

In vitro Chloroquine and Quinine Sensitivity of a Thai
Strain of *Plasmodium falciparum*

Investigators : Chaiphorn Teerakiartkamjorn, M.D.
Edward B. Doberstyn, LTC, MC
Richard G. Andre, MAJ, MSC
Aumpon Na Nakorn

OBJECTIVE : To document chloroquine susceptibility of *P. falciparum* using the Rieckmann *in vitro* technique in Phrabuddhabat, the site of current therapeutic drug trials.

BACKGROUND : The problem of chloroquine-resistant *P. falciparum* was first described in Thailand in 1962. It is currently believed that sensitive strains have all but disappeared from Thailand. The determination of drug resistance in *P. falciparum* based on the clinical response in antimalarial agent has several limitations. Rieckmann, *et al.*, in 1968 described a relatively simple and rapid technique for the *in vitro* detection of chloroquine resistant falciparum malaria. In Thailand, Colwell in 1972 used this technique in describing chloroquine-resistant strains of *P. falciparum* in several parts of Thailand.

METHODS : Subjects for this study were selected from patients presenting to the hospital out-patient department or to the Malaria Eradication Project Clinic in Phrabuddhabat District, Saraburi Province, Central Thailand. Twelve to fifteen milliliters of blood was obtained from suitable patients by venipuncture and ejected into a sterile Erlenmeyer flask containing a number of glass beads. This specimen was swirled for 15 minutes in order to defibrinate the blood. One milliliter of the blood was pipetted into each of several screw capped flat bottom glass vials containing glucose and varying concentrations of chloroquine or quinine. The contents were mixed and incubated in a water bath for 24 hours. At the end of incubation thick blood films were made, coded and dried overnight. The films were stained with Giemsa for 30 minutes. Examination of the smears was accomplished without the reader's knowledge of the relative position on the slide of smears from control or drug-treated vials. The degree of maturation was assessed by counting the number of schizonts with two or more nuclei per 200 consecutive asexual parasites. Values obtained for the vials were divided by the values obtained for corresponding control vials, and the results expressed as percentage maturation.

RESULTS : Twenty-nine samples in each group were successfully cultured.

a. Quinine. Samples of blood containing two nanomoles of quinine showed little or no inhibition of the formation of schizonts but when the samples contained four nanomoles or more slight to complete inhibition of maturation was observed. Complete arrest of the formation of schizonts was observed in all samples containing ten nanomoles of quinine per milliliter of blood. Comparison of quinine sensitivity of the Phrabuddhabat strain with the reference sensitive Uganda I and resistant Malayan (Camp) strains of *P. falciparum* shows nearly identical susceptibility to quinine (Figure 1).

b. Chloroquine. Samples of blood containing 0.4 to 1.35 nanomoles of chloroquine showed little or no inhibition of maturation. When the samples contained 2.03 and 3.04 nanomoles marked inhibition of maturation was observed but the formation of schizonts was not completely inhibited. Only 44.8 and 72.4 percent of samples containing 2.03 and 3.04 nanomoles of chloroquine respectively were completely inhibited. In comparison with the sensitive Uganda I strain and the moderately resistant Malayan (Camp) strain, the Thai parasite was highly resistant (Figure 2).

This study is complete, however, it is planned that *in vitro* susceptibility of *P. falciparum* to amodiaquine and mefloquine will be evaluated in the future, and that the effect of chloroquine will be monitored continually in areas where drug trials are underway.

