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Studies on a Strain of Chloroquine-Resistant *Plasmodium falciparum* from Thailand *

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Infections with a strain of Plasmodium falciparum *from Thailand, termed the Thailand (JHK) strain, were established in 25 non-immune volunteers in a non-endemic area under conditions precluding reinfection. Eleven volunteers received chloroquine in usually curative doses on a three-day schedule during acute clinical malaria attacks. Volunteers also received (again during acute clinical attacks) hydroxychloroquine, amodiaquine, mepacrine, pyrimethamine, proguanil or 377-C-54, alone or in combination. These regimens failed, both before and after passage of the strain through mosquitos, to effect radical cure of the infection. Radical cure was achieved by administration of 1350 mg or 1620 mg of quinine base daily for seven days.*

The authors point out that resistance to chloroquine by P. falciparum *is being recognized with increasing frequency in South America and South-East Asia, and that the effect of this on global chemotherapy of malaria may be serious.*

This paper presents the results of chemotherapeutic studies on a strain of *Plasmodium falciparum* from Thailand. This strain is termed the Thailand (JHK) strain. J.H.K., a member of the United States Armed Forces, was stationed in Thailand from 11 November 1961 to 21 November 1961. He had no previous history of malaria and was not in a malarious area immediately prior to his assignment in Thailand. He developed symptoms of malaria on 26 November 1961. Blood smears on 4 December 1961 revealed asexual erythrocytic forms of *Plasmodium falciparum*. During the next two weeks 2100 mg of chloroquine and 90 mg of primaquine were administered. The symptoms and parasitaemia abated temporarily, but overt parasitaemia and fever recurred on 26 December 1961. Between 28 December 1961 and 17 January 1962, the patient received 4200 mg of chloroquine. The symptoms were relieved, but blood smears remained positive.

On 28 January 1962, J.H.K. was transferred to the United States Naval Hospital, National Naval Medical Center, Bethesda, Md., USA. Blood smears revealed asexual erythrocytic forms of *Plasmodium falciparum*. From 28 January 1962 to 30 January 1962, 1500 mg of chloroquine were administered. The symptoms and parasitaemia abated, but a recrudescence (fever and overt parasitaemia) occurred one month later. On 8 March 1962, infected blood was obtained from J.H.K. and the studies presented in this report were initiated by intravenous inoculation of this sample of blood into inmate volunteers at Stateville Penitentiary, Joliet, Ill., USA.[4]

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[4] Dr Martin D. Young and his co-workers carried out extensive investigations, with respect to J.H.K., prior to 8 March 1962 and subsequently. They also transferred the strain to volunteers. Their studies with volunteers were carried out independently at approximately the same time as ours. The results of Dr Young and co-workers' studies and ours were presented, in part, on 31 October 1962 at the meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, Ga., USA. A report of Dr Young and co-workers' studies, including a detailed description of the course of J.H.K., has been published (Young et al., 1963).

MATERIALS AND METHODS

All studies were carried out in a non-endemic area (Joliet, Ill.) under conditions precluding reinfection.

Volunteers

All volunteers were healthy, adult, American males between 21 and 46 years old (mean age: 30 years). They weighed between 61 kg and 88 kg (mean weight: 73 kg). Twenty-two volunteers were Caucasians; two were Negroes. All volunteers were non-immune. The non-immune status of every volunteer was determined by adherence to rigorous criteria for acceptance for study. We excluded from study: (1) all volunteers who, prior to entering the institution, had a history of malaria or of an illness even remotely suggesting malaria; (2) all volunteers who were born in or who had ever lived in or visited a malarious area; and (3) all volunteers who had previously had *P. falciparum* malaria as a part of studies at the institution.

Procedures

Blood-induced infections were obtained by intravenous inoculation of infected blood, containing approximately 500 000 asexual parasites, into the volunteers. Volunteers having patent infections were hospitalized. Parasite counts were performed at least daily, and frequently more often, during the entire course of each study: (1) films of capillary blood, 0.11 mm thick, were examined by the method of Earle & Perez (1932); (2) 90 oil-immersion fields (each 0.01 mm^2) were examined microscopically to ensure counting all parasites contained in 0.1 mm^3 of whole blood; and (3) counts were expressed in terms of 1 mm^3 of whole blood (Earle & Perez, 1932).

Early during these investigations it became apparent that drugs usually effective in eradication of *falciparum* infections could not be depended upon. Periodic administration of quinine proved necessary to prevent dangerously high levels of parasitaemia. By this means, the investigations were carried out without the occurrence of life-threatening complications.

The strain was passed through *Anopheles quadrimaculatus* and *Anopheles stephensi*. Methods of obtaining sporozoite-induced infections and other methods of follow-up have been described previously by Alving and co-workers (1948).

Samples of urine from patients receiving chloroquine were tested frequently for the presence of chloroquine by the method described by Haskins (1958). Quinine and 377-C-54 give positive Haskins tests; urine from patients receiving chloroquine and quinine or 377-C-54 concurrently was not examined. Urine from a normal individual receiving no drug served as the control sample for each test. All control samples and samples taken prior to drug administration were negative. Samples of urine from patients receiving only chloroquine (Table 1) were positive both during drug administration and also five to seven days after initiation of therapy.

Administration of drugs

All drugs were administered orally and under extremely close supervision. Insertion of the drug into the volunteer's mouth and swallowing were observed. The volunteer's mouth was then inspected to be certain that the drug had been swallowed. It is doubtful that there was a failure to swallow even one dose of medication during the entire study.

Dosages of drugs

Chloroquine diphosphate (Aralen), hydroxychloroquine sulfate (Plaquenil), amodiaquine dihydrochloride (Camoquin), mepacrine dihydrochloride (Atabrine), pyrimethamine (Daraprim), proguanil hydrochloride (Paludrine), 377-C-54 (2:5-bis (*cyclo*-hexylaminoethyl) naphthalene-1:6-diol) dihydrochloride, and quinine sulfate were used. Doses of all drugs are expressed in terms of free base. The regimens were as follows:

(1) Chloroquine: 900 mg (600 mg initially, 300 mg six hours later) the first day; 300 mg daily for the next two days (total dose: 1500 mg). One volunteer (Table 1, Volunteer 8) received doses of chloroquine twice these amounts (total dose: 3000 mg).

(2) Hydroxychloroquine: schedule identical to that of chloroquine (total dose: 1500 mg).

(3) Amodiaquine: 600 mg the first day; 400 mg daily for the next two days (total dose: 1400 mg).

(4) Mepacrine: 785 mg (5 doses of 157 mg) the first day; 78.5 mg thrice daily for the next six days (total dose: 2198 mg).

(5) Pyrimethamine: 50 mg daily for three days (total dose: 150 mg).

(6) Proguanil: 87 mg thrice daily for 10 days (total dose: 2610 mg).

