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Research Article

STUDIES ON THE CHEMOTHERAPY OF THE HUMAN MALARIAS. I. METHOD FOR THE QUANTITATIVE ASSAY OF SUPPRESSIVE ANTIMALARIAL ACTION IN VIVAX MALARIA [1, 2, 3]

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INTRODUCTION

The wartime program for the development of new antimalarial drugs made apparent the need for satisfactory techniques for the assay of their activities in the human malarias. It seemed likely that the use of blood-induced malaria would partly satisfy this need. Information which has accrued since the beginning of these studies now permits the interpretation of results obtained with this type of infection in terms of a reasonable hypothesis concerning the biology of the malarias.

It is now believed that in mosquito-transmitted vivax malaria the sporozoites are localized initially in macrophage cells where they undergo growth, segmentation, and sporulation. This initial tissue phase yields forms of the plasmodia capable of invading and multiplying in the erythrocytes, thereby initiating a clinical attack of malaria. A portion of the tissue phase is believed to persist and to release periodically forms capable of erythrocytic invasion, thereby being responsible for the repeated, true relapses of vivax malaria. On the other hand, it is now quite clear that vivax malaria induced by the simple transfer of parasitized blood is devoid of a tissue phase and, therefore, does not relapse.

In accordance with this general view of the biology of vivax malaria, there are at least three different types of antimalarial activity (1). Activity is considered to be *prophylactic* if the action is exerted against the sporozoites or the parasites of the initial tissue phase of the disease; *suppressive,* if against the parasites of the asexual erythrocytic phase of the disease; and *curative,* if against the parasites of the persisting tissue phase. Since the primary need in wartime was for agents with suppressive activity, *i.e.,* with a quinine- or quinacrine-like action, it was believed that the blood-induced infection, consisting entirely of the erythrocytic phase of the plasmodium, afforded the best test object with which to evaluate this type of antimalarial activity.

Therefore, studies were undertaken to determine whether blood-induced malaria is suitable for the quantitative appraisal of drug activity, to compare the susceptibility of erythrocytic parasites derived from simple blood transfer and from sporozoite inoculation, and to determine whether information obtained with a single strain of plasmodium is applicable to problems involving the treatment of malaria due to other strains.

BLOOD-INDUCED MCCOY STRAIN VIVAX MALARIA

Material and methods

The comparative assay of potential antimalarial drugs requires a highly standardized infection and testing procedure. The reliability of the therapeutic test depends largely upon a uniform host-susceptibility and the use of

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[2] A part of the material in this paper has been presented in a Harvey Lecture by Dr. James A. Shannon (1) and also appeared in "Survey of Antimalarial Drugs, 1941–1945," p. 177, J. W. Edwards, Ann Arbor, Mich., 1946. Permission to use Tables I, II, IV and VI and Figures 1, 2, 3 and 4, has been obtained from the Harvey Society and from the editors of the Survey.

[3] The authors express their thanks to the Malaria Study Section of the National Institute of Health for editorial assistance and for arrangements in regard to the publication of this paper. They are also grateful to the Abbott Laboratories, E. I. du Pont de Nemours and Company, Inc., E. R. Squibb and Sons, Eli Lilly and Company, Sharp and Dohme, and Wyeth, Inc., for contributing toward the publication costs.

an infective organism with stable characteristics. These considerations are important in the selection of experimental subjects and in the design of the therapeutic test.

The subjects used were individuals with central nervous system syphilis who presented no medical contraindications to induced malaria. Special attention was directed, in obtaining the history, to the place of birth, racial extraction, residence in malarious areas, and previous malaria. Only those white patients who had never lived in a malarious area and who gave no history of previous malaria were considered suitable for vivax infections. These criteria specifically exclude all colored races and all persons born in Italy, the Eastern Mediterranean countries, Central and South America, and the West Indies, in addition to other recognized malarious areas.

These studies were performed with the McCoy strain of *P. vivax* originally isolated by Boyd in 1931 (2). The character of the infection which it produces has been the subject of extensive studies by Boyd and his associates (3). The off-shoot of the strain used in the present work has been in continuous human passage by blood transfer since 1936.

To insure maximal stability of the strain in its virulence and response to chemotherapeutic agents, its passage was restricted to individuals presumed to be completely susceptible and was performed before the beginning of therapy, *i.e.*, before the fourth or fifth day after the onset of fever. Exposure of the strain to acquired immune bodies in the host, which might in time modify the characteristics of the parasite, was thereby minimized. Transfer prior to therapy removed the theoretical hazard of developing drug resistance.