Figure 1. Effects of Quinine *in vitro* upon Uganda I, Malayan (Camp), and Thai strains of *P. falciparum*

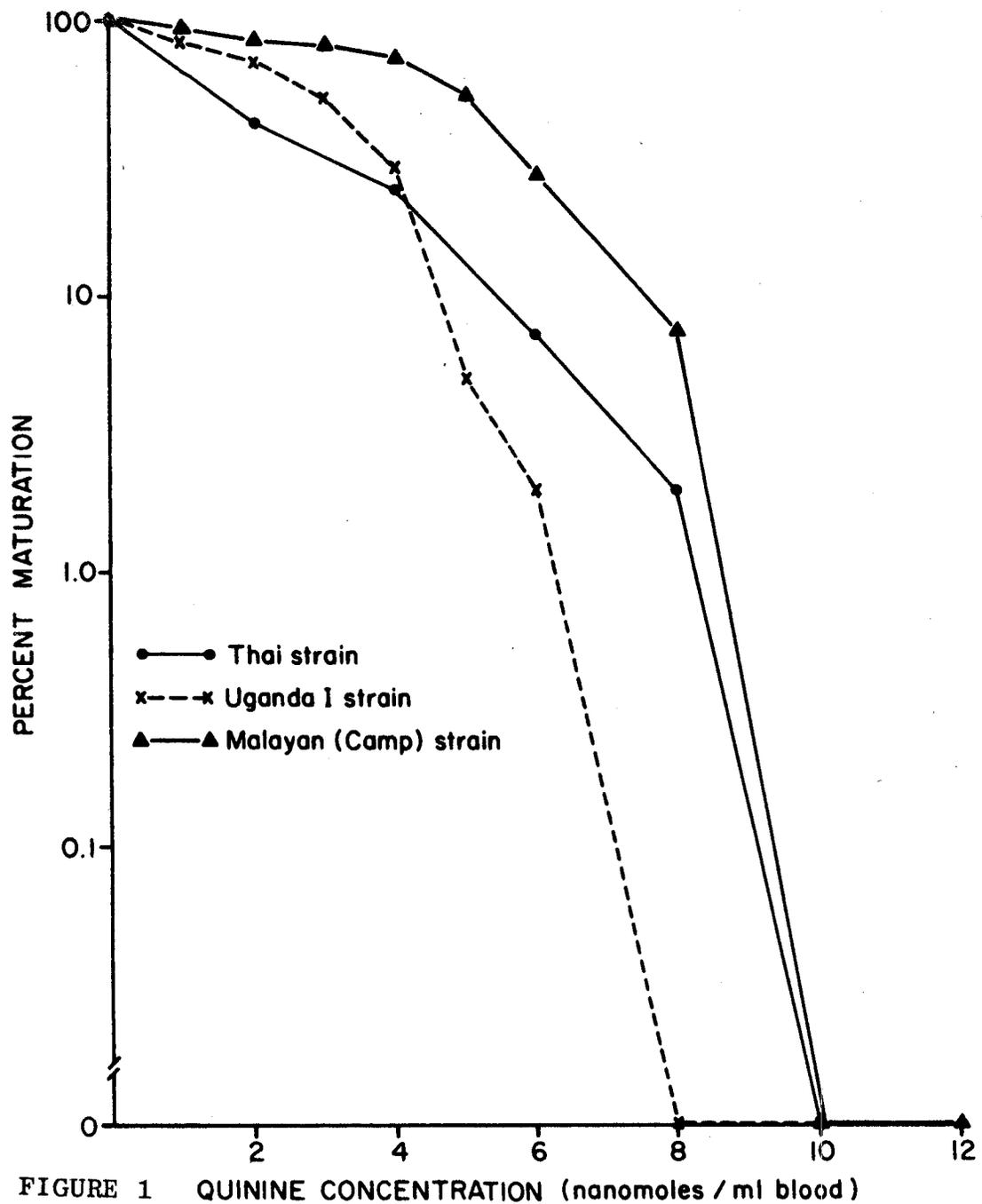


Figure 2 Effects of Chloroquine *in vitro* upon Uganda I, Malayan (Camp), and Thai strains of *P. falciparum*