(7) 377-C-54: 1500 mg (1000 mg initially, 500 mg six hours later) the first day; 500 mg daily for the next two days (total dose: 2500 mg).

(8) Quinine: (three regimens were employed): (*a*) 1350 mg (five doses of 270 mg) daily for seven days (total dose: 9450 mg); (*b*) 1620 mg (three

doses of 540 mg) daily for seven days (total dose: 11 340 mg); and (*c*) 1620 mg (three doses of 540 mg) daily for 10 days (total dose: 16 200 mg). The third regimen of quinine (total dose: 16 200 mg) was employed when termination of an infection was required for administrative reasons, at which time it was necessary to be as certain as possible that radical cure would be effected.

RESULTS

Pertinent data are presented in Table 1; the results are summarized in Table 2. Courses of medication were initiated during acute attacks of malaria when parasitaemia and fever (oral temperature at least 38.5° C) were present. Drugs were administered according to the schedules outlined previously.

Effect of chloroquine

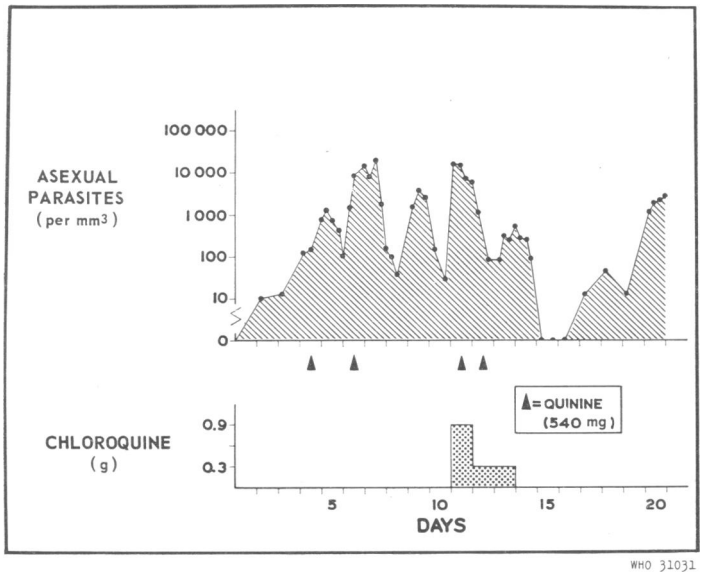
Eight volunteers were treated with a three-day course of chloroquine (total dose: 1500 mg) (Table 1). The infections in Volunteers 1, 2, 3, 5, 6 and 10 had been established before passage of the strain through mosquitos; the infections in Volunteers 12 and 16 had been established after passage through mosquitos.

Volunteer 1 received infected blood from patient J.H.K. on 8 March 1962 and developed overt parasitaemia and fever on 13 March 1962. Chloroquine was administered. Spiking fever (40° C initially) abated, but low-grade fever (38°-38.5° C) persisted. The parasite count (420 per mm³ initially) decreased. Blood smears proved negative the day after therapy had been completed, but remained negative for only four days (Table 1). Rapidly increasing parasitaemia and spiking fever (39°-40°C) ensued.

Chloroquine was administered to Volunteer 2 during a severe acute attack of malaria. The course of parasitaemia in this volunteer is shown in Fig. 1. Fever (41° C initially) decreased slightly, but a lower-grade fever (39°-39.5° C) persisted. Parasitaemia (11 600 per mm³ initially) decreased. Blood smears proved negative two days after completion of the course of chloroquine, but remained negative for less than one day (Fig. 1). Rapidly increasing parasitaemia and high spiking fever developed.

Similar sequences of events attended the administration of chloroquine to Volunteers 3, 5, 6, 10, 12 and 16 (Table 1). Spiking fever decreased; a low-

FIG. 1
EFFECT OF CHLOROQUINE ON BLOOD-INDUCED *P. FALCIPARUM* MALARIA WITH THAILAND (JHK) STRAIN [a]



[a] Total dose: 1500 mg base. Inoculation of infected blood had been carried out four days prior to day 1 of this study. Day 1 represents the last day on which thick blood smears were negative prior to patency. Volunteer 2.

TABLE 1

RESULTS OF CHEMOTHERAPEUTIC STUDIES WITH THE THAILAND (JHK) STRAIN OF *PLASMODIUM FALCIPARUM* [a]

Volunteer and type of infection [b]	Race [c]	Age (years)	Weight (kg)	Findings of first day of therapy [d]		Drug [e] (duration of therapy)	Total dose (mg)	Quinine given concurrently [f] (mg)	Parasite clearance time [g] (days)	Days to normal temp. [h]	Day of recrudescence [i]		Remarks [j]
				Parasite count (per mm³)	Temp. (°C)						Parasites	Fever	
1. BI	C	24	68	420	38.6	Chl. (3 days)	1 500	None	3	FP	7		
				1 050	40	Quin. (7 days)	9 450		3	5			Radical cure
2. BI	C	23	73	11 600	40.8	Chl. (3 days)	1 500	1 080	4	FP	5		See Fig. 1
3. BI	C	29	71	1 290	39	Chl. (3 days)	1 500	135	4	FP	4		See Fig. 2
				5 840	39.2	Hy. (3 days)	1 500	540	5	FP	5		See Fig. 2
				820	38.6	Py. (3 days)	150	135	NC	FP			
				1 280	38.8	Quin. (7 days)	9 450		5	6			Radical cure See Fig. 8
4. BI	C	34	72	3 320	39.5	Amo. (3 days)	1 400	135	NC	FP			See Fig. 3
5. BI	C	27	76	650	38.5	Chl. (3 days)	1 500	None	3	5	6	7	
				2 380	39.6	Py. (3 days)	150	540	NC	FP			See Fig. 5
6. BI	C	25	69	900	40	Chl. (3 days)	1 500	None	3	5	6	7	
				1 230	39.4	Py. (3 days)	150	810	NC	FP			
				340	38.5	Mep. (7 days)	2 198	None	4	5	18	19	See Fig. 4
				1 370	40	Prog. (10 days)	2 610	945	2	3	18	20	See Fig. 6
				820	38.6	Quin. (7 days)	9 450		3	4			Radical cure
7. BI	C	32	70	2 526	39.5	377-C-54 (3 days)	2 500	None	4	FP	9		See Fig. 7
				2 850	40.4	Chl. (3 days) plus 377-C-54 (7 days)	1 500 2 500	None	4	6	15	16	
				2 190	39	Quin. (10 days)	16 200		3	4			Radical cure

TABLE 1

RESULTS OF CHEMOTHERAPEUTIC STUDIES WITH THE THAILAND (JHK) STRAIN OF *PLASMODIUM FALCIPARUM* [a]

(continued)