Biological characteristics of blood-induced McCoy vivax malaria

The biological characteristics of blood-induced McCoy vivax malaria will be described briefly in terms of parasitemia and fever. The number of parasites in the circulating blood is generally too low, during the first few days after inoculation with 500,000 parasites, to permit their ready demonstration by the usual thick blood smear technique. This *prepatent* period varies from two to 12 days, with an average of about six days. The onset of fever usually bears a close relationship with the appearance of a demonstrable number of parasites in the blood. Thus, on the first day of fever above 100.6°, a negative thick smear is found in about one-quarter of non-immune individuals and the parasite count is only occasionally above 1000 per cu. mm.

The first week of a primary attack is characterized by an irregular, sustained fever of moderate degree and a rapidly increasing density of parasites in the blood. By the fourth day of fever, a negative thick smear is a rare finding and approximately one-half of the individuals show parasite counts in excess of 1000 per cu. mm.

During the second week of an uninterrupted infection, the fever assumes the usual intermittent pattern of malaria with regular tertian or quotidian paroxysms. The schizogonous cycle covers a period of 40 hours. The parasite count tends to become relatively stable between 2000 to 10,000 per cu. mm., although it has been observed to rise to as high as 75,000.

The spontaneous termination of fever in the uninterrupted infection usually occurs between the 12th and 25th days after the onset, the average being 17 days. Although the parasite count may fall rather rapidly at the time of spontaneous termination, positive thick blood smears persist for a period of days or weeks thereafter. Spontaneous termination has not been observed earlier than the 12th day in any white patient with a completely negative history of previous contact with the disease.

Therapeutic interruption of the infection early in its course may modify, in certain respects, a second experience with the disease. The onset of fever during a recrudescence, or following reinoculation, usually coincides with a higher density of parasites than was the case during the primary attack. This increased tolerance to the parasite may be interpreted as a manifestation of immunity acquired during the initial bout of fever. It would appear that the development of immunity during a period of clinical latency following therapy is not sufficient to render the patient resistant to a second infection, since interruptions up to 30 days in length do not diminish the average total duration of fever by more than two or three days.

Routine of the therapeutic test

Each subject was inoculated with 500,000 parasites from a patient in the fourth or fifth day of fever. Blood smears were obtained daily throughout the period of observation. The usual thick blood smears were used until the parasite count reached approximately 50 per cu. mm. Thereafter, a technique (4) involving the delivery of a definite volume of blood onto a measured area of the slide was utilized.

Therapy was started on the fourth or fifth day after the onset of fever above 100.6° (rectal). The test drug was administered by a regimen designed to achieve a fairly stable plasma drug concentration of the desired level for four days. Each course of therapy was in-

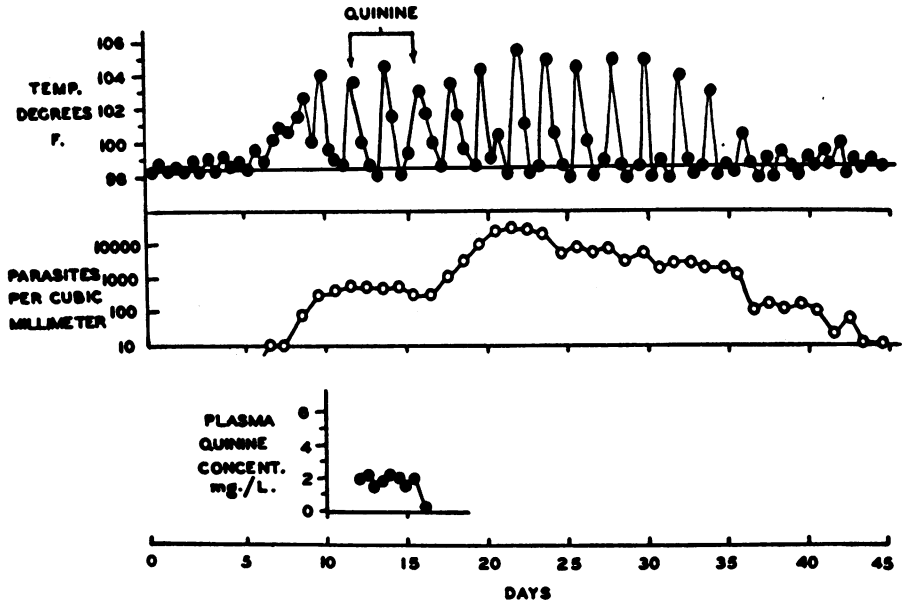

FIG. 1. CLASS I RESULT—NO EFFECT

itiated by a priming dose and continued with smaller doses at four- to six-hour intervals; all doses are recorded in terms of quinine base. Blood samples for the estimation of the plasma quinine concentration (5) were obtained at sufficiently short intervals to permit an appraisal of the mean concentration each day. The mean plasma drug concentrations referred to throughout this paper are the averages of the individual daily mean concentrations.