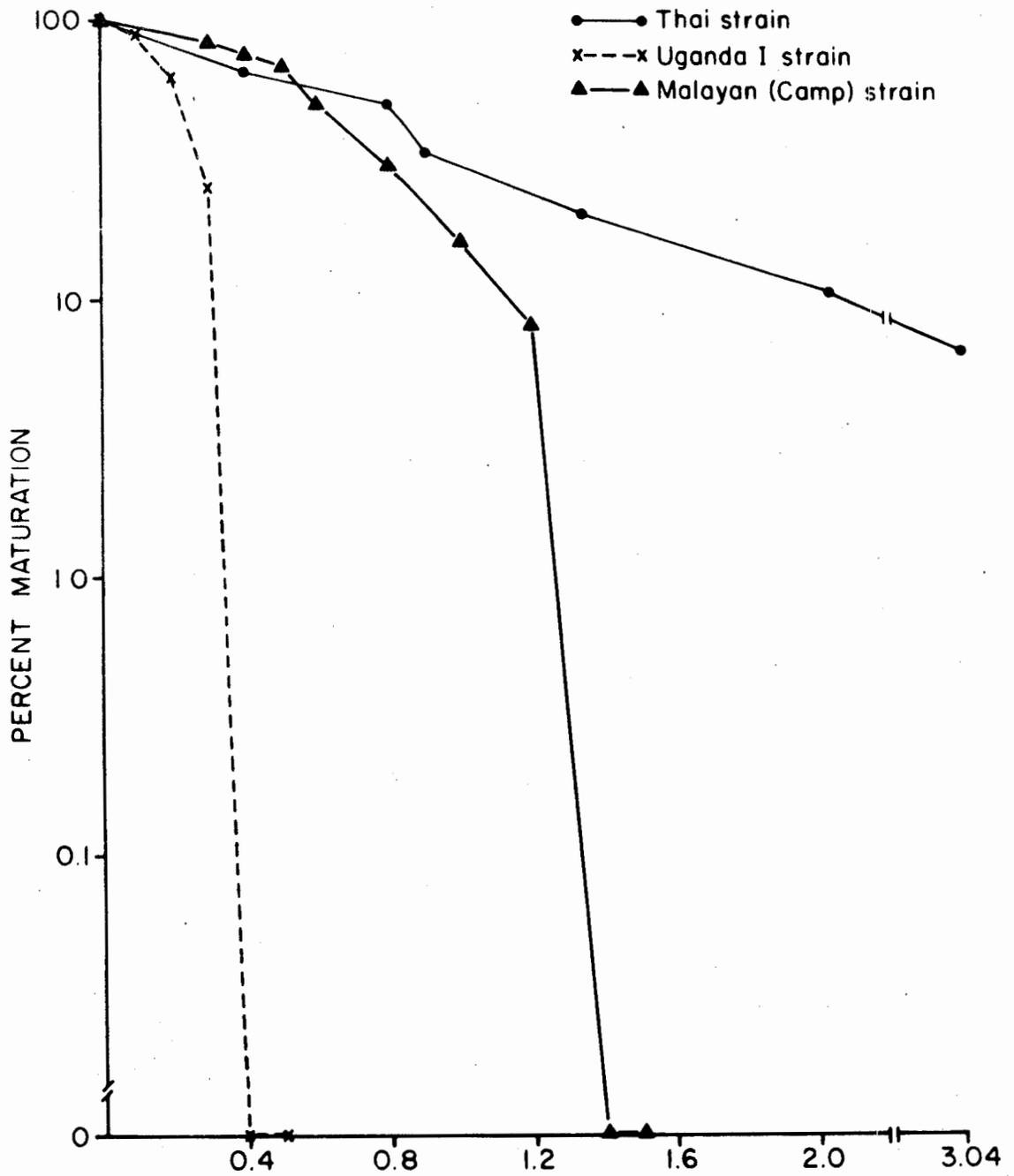


FIGURE 2 CHLOROQUINE CONCENTRATION (nanomoles/ml blood)