Volunteer and type of infection [b]	Race [c]	Age (years)	Weight (kg)	Findings of first day of therapy [d]		Drug [e] (duration of therapy)	Total dose (mg)	Quinine given concurrently [f] (mg)	Parasite clearance time [g] (days)	Days to normal temp. [h]	Day of recrudescence [i]		Remarks [j]
				Parasite count (per mm³)	Temp. (°C)						Parasites	Fever	
8. BI	C	32	64	6 540	40.4	Chl. (3 days)	3 000	None	4	5	11	21	
				880	40	Quin. (10 days)	16 200		3	5			Radical cure
9. BI	C	24	80	5 700	40.5	377-C-54 (3 days)	2 500	None	NC	FP			
				6 040	40.5	Chl. (3 days) plus 377-C-54 (3 days)	1 500 2 500	None	4	4	10	7	
10. BI	C	41	84	600	41	Chl. (3 days)	1 500	135	4	FP	6		
				40	38.8	Quin. (7 days)	9 450		3	5			Radical cure
11. BI	C	45	75	4 540	40.5	Quin. (10 days)	16 200		3	4			Radical cure
12. MI	C	22	77	5 540	40	Chl. (3 days)	1 500	None	NC	FP			
				6 900	40.1	Py. (3 days)	150	270	NC	FP			
				1 120	40.4	Quin. (7 days)	11 340		3	5			Radical cure
13. MI	C	27	72	10 600	40	Prog. (10 days)	2 610	540	NC	FP			
				5 000	38.8	Hy. (3 days)	1 500	None	NC	FP			
				15 840	40.1	Amo. (3 days)	1 400	540	NC	FP			
				13 600	39	Quin. (7 days)	11 340		4	6			Radical cure
14. BIA	C	29	66	200	38.6	Amo. (3 days)	1 400	None	4	6	8	9	
				5 460	39.4	Quin. (7 days)	11 340		5	6			Radical cure

TABLE 1

RESULTS OF CHEMOTHERAPEUTIC STUDIES WITH THE THAILAND (JHK) STRAIN OF *PLASMODIUM FALCIPARUM* [a] *(concluded)*

Volunteer and type of infection [b]	Race [c]	Age (years)	Weight (kg)	Findings of first day of therapy [d]		Drug [e] (duration of therapy)	Total dose (mg)	Quinine given concurrently [f] (mg)	Parasite clearance time [g] (days)	Days to normal temp. [h]	Day of recrudescence [i]		Remarks [j]
				Parasite count (per mm³)	Temp. (°C)						Parasites	Fever	
15. BIA	CN	30	86	3 760	40.6	Mep. (7 days)	2 198	None	5	6	13	13	
16. BIA	N	37	88	21 080	38.9	Chl. (3 days)	1 500	None	4	4	5	6	
17. BIA	N	28	75	45 840	41	377-C-54 (3 days)	2 500	810	NC	FP			
				4 180	38.6	Quin. (7 days)	11 340		3	5			Radical cure
18. MI	C	26	70	500	39.5	Quin. (7 days)	11 340		3	5			Radical cure
19. MI	C	25	75	910	38.9	Quin. (7 days)	11 340		2	3			Radical cure
20. MI	C	22	61	6 220	40.8	Quin. (7 days)	11 340		4	7			Radical cure
21. MI	C	41	70	1 380	40.5	Quin. (7 days)	11 340		3	6			Radical cure
22. MI	C	32	74	240	39.5	Quin. (7 days)	11 340		3	5			Radical cure
23. MI	C	28	78	1 850	38.8	Quin. (7 days)	11 340		3	4			Radical cure
24. MI	C	31	66	5 260	41.1	Quin. (7 days	11 340		4	7			Radical cure

[a] Studies are listed in the order in which they were carried out. Data horizontally in line with each volunteer's number represent studies carried out during the volunteer's initial acute attack of malaria.

[b] BI = blood-induced; MI = mosquito-induced; BIA = blood-induced after passage of the strain through mosquitos. Prior to passage through mosquitos (Volunteers 12 and 13), the strain had been exposed, during the studies, only to 4-aminoquinolines and small doses of quinine.

[c] C = Caucasian; N = Negro.

[d] Parasite count and oral temperature at the time of initiation of drug administration.

[e] Chl. = chloroquine; Hy. = hydroxychloroquine; Amo. = amodiaquine; Py. = pyrimethamine; Mep. = mepacrine; Prog. = proguanil; Quin. = quinine.

[f] The concurrent administration of quinine was in order to prevent too high a level of parasitaemia.

[g] Parasite clearance time denotes the number of days elapsing from the first day of drug administration to the first day on which no parasites could be demonstrated in the blood. NC (not cleared) indicates persistence of overt parasitaemia.

[h] Days to normal temperature denotes the number of days elapsing from the first day of drug administration to the first day on which temperature returned to and remained normal the entire day. FP indicates that fever persisted.

[i] Day of recrudescence denotes the number of days elapsing from the first day of drug administration to the first day of recurrence of overt parasitaemia (positive blood smears) or fever (38.5°C). The term " relapse " is not applicable in this situation.

[j] Radical cure denotes complete elimination of the parasite from the body, as evidenced by a lack of recrudescence during a follow-up of at least 60 days. Radical cure of infections in Volunteers 2, 4, 5, 9, 15 and 16 was achieved by administration of quinine. However, in these six instances, quinine was not administered during an acute attack; these latter data, therefore, are not included in the report. After no recrudescence occurred during at least *three* months' follow-up in Volunteers 1, 3, 6 and 10, who received a total dose of 9450 mg of quinine base, continuing host-susceptibility in these four volunteers was demonstrated by reinoculation of blood infected with the Thailand (JHK) strain of *P. falciparum*; all four volunteers promptly developed acute attacks of malaria.

TABLE 2

SUMMARY OF CHEMOTHERAPEUTIC STUDIES WITH THE THAILAND (JHK) STRAIN OF *PLASMODIUM FALCIPARUM*

Drug	Total dose (mg base)	Duration of therapy (days)	Number of volunteers treated	Results [a]			
				No effect	Temporary effect		Radical cure
					Partial	Complete	
Chloroquine	1 500	3	8		6	2	
	3 000	3	1			1	
Hydroxychloroquine	1 500	3	2		2		
Amodiaquine	1 400	3	3		2	1	
Mepacrine	2 198	7	2			2	
Pyrimethamine	150	3	4	1	3		
Proguanil	2 610	10	2		1	1	
377-C-54	2 500	3	3		2	1	
377-C-54 and chloroquine (concurrently)	2 500	3	2			2	
	1 500	3					
Quinine	9 450	7	4				4
	11 340	7	11				11
	16 250	10	3				3

[a] No effect: no discernible abatement of parasitaemia or fever attributable to drug administration.
Partial temporary effect: temporary decrease in fever or parasitaemia attributable to drug administration, but without occurrence of both normal temperature and negative blood smears.
Complete temporary effect: abatement of fever and parasitaemia attributable to drug administration with temporary development of both normal temperature and negative blood smears.
Radical cure: see footnote *j* to Table 1.

grade fever (38.5°C) persisted in four of the six volunteers. Parasitaemia decreased, but radical cure was not achieved. Smears of blood from Volunteer12 remained positive. Smears of blood from Volunteers 3, 5, 6, 10 and 16 proved negative one to two days after completion of administration of chloroquine. Smears of blood from Volunteer 3 remained negative for less than one day (see Fig. 2); smears of blood from the other volunteers remained negative for from one to three days. Recrudescence (overt and rapidly increasing parasitaemia and spiking fever) occurred consistently five to seven days after initiation of therapy.

