



as a solvent was used to prepare the serum samples for the spectrophotofluorometer. The recovery obtained was not satisfactory, and the procedure of McChesney, Banks and McAuliff (Antibiotics and Chemotherapy, 1962) was substituted. The method presently used is a further modification of that technique. Serum samples were obtained from several groups of participating Thais and Americans, according to schedules which will be outlined below. Fresh serum was extracted when possible, but some samples were frozen before extraction. The parasite counts referred to were made by counting the number of parasites in a stained (Giemsa) thick smear, in relation to a standard count of 500 leucocytes.

Chloroquine Serum Levels in Normal Individuals: Before attempting to correlate serum levels of chloroquine with malaria infections, information was sought on the serum levels of the drug which would be reached in healthy Thais and Americans. This was designed to obtain baseline data where the problem of treatment and severe illness would not interfere with the drug administration and blood sampling. Several treatment schedules were used, and the three phases of the study of normal individuals were as follows:

Phase I: Fifteen Thai adults, not on chloroquine therapy, were given a full therapeutic dose of 1500 mg of chloroquine base. The drug was administered orally as the phosphate salt in two doses of 450 mg (four hours apart) on the first day, and 300 mg on each of the two following days. The first day dosage schedule is slightly different from the World Health Organization recommendation of 600 mg followed by 300 mg after an interval of six hours. It had been observed by a number of workers that Thais showed slight side effects from the initial 600 mg dose. These side effects (headache, dizziness) were minimized by the schedule used. Blood was drawn just before the first ingestion of drug and at 2, 4, 12, 24, 48, 72, 96, 216 and 336 hours thereafter. Figure 1 illustrates the average serum level of chloroquine for this group at the times indicated. The highest level of chloroquine in the serum was reached at hour 12. At that time 900 mg of the drug had been ingested, and 8 hours had elapsed since the last dose. The subsequent administration of two 300 mg doses established a maintenance level of chloroquine in excess of 20  $\mu\text{g}/\text{L}$ . This level persisted for 14 days. A serum chloroquine concentration of 10-20  $\mu\text{g}/\text{L}$  is adequate for suppression of malaria parasites according to Berliner et. al. (Journal of Clinical Investigation, 1948).

Phase II: Fifteen U.S. military personnel were placed on a therapeutic schedule of 1500 mg chloroquine base. The initial dose was 600 mg, followed by 300 mg six hours later. An additional 300 mg dose was administered on each of the succeeding two days. The participants were observed for any possible side effects, but these were minimal, and all participants carried on their normal military duties. Blood was drawn on the same time schedule as in Phase I. The full series was completed on two participants, but technical difficulties were encountered in obtaining readings at the required fluorescent wavelengths of 390-400  $\text{m}\mu$  during the processing of the remaining thirteen participants. Attempts to obtain readings on these sera after repair of the instrument were hampered by hemolysis of the sera which had been frozen in the interim.

