquine was administered to *P. vivax* patients and the oral course of chloroquine were repeated upon relapse.

Blood specimens were collected in number and volume permitted by the patients. Specimens were obtained, each day during the first week, on the 7th, 10th or 14th day, and then every 14 days for as long as possible. Serum was separated from all blood specimens after clotting and immediately frozen. The micro-complement fixation technic was used. The extent of sheep erythrocytes sensitized with rabbit antibody to yield 80 to 90% lysis after 30 minutes at 37°C was estimated by absorption measurements at 412 m μ = OD₄₁₂ spectrophotometrically on supernatant fluids from the reaction mixtures. The results are expressed as logarithmic values at OD₄₁₂.

The distribution of C' activity levels in malarial patients during acute illness, after radical cure and recrudescence are shown in Fig. 1. Normal Thai values and those of patients with other diseases are shown for comparison. In one control group of 10 normal Thai randomly selected and followed during the same period as some of the patients, and 15 normal Thais randomly selected for determination of activity by one test range from 3.0–9.989 (75–92.2% hemolysis of sensitized sheep erythrocytes). The sera from patients with infections other than malaria did not produce low C' values.

In Figure 2 are shown the mean C' activity values for patients who were radically cured, some who were incompletely cured (i.e. the parasitemia was persistent) and of normal patients who were followed extensively. The OD₄₁₂ values of the C' activity on the day of admission or during the acute attack were between 0.0 and 0.433 (7%–30% hemolysis) with a mean value of 0.232 for P. falciparum and 0.267 in P. vivax infections. The ranges in parasitemia were from 0.2 to 1.4% in P. falciparum cases and from .11 to .35% in P. vivax infections. The C' levels rose rapidly in response to chloroquine chemotherapy, and returned to normal in 3–5 days in P. vivax patients and in about 7 days in P. falciparum cases. After that time the levels were consistently above 3.0, and equivalent to normal.

In those demonstrating chloroquine resistance, the levels did not return to normal for several weeks. When such activity did increase to beyond 3.0 it remained at the higher level. Whether the infection relapsed or remained patent at a very low parasitemia, in those patients for whom the chemotherapy was inadequate the C' activity level persisted below 3.0.

The studies performed to detect hemolytic factors associated with an immunological phenomenon yielded no abnormal results. None of the patients examined presented with chronic infections similar to those observed in previously studied experimental monkeys. No cases of blackwater fever were discovered during the reporting period.

SUMMARY: The measurement of C' activity levels during and following infections with P. falciparum and P. vivax has been performed on patients for as long as 150 days. During the infection the complement level was markedly decreased and returned to normal rapidly after successful chemotherapy. Throughout persistent infections and recrudescence the C' activity remained depressed, regardless of the species of malaria. In other infectious diseases the complement was not detectably reduced.