Investigations carried out with Volunteer 8 are of especial interest because this volunteer received doses of chloroquine twice those utilized in the other studies. At the outset of therapy, high spiking fever (41°C) was occurring and the parasite count was 6540 per mm^3. A total of 3000 mg of chloroquine was administered during a three-day period and fever and parasitaemia decreased. Blood smears proved negative two days after completion of therapy. The volunteer's temperature returned to normal and remained so the following day. Blood smears remained negative for seven days, after which overt parasitaemia returned. Increasing parasitaemia and fever, however, did not commence immediately thereafter, but developed only after an additional interval of 10 days (21st day after initiation of therapy) (Table 1).

To summarize, nine volunteers were treated, during acute attacks, with a three-day course of chloroquine;

eight received 1500 mg and one received 3000 mg. Radical cure was achieved in none of the individuals (Tables 1 and 2).

Control studies with chloroquine

Unexpected ineffectiveness of a drug may be due to " drug failure " or " drug resistance "; it is important to distinguish between the two. " Drug failure " has been defined as: " Absence of drug action after ingestion of an effective dose, due to deficient absorption, unusual rates of metabolism or of excretion of the drug ". " Drug resistance ", on the other hand, has been defined as " The ability of a parasite strain to survive in the body of the host in the presence of concentrations of the drug that normally destroy or prevent multiplication of this parasite species " (WHO Technical Meeting on Chemotherapy of Malaria, 1961). The results of studies such as those presented above, with respect to any one individual, might represent either " drug failure " or " drug resistance ". Even in the presence of high plasma levels of chloroquine, " drug failure ", due to abnormal metabolism of the drug in a particular individual, may occur (L. T. Coggeshall—personal communication). Plasma levels of chloroquine were not determined. Haskins tests of urine from volunteers receiving chloroquine were positive, indicating the presence of chloroquine in the urine, both during drug administration and five to seven days after initiation of therapy.

The consistent ineffectiveness of chloroquine makes it extremely likely that the results presented above represent drug resistance and not drug failure. However, to exclude with certainty the possibility of drug failure, control investigations were carried out (at the same time as the studies described above) with 15 additional non-immune volunteers, chosen at random, infected with the McLendon strain of *Plasmodium falciparum*. This strain is known to be susceptible to chloroquine. The 15 volunteers were treated, during acute attacks, with a three-day regimen of chloroquine (total dose: 1500 mg) administered according to the schedule outlined previously. Rapid elimination of parasitaemia and symptoms, with radical cure of the infection, resulted in all 15. These control investigations, demonstrating, under the conditions employed in this study, the effectiveness of the " standard dosage " of chloroquine against a susceptible strain of *Plasmodium falciparum*, together with the results presented above demonstrating the consistent relevant ineffectiveness of chloroquine against the Thailand (JHK) strain, conclusively exclude drug failure as a possible explanation for the ineffectiveness of chloroquine against the Thailand (JHK) strain.

Effect of hydroxychloroquine

Hydroxychloroquine (1500 mg given over three days) was administered to Volunteer 3 (infection established before passage of the strain through mosquitos) and to Volunteer 13 (infection established after passage through mosquitos) (Table 1). The course of parasitaemia in Volunteer 3 is shown in Fig. 2. The results were similar to those obtained with chloroquine. Administration of hydroxychloroquine resulted in a temporary decrease in both parasitaemia and fever. A lower-grade fever (38.5°-39° C) persisted. Smears of blood from Volunteer 13 remained positive. One blood smear from Volunteer 3 was negative the day following completion of administration of chloroquine (Fig. 2). Rapidly increasing parasitaemia and spiking fever developed in both volunteers five to seven days after initiation of therapy.

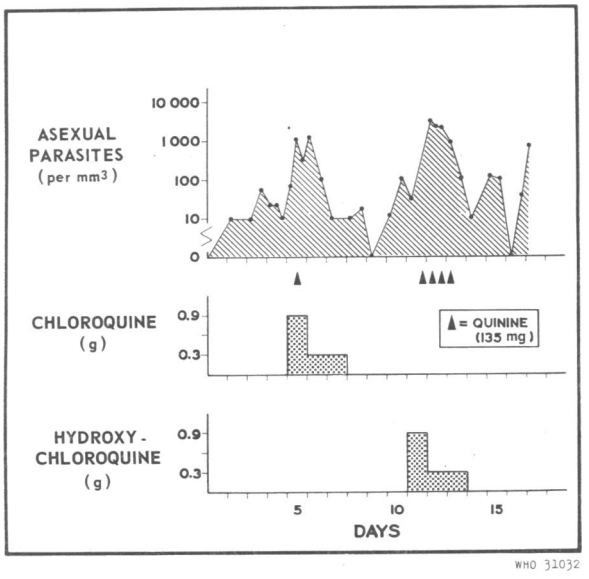
Effect of amodiaquine

Amodiaquine (1400 mg base given over three days) was administered to Volunteer 4 (infection established before passage of the strain through mosquitos) and to Volunteers 13 and 14 (infections established after passage through mosquitos) (Table 1). The course of parasitaemia in Volunteer 4 is shown in Fig. 3. Fever and parasitaemia in Volunteers 4 and 13 decreased slightly between the third and sixth days following initiation of therapy, but low-grade fever persisted and blood smears remained positive. Smears of blood from Volunteer 14 proved negative from the fourth to the eighth day after initiation of therapy; his temperature was normal from the sixth to the ninth day. All three volunteers developed rapidly increasing parasitaemia and spiking fever nine to 10 days after initiation of therapy.