FIGURE I :  
 AVERAGE SERUM CHLOROQUINE LEVELS OF THAI VOLUNTEERS  
 AFTER ORAL DOSAGE OF 1500 MG BASE

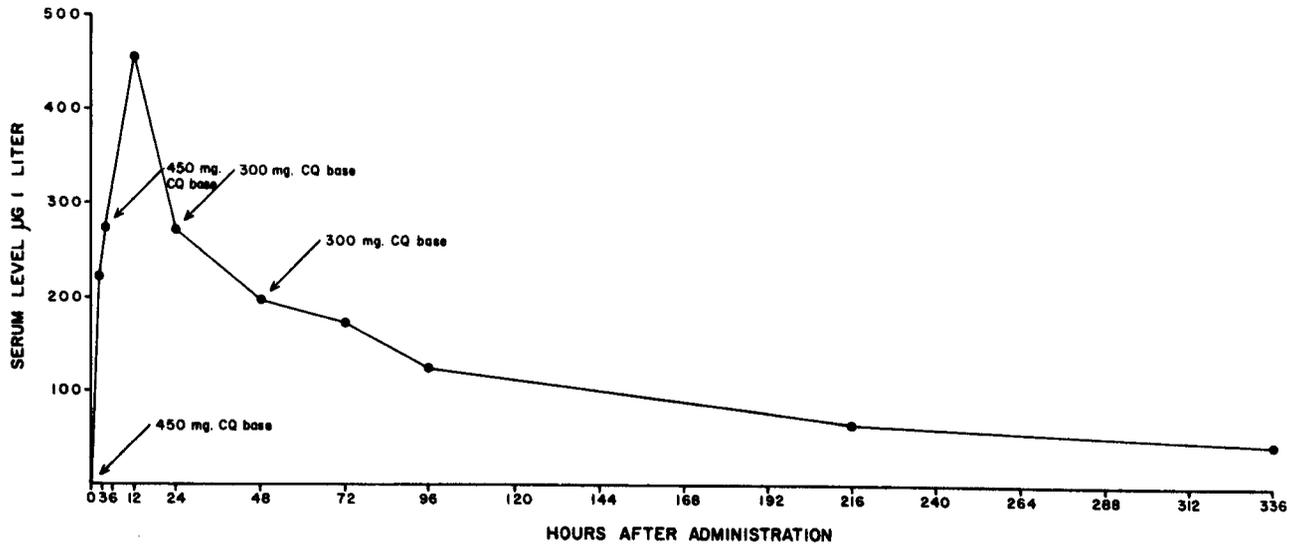
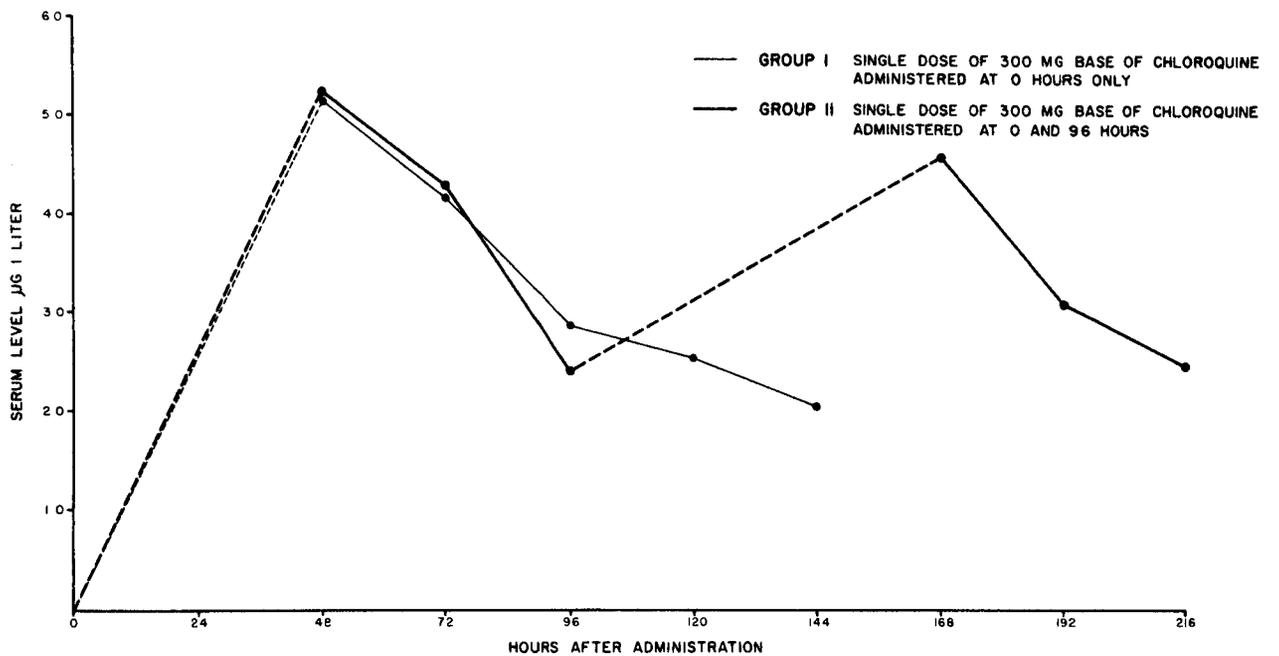


FIGURE II :  
 AVERAGE SERUM CHLOROQUINE LEVEL IN AMERICAN VOLUNTEERS  
 AFTER ORAL DOSAGE OF 300 MG. BASE



Phase III - Thirteen Thai adults were administered a single tablet containing 300 mg of chloroquine base and 45 mg of primaquine base. A blood sample was obtained just before administration of the drug and additional samples were obtained at 24 hour intervals for the following four days. This is the standard U.S. Army prophylactic drug designed to be taken once a week. The results obtained from this group revealed that there are great variations of the levels among this group possibly due to gastrointestinal disturbances in many volunteers. This phase of study was again repeated on American volunteers as follows:

Group I - Sixteen Americans were placed on a single oral tablet of combined chloroquine and primaquine. Blood specimens were obtained at similar intervals as in the previous Thai volunteers.

Group II - The same number of American volunteers were placed on oral tablet schedule as in group I, an additional dose of 300 mg base of chloroquine was administered 96 hours after the first dose. Blood specimens were obtained at 0, 48, 72, 96, 168, 192 and 216 hours.

Due to the side effects observed in a previous group of Thai volunteers, the drug was administered after breakfast. This minimized the side effects and no vomiting was observed in any volunteers, although diarrhea occurred in about 9%.

Average serum levels of these groups are shown in Figure II.