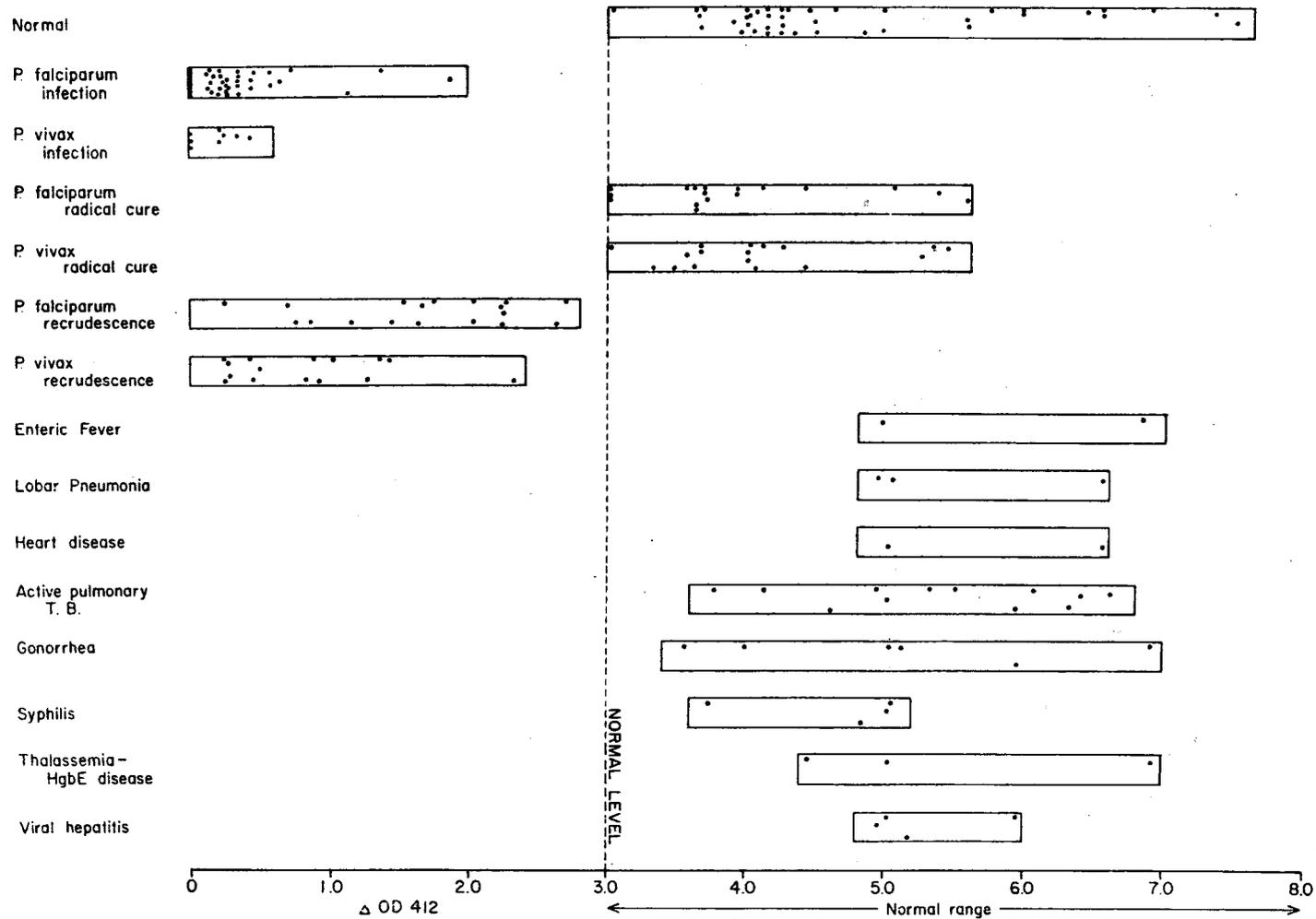


FIGURE 1. COMPARISON OF DISTRIBUTION OF C' ACTIVITY IN RANDOMLY SELECTED NORMAL THAI, MALARIAL PATIENTS AND PATIENTS WITH OTHER DISEASES.

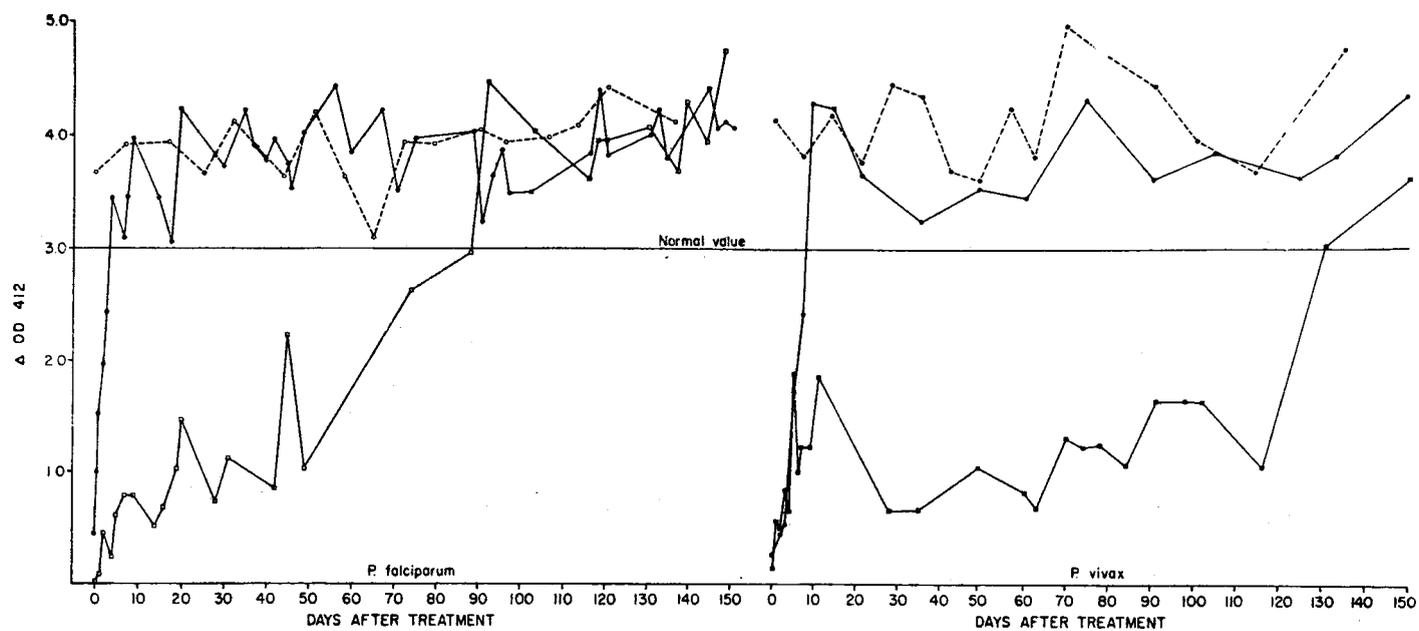


Figure 2. Mean C' Activity in Patients with malaria. _____ = Radical Cure (11 P.f. and 2 P.v.) and Incomplete cure (3 P.f. and 2 P.v.)
 ----- = Normal controls (6 and 6).