Effect of mepacrine

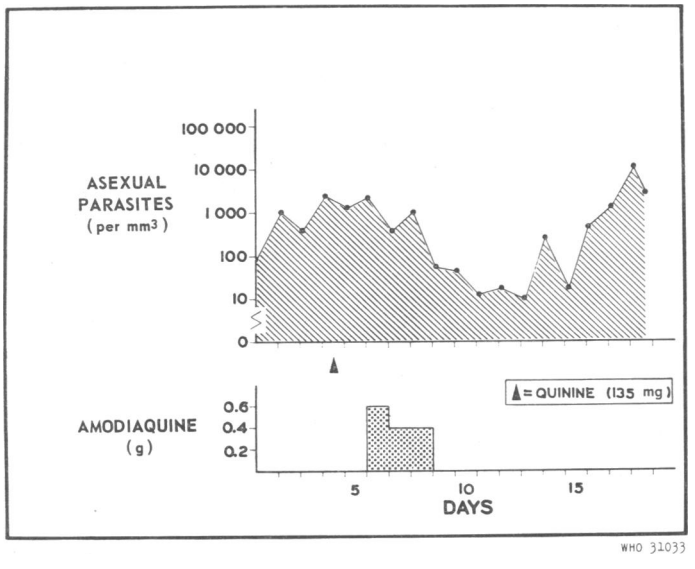
Mepacrine (2198 mg given over seven days) was administered to Volunteer 6 (infection established before passage of the strain through mosquitos) and Volunteer 15 (infection established after passage through mosquitos) (Table 1). The course of parasitaemia in Volunteer 6 is shown in Fig. 4. In both instances, (*a*) spiking fever (39°-40°C) gradually subsided and oral temperature returned to normal five to six days after initiation of therapy, and (*b*) parasitaemia decreased and blood smears proved

FIG. 2
EFFECT OF HYDROXYCHLOROQUINE COMPARED WITH THAT OF CHLOROQUINE ON BLOOD-INDUCED *P. FALCIPARUM* MALARIA WITH THAILAND (JHK) STRAIN [a]



[a] Total dose of each drug: 1500 mg base. Inoculation of infected blood had been carried out five days prior to day 1 of this study. Day 1 represents the last day on which thick blood smears were negative prior to patency. Volunteer 3.

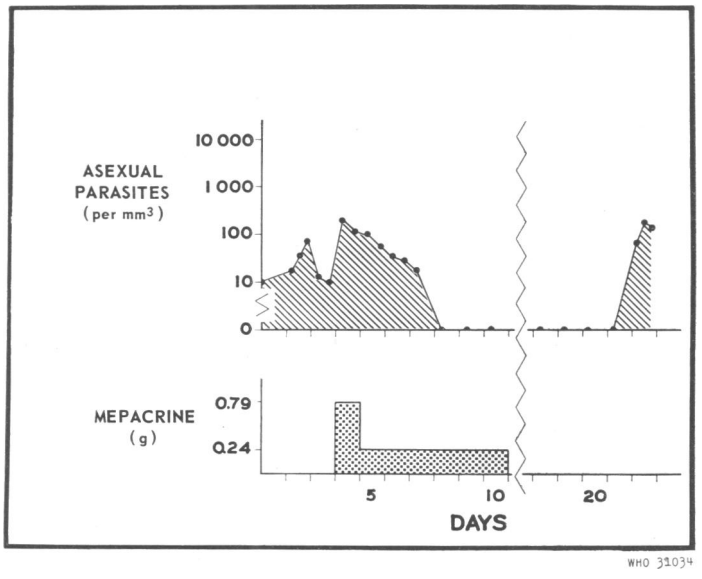
FIG. 3
EFFECT OF AMODIAQUINE ON BLOOD-INDUCED *P. FALCIPARUM* MALARIA WITH THAILAND (JHK) STRAIN [a]



[a] Total dose: 1400 mg base. A patent infection had been present intermittently (see Table 1) for 20 days prior to day 1 of this study. Volunteer 4.

FIG. 4

EFFECT OF MEPACRINE ON BLOOD-INDUCED *P. FALCIPARUM* MALARIA WITH THAILAND (JHK) STRAIN [a]



[a] Total dose: 2198 mg base. (The total amount of mepacrine hydrochloride administered was 2800 mg.) A patent infection had been present for 12 days prior to day 1 of this study. Volunteer 6.

negative four to five days after initiation of therapy. Volunteer 6 developed overt parasitaemia 18 days after initiation of therapy (Table 1); rapidly increasing parasitaemia and spiking fever developed during the next two days. Volunteer 15 developed overt parasitaemia and fever 13 days after initiation of therapy, following which parasitaemia increased rapidly and spiking fever developed.

Effect of pyrimethamine

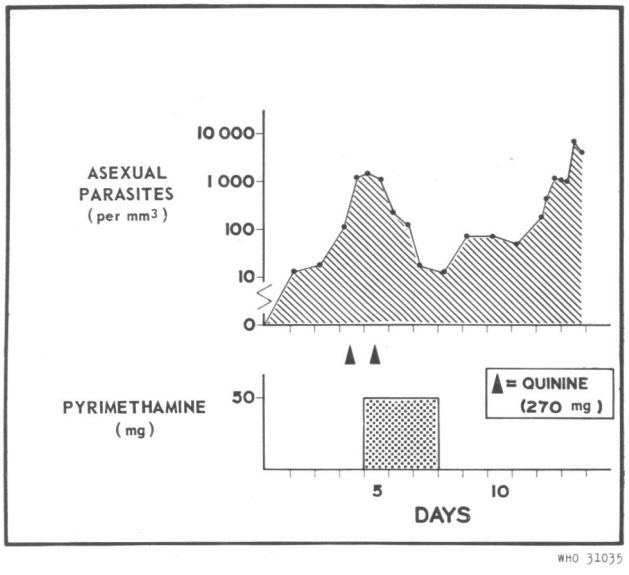
Pyrimethamine (150 mg base given over three days) was administered to Volunteers 3, 5 and 6 (infections established after passage through mosquitos) and to Volunteer 12 (infection established after passage through mosquitos) (Table 1). The course of parasitaemia in Volunteer 5 is shown in Fig. 5. Intermittent fever (39°-40°C) persisted in all four volunteers, during and subsequent to the administration of pyrimethamine. Parasitaemia in Volunteer 3 continued to increase. In the other three volunteers there was a transient decrease in parasitaemia three to four days after initiation of therapy, following which increasing parasitaemia developed (Fig. 5).

Effect of proguanil

Proguanil (2610 mg over 10 days) was administered to Volunteer 6 (infection established before passage through mosquitos) and Volunteer 13 (infection established after passage through mosquitos) (Table 1). The course of parasitaemia in Volunteer 6 is shown in Fig. 6. The spiking fever (40°C) in Volunteer 6 subsided rapidly and his temperature returned to normal three days after initiation of therapy. Parasitaemia in this volunteer also decreased rapidly and blood smears proved negative two days after initiation of therapy (Fig. 6); 18 days after initiation of therapy, overt parasitaemia recurred and, two days later, spiking fever and rapidly increasing parasitaemia developed (Fig. 6). The concurrent administration of quinine (945 mg) to Volunteer 6 (Fig. 6) may have influenced appreciably the course of parasitaemia and fever in this volunteer. The spiking fever (40°C) in Volunteer 13 decreased, but an intermittent lower-grade fever (39°-39.5°C) persisted; blood smears remained positive and, 13 days after initiation of therapy, rapidly increasing parasitaemia and high spiking fever (41°C) recurred.