Chloroquine Serum Levels and Malaria Parasite Density: Clinical data, blood specimens for chloroquine determination and finger tip blood smears for parasite counts were obtained from a group of patients admitted to Cholburi Provincial Hospital with a confirmed diagnosis of malaria. The study was conducted with the cooperation of the Director of the hospital. This hospital is located southeast of Bangkok on the gulf of Thailand. It serves a region which contains a number of highly malarious areas. A total of 126 patients was included in our study. During the first part of this study, blood specimens were obtained from 60 patients at 0, 12, 24, 48, 72 and 96 hours. It was observed that the majority of these patients were from different parts of Thailand and came to Cholburi for temporary jobs. They objected to the numbers of bleedings required, especially those patients who showed a rapid response to chloroquine treatment. Many of them left the Hospital before the entire series of bleedings and other observations could be completed. Under the circumstances a number of elements in the original design could not be completed. These included: collection of urine samples, observations on relapse following apparently successful treatment and blood samples on all patients after the last bleeding which was 48 hours after completion of a standard therapeutic dose.

In the latter part of the study, the bleeding schedule was reduced to 0, 72, 96 and 120 hours. By this means the patients were encouraged to complete the series and the length of study on the course of parasitemia was increased. A total number of 66 patients were included in this part of the study.

All the serum specimens were separated at the Hospital and kept frozen until the biochemical determination could be performed at the main laboratory in Bangkok. At each bleeding, peripheral blood smears (thick and thin films) were made for parasite counts and identification. The summary of the data obtained follows:

a. Plasmodium falciparum is the predominant parasite species in the Province and all the selected cases were parasitized by this species. Apparently the less serious cases caused by P. vivax do not appear for hospitalization in very great numbers. No resistance to chloroquine has yet been observed in vivax malaria.

b. About 25% of patients originally included in our study, refused to cooperate. Of the remaining number, 56 cases were found with parasitemia 48 hours after a full therapeutic dose of 1500 mg base of chloroquine.

c. Of 37 patients with parasitemia at 48 hours, 21 continued with parasitemia up to 96 hours after completion of the full therapeutic course of chloroquine.

d. Serum chloroquine levels in all resistant cases were much greater than the presumed therapeutic level required. In the group with parasitemia at 96 hours after completion of a full therapeutic dose, none exhibited a serum level below 40 µg/L.

SERUM CHLOROQUINE LEVELS AT INTERVALS AFTER THERAPEUTIC DOSE  
OF 1500 mg

		48 hours	72 hours	96 hours
Normal Thais	Mean	198.7	173.7	125.1
	Range	372-92	360-90	194-68
Thai patients with Asexual parasitemia	Mean	253.37	174.9	119.49
	Range	502-93	402.8-40	321-40

Details of this study are being prepared for publication.

Case Report of Drug Resistant Malaria. Intensive studies were made on a young Caucasian who contracted Plasmodium falciparum despite chloroquine and combined chloroquine-primaquine prophylaxis. The primary attack occurred 14 days after exposure in a hyperendemic malarious area in Southeast Thailand. He was first treated with camoquine, atabrine, quinine and primaquine. The first relapse occurred three weeks after his first attack. Detailed studies were made during his first relapse. The parasites proved to be resistant to supposedly therapeutic doses of chloroquine and pyrimethamine. The same pattern of resist-

ance was observed in a healthy Thai volunteer artificially infected with his blood. Studies on parasitemia as well as serum levels of chloroquine were made on both cases. Radical cure followed in both cases from quinine therapy.

Summary: The biochemical procedure described by McChesney, Banks and McAuliff (1962) was used to study the levels of chloroquine in sera of Thai and U.S. personnel after taking various doses of the drug. In normal individuals average serum levels of 44.3 µg/L were found 11 days after the administration of 1500 mg of chloroquine. When taking the prophylactic dose of 300 mg chloroquine base, Thai volunteers show an average serum level of 24 µg/L while in American volunteers 20.5 µg/L was observed in two groups 144 hours after administration of the drug. Berliner et. al. (1948) stated that 10-20 µg/L is a therapeutic level.

Patients infected with chloroquine resistant strains of P. falciparum were found among malaria cases admitted to Cholburi Provincial Hospital. Studies of serum chloroquine levels indicated that the resistance was due to the strain of parasites.

Conclusion: Administration of chloroquine at different doses and time schedules established different serum levels and maintenance periods. In healthy individuals taking a full therapeutic dose of 1500 mg of chloroquine base in a three-day period, serum levels 11 days afterwards were greater than the therapeutic level stated by Berliner et. al.

Strains of P. falciparum resistant to chloroquine were detected in malaria patients admitted to Cholburi Provincial Hospital.

#### Publications:

1. Sandhinand, U., Pinswasdi, K., and Neely, J. M. 1965. Chloroquine Resistant Strain of P. falciparum from Khao Mai Khaeo, Thailand. (In press).
2. Sandhinand, U., and Pinswasdi, K., 1965. Morphological evidence of Chloroquine-Resistant Strain of P. falciparum (to be published).
3. Pinswasdi, K., and Sandhinand, U., 1965. Serum levels of Chloroquine after Various Oral Dosage Regimens (to be published).
4. Pinswasdi, K., and Sandhinand, U., 1965. Serum Levels of Chloroquine in Thai Patients with Chloroquine-Resistant P. falciparum (to be published).