FIG. 5

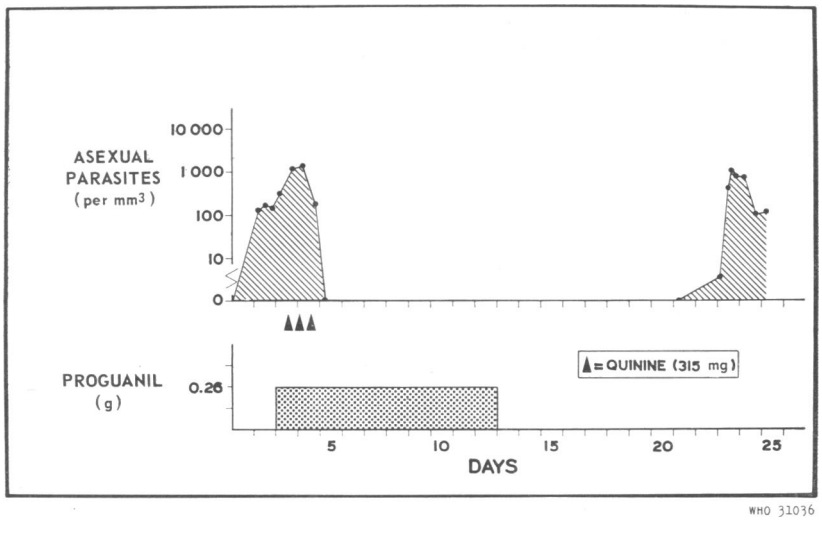
EFFECT OF PYRIMETHAMINE ON BLOOD-INDUCED *P. FALCIPARUM* MALARIA WITH THAILAND (JHK) STRAIN [a]



[a] Total dose: 150 mg base. A patent infection had been present for 11 days (see Table 1) prior to day 1 of this study. Volunteer 5.

FIG. 6

EFFECT OF PROGUANIL ON BLOOD-INDUCED *P. FALCIPARUM* MALARIA WITH THAILAND (JHK) STRAIN [a]



[a] Total dose: 2610 mg base. (The total amount of proguanil monohydrochloride administered was 3000 mg.) A patent infection had been present intermittently (see Table 1) for 34 days prior to day 1 of this study. Blood smears examined daily between day 5 and day 20 were negative. Volunteer 6.

Effect of 377-C-54

377-C-54 (2500 mg given over three days) was administered to Volunteers 7 and 9 (infections established before passage of the strain through mosquitos) and to Volunteer 17 (infection established after passage through mosquitos) (Table 1). The course of parasitaemia in Volunteer 7 is shown in Fig. 7. Fever and parasitaemia in Volunteers 7 and 17 decreased, but low-grade fever (38°-38.5°C) persisted in both. Smears of blood from Volunteer 7 proved negative from the fourth to the eighth day after initiation of therapy, following which rapidly increasing parasitaemia and spiking fever occurred. Smears of blood from Volunteer 17 remained positive; spiking fever and rapidly increasing parasitaemia recurred 13 days after initiation of therapy. Spiking fever (39°-40°C) persisted in Volunteer 9; parasitaemia decreased transiently, but blood smears remained positive and rapidly increasing parasitaemia developed seven days after initiation of therapy.

Effect of concurrent administration of chloroquine and 377-C-54

Three-day courses of chloroquine (1500 mg) and 377-C-54 (2500 mg) were administered concurrently to Volunteers 7 and 9 (Table 1). Parasitaemia and fever decreased. Blood smears, in both instances, proved negative four days after initiation of therapy. Volunteer 7 became afebrile the same day and Volunteer 7 became afebrile two days later. Smears of blood from Volunteer 7 remained negative for 11 days; smears of blood from Volunteer 9 remained negative for six days. Both volunteers then developed rapidly increasing parasitaemia and spiking fever.

Effects of quinine

Quinine was administered as follows: (1) 1350 mg daily for seven days (total dose: 9450 mg) to Volunteers 1, 3, 6 and 10; (2) 1620 mg daily for seven days (total dose: 11 340 mg) to Volunteers 12, 13, 14 and 17 to 24; and (3) 1620 mg daily for 10 days (total dose: 16 200 mg) to Volunteers 7, 8 and 11 (Table 1). The course of parasitaemia in Volunteer 3 is shown in Fig. 8. Fever subsided three to seven days after initiation of therapy; blood smears proved negative after two to five days. Radical cure resulted in all instances (Tables 1 and 2).

DISCUSSION

Chloroquine occupies a key position in the chemotherapy of malaria. It is widely used and depended upon both for treatment of acute attacks and for suppression. The "standard treatment" recommended for therapy of acute attacks of moderate severity in non-immune adults is 1500 mg of chloroquine base, given orally over three days (Covell et al., 1955).

This regimen was administered to eight non-immune volunteers infected with the Thailand (JHK) strain of *Plasmodium falciparum*. Radical cure of the infections did not result. The asexual erythrocytic forms of the Thailand (JHK) strain are resistant to this dosage of chloroquine. Twice the recommended amount of chloroquine (3000 mg base) was administered, over three days, to one volunteer. Even this high dosage of chloroquine failed to effect radical cure of the infection.

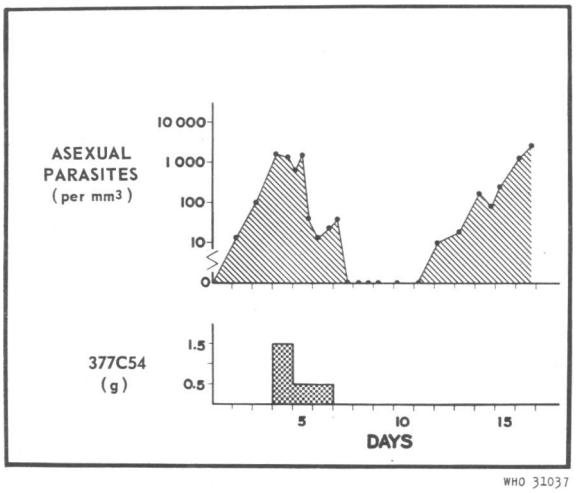
Previously, the asexual erythrocytic forms of only one strain of *P. falciparum*—a strain from Colombia, South America—have been shown conclusively to be resistant to 1500 mg of chloroquine base given over three days (Moore & Lanier, 1961; Young & Moore, 1961). Important observations concerning other possibly chloroquine-resistant strains of *P. falciparum* have been presented (Rodrigues, 1961; Box et al., 1963), but await substantiation by studies that exclude reinfection and include transfer of the strains to uninfected, non-immune hosts.

Three volunteers infected with the Thailand (JHK) strain received a three-day course of amodiaquine (1400 mg base), two received a three-day regimen of hydroxychloroquine (1500 mg base), and two received a seven-day course of mepacrine (2198 mg base). Radical cure did not result. The regimens employed were those recommended for treatment of acute attacks in non-immune adults (Covell et al., 1955; WHO Technical Meeting on Chemotherapy of Malaria, 1961). The data indicate that the asexual erythrocytic forms of the Thailand (JHK) strain are resistant to recommended doses of amodiaquine, hydroxychloroquine and mepacrine. In this respect, the characteristics of the Thailand (JHK) strain appear similar to those of the strain from Colombia (Young, 1961, 1962).

Radical cure of infections with the strain from Colombia, in non-immune volunteers, has been achieved by the administration of proguanil (Young, 1962) and pyrimethamine (Young, 1961, 1962; Powell et al., 1963). Two volunteers infected with the Thailand (JHK) strain received a 10-day regimen of proguanil (2610 mg base) and four volunteers received a three-day course of pyrimethamine (150 mg base). Radical cure did not result. The dosages of

FIG. 7

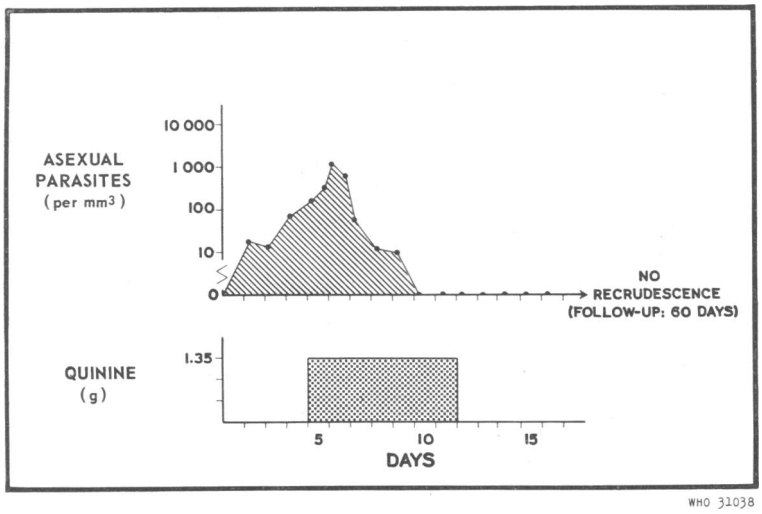
EFFECT OF 377-C-54 ON BLOOD-INDUCED *P. FALCIPARUM* MALARIA WITH THAILAND (JHK) STRAIN [a]



[a] Total dose: 2500 mg base. Inoculation of infected blood had been carried out 3 days prior to day 1 of this study. Day 1 represents the last day on which thick blood smears were negative prior to patency. Volunteer 7.

FIG. 8

EFFECT OF QUININE ON BLOOD-INDUCED *P. FALCIPARUM* MALARIA WITH THAILAND (JHK) STRAIN [a]



[a] Total dose: 9450 mg base. (The total amount of quinine sulfate administered was 11 375 mg.) A patent infection had been present intermittently (see Table 1) for 20 days prior to day 1 of this study. Volunteer 3.

proguanil and pyrimethamine employed were equal to or exceeded the dosages of these two drugs that have effected radical cure of infections with the strain from Colombia. The data suggest an important difference between the strain from Colombia and the Thailand (JHK) strain. The asexual erythrocytic forms of the Thailand (JHK) strain appear resistant to both proguanil and pyrimethamine. Proguanil-resistant and pyrimethamine-resistant *P. falciparum* has been detected previously in countries near Thailand (WHO Technical Meeting on Chemotherapy of Malaria, 1961).

377-C-54, a hydroxynaphthalene derivative, has been reported to have blood schizontocidal activity (approximately one-half to one-third that of chloroquine) against strains of *P. falciparum* infecting humans in Africa and in India (Bruce-Chwatt & Charles, 1957; Ray et al., 1959). In addition, 377-C-54 has been found to have blood schizontocidal activity against a strain of chloroquine-resistant *P. berghei* in mice (Hawking & Gammage, 1962). The latter findings suggested that 377-C-54 might be of value in the therapy of humans infected with chloroquine-resistant *P. falciparum*. Three volunteers infected with the Thailand (JHK) strain of P. *falciparum* received a three-day course of 377-C-54, the total dose being 2500 mg base. This dose is at least fivefold greater than the dosages employed in the studies of 377-C-54 carried out in Africa and in India. Two volunteers received three-day courses of chloroquine (1500 mg base) and 377-C-54 (2500 mg base) concurrently. Radical cure did not result. 377-C-54 (2500 mg base) administered to volunteers infected with the strain of chloroquine-resistant *P. falciparum* from Colombia failed to eradicate infections with this strain. The data do not suggest that 377-C-54 may be of value in the treatment of humans infected with chloroquine-resistant *P. falciparum*.

Quinine consistently effected radical cure of infections with the Thailand (JHK) strain of *P. falciparum*. The asexual erythrocytic forms of this strain are very susceptible to quinine. We have consistently observed that a single dose of quinine (e.g., 540 mg base), although not curative, exerts a more pronounced blood schizontocidal effect against the Thailand (JHK) strain than against the strain of chloroquine-resistant *P. falciparum* from Colombia or the McLendon strain of *P. falciparum*, a strain not chloroquine-resistant. Quinine is the only drug found thus far that effects radical cure of infections with the Thailand (JHK) strain of *P. falciparum*.

The asexual erythrocytic forms of the Thailand (JHK) strain are resistant to a remarkable spectrum of antimalarial drugs, including drugs of remarkably diverse structure that have, in all likelihood, remarkably diverse mechanisms of action, and including all antimalarials currently used widely for treatment of *P. falciparum* malaria. Resistance to these drugs persists after passage of the strain through mosquitos.

Field epidemiological reconnaissance in the area of the possible focus of chloroquine-resistant *P. falciparum* in Colombia failed to disclose evidence of any such focus in that country (WHO Technical Meeting on the Chemotherapy of Malaria, 1961). Chloroquine-resistant *P. falciparum* malaria has been detected recently in at least three additional members of the United States Armed Forces. Two of the three acquired their infections in Thailand, the third in Viet Nam. Studies on *P. falciparum* from these three individuals are currently under way in our laboratory. Blood-induced infections with *P. falciparum* from two of the individuals (And., infected in Thailand; Sn, infected in Viet Nam) have been established in non-immune Caucasian volunteers. In both instances, the administration of 1500 mg of chloroquine base over three days failed to effect radical cure of the infection. Identical results have recently been obtained in our laboratory with a strain of *P. falciparum* that infected an individual (Camp.) in Malaya. Chloroquine-resistant *P. falciparum* malaria is apparently widespread in South-East Asia. It is quite likely that chloroquine-resistant *P. falciparum* exists in Brazil (Rodrigues, 1961; Box et al., 1963). Chloroquine-resistance in *P. falciparum* is being reported with increasing frequency and in widely separated sectors of the globe and the impact upon global chemotherapy of malaria could be serious.

POSTSCRIPT

Two additional pertinent studies have been carried out since completion of this manuscript.

1. One additional non-immune Caucasian volunteer (age: 31 years; weight: 69 kg), infected with the Thailand (JHK) strain of *P. falciparum*, was treated, during an acute clinical attack, with 3000 mg of chloroquine base administered orally over three days. The regimen employed was identical to that administered to Volunteer 8 (Table 1). At the outset of therapy, the parasite count was 6820 per mm^3. Thick blood smears proved negative two days after initia-

tion of therapy; fever (39.5° C initially) subsided the following day. The volunteer remained afebrile and thick blood smears were negative until the seventeenth day after initiation of therapy, at which time thick blood smears again revealed asexual erythrocytic forms of *P. falciparum*. Rapidly increasing parasitaemia and spiking fever occurred during the next three days.

2. Volunteer 6 (Table 1), who was reinoculated with the Thailand (JHK) strain of *P. falciparum* to demonstrate continuing susceptibility to infection (see footnote *j* to Table 1), was treated, during an acute clinical attack, with 240 mg of chloroquine base (300 mg of chloroquine dihydrochloride) administered intramuscularly thrice daily for three days (total intramuscular dose: 2160 mg chloroquine base). At the outset of therapy, the parasite count was 4820 per mm^3. Thick blood smears proved negative and fever (39° C initially) subsided two days after therapy was initiated. The patient was afebrile and thick blood smears were negative until the twentieth day after initiation of therapy, at which time thick blood smears revealed asexual erythrocytic forms of *P. falciparum*. Increasing parasitaemia and spiking fever ensued during the next two days.

Haskins tests of urine from both volunteers were negative prior to administration of chloroquine, strongly positive during drug administration, and still positive at the time of recrudescence. These data provide strong additional evidence indicating that the asexual erythrocytic forms of the Thailand (JHK) strain of *P. falciparum* are highly resistant to chloroquine.

RÉSUMÉ

Des études de chimiothérapie ont été effectuées à l'aide d'une souche de *Plasmodium falciparum* originaire de Thaïlande et appelée souche JHK. Des infections expérimentales ont été réalisées chez 25 volontaires non immunisés, dans des conditions excluant toute possibilité de réinfection. Onze volontaires ont reçu lors des accès aigus des traitements de trois jours de chloroquine: huit ont reçu par voie orale 1500 mg de chloroquine-base, deux autres 3000 mg, le onzième a reçu 2160 mg de chloroquine-base par voie intramusculaire. Le test urinaire de Haskins a été, chez ces volontaires, négatif avant toute administration de chloroquine et fortement positif pendant et après le traitement. Les volontaires ont également reçu, au cours des accès, de l'hydroxychloroquine (1500 mg de base pendant trois jours), de l'amodiaquine (1400 mg de base pendant trois jours), de la mépacrine (2198 mg de base pendant sept jours), du proguanil (2610 mg de base pendant dix jours) ou de la pyriméthamine (150 mg de base pendant trois jours). Les traitements n'ont pas réussi, aussi bien avant qu'après passage de la souche chez les moustiques, à guérir les infections de manière radicale.

Ces recherches montrent que les formes asexuées érythrocytaires de la souche thaïlandaise (JHK) de *P. falciparum* résistent aux doses habituellement curatives de chloroquine, d'hydroxychloroquine, d'amodiaquine, de mépacrine, de proguanil et de pyriméthamine. Après administration, sous forme de traitements de trois jours (2500 mg), du composé 377-C-54, dérivé de l'hydroxynaphtaline et connu pour son efficacité contre une souche de *P. berghei* résistante à la chloroquine chez la souris, l'on n'a pu obtenir la cure radicale d'infections avec la souche thaïlandaise (JHK). L'administration simultanée, pendant 3 jours de chloroquine (1500 mg) et de 377-C-54 (2500 mg) fut également inefficace. Par contre, les formes asexuées érythrocytaires de la souche thaïlandaise (JHK) se sont montrées très sensibles à la quinine. Une cure radicale de l'infection a pu être obtenue après administration de 1350 ou de 1620 mg de quinine-base (c'est-à-dire 1,60 g ou 1,95 g de sulfate de quinine) chaque jour pendant sept jours.

La résistance à la chloroquine de souches de *P. falciparum* est de plus en plus observée en Amérique du Sud et en Asie du Sud-Est. Les conséquences sur la chimiothérapie d'ensemble du paludisme pourraient être sérieuses.

REFERENCES

Alving, A. S., Craige, B., Jr., Pullman, T. N., Whorton, C. M., Jones, R., Jr & Eichelberger, L. (1948) *J. clin. Invest.*, **27**, 2

Box, E. D., Box Q. T. & Young M. D. (1963) *Amer. J. trop. Med. Hyg.*, **12**, 300

Bruce-Chwatt, L. J. & Charles, L. J. (1957) *Brit. med. J.*, **2**, 23

Covell, G., Coatney, G. R., Field, J. W. & Singh, J. (1955) *Chemotherapy of malaria*, Geneva (*World Health Organization : Monograph Series*, No. 27)

Earle, W. C. & Perez, M. (1932) *J. Lab. clin. Med.*, **17**, 1124

Haskins, W. T. (1958) *Amer. J. trop. Med. Hyg.*, **7**, 199

Hawking, F. & Gammage, K. (1962) *Trans. roy. Soc. trop. Med. Hyg.*, **56**, 263

Moore, D. V. & Lanier, J. E. (1961) *Amer. J. trop. Med. Hyg.*, **10**, 5

Powell, R. D., Brewer, G. J. & Alving, A. S. (1963) *Amer. J. trop. Med. Hyg.*, **12**, 509

Ray, A. P., Singh, N., Sen Gupta, G. P. & Misra, B. B. (1959) *J. Indian med. Ass.*, **32**, 398

Rodrigues, D. C. (1961) *Arch. Hig. (S. Paulo)*, **26**, 231

World Health Organization, Technical Meeting on Chemotherapy of Malaria (1961) *Wld Hlth Org. techn. Rep. Ser.*, **226**

Young, M. D. (1961) *Amer. J. trop. Med. Hyg.*, **10**, 689

Young, M. D. (1962) *Trans. roy. Soc. trop. Med. Hyg.*, **56**, 252

Young, M. D., Contacos, P. G., Stitcher, J. E. & Millar, J. W. (1963) *Amer. J. trop. Med. Hyg.*, **12**, 305

Young, M. D. & Moore, D. V. (1961) *Amer. J. trop. Med. Hyg.*, **10**, 317