The observation period subsequent to therapy began on the day following the last effective plasma drug level. In the event of a complete disappearance of parasites and fever, the patient was observed for at least 14 days. If, at the end of this period, there had been no evidence of renewed activity of the infection, reinoculation with one million parasites was performed and the patient followed for an additional 14 days.

Classification of therapeutic results

The therapeutic results have been classified in three groups on the basis of the course of the parasitemia and fever following therapy.

Class I (No effect). The results falling into this group are those in which the administration of drug had no certain effect upon either the parasite density in the blood or the course of the febrile paroxysms (Figure 1).

Class II (Temporary effect). This group includes those results in which there was at least a temporary, partial, or complete, suppression of parasitemia or fever. Disappearance of fever and parasites, followed by spontaneous recurrence within 14 days after the last effective plasma drug level, was considered to be a complete, temporary effect (Figure 2). If the parasite count on the fifth day after starting therapy was less than half the maximum count during drug administration, and subsequently increased, the result was classified as a partial effect. A reduction of the febrile elevation to less than half of that of the preceding and subsequent paroxysms was also considered to be a partial effect.

Class III (Permanent effect). The therapeutic results fall into this group when parasites were absent from thick blood smears for at least 14 days after the last effective plasma drug level and when continued susceptibility of the host to the infection was established by the appearance of parasitemia and fever following reinoculation (Figure 3).[4]

[4] A review of the data on patients in whom reinoculation was delayed beyond the usual observation period indicates that an occasional individual may manifest a spontaneous recurrence after the 14th day. Since prepatent periods of less than two days have not been observed in patients receiving inocula of the size used in these studies, it has been assumed that parasitemia observed within 48 hours after reinoculation can be attributed to parasites persisting from the primary attack. These cases have therefore been placed in the Class II category of therapeutic effects. Such an occurrence has not been sufficiently frequent to prejudice the data on any given drug.

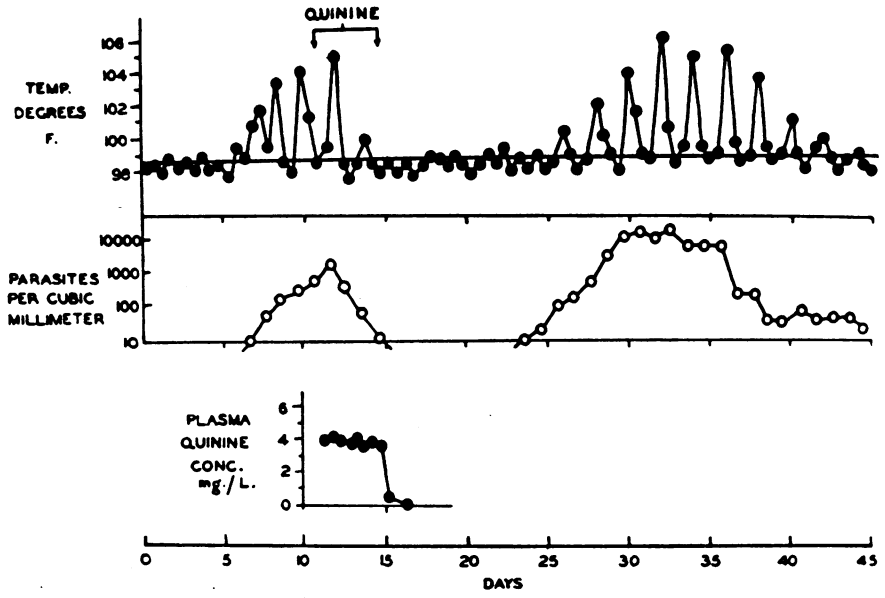

FIG. 2. CLASS II RESULT—COMPLETE TEMPORARY EFFECT

The susceptibility of blood-induced McCoy vivax malaria to quinine

The first series of observations (1942) involved the administration of quinine to 12 patients with blood-induced McCoy strain vivax malaria. The experimental results are summarized in Table I. The mean plasma quinine concentrations ranged from 1.4 to 6.2 mg. per liter. On the basis of the criteria established for this test, it was found that plasma concentrations of 2.0 mg. per liter or less exerted no certain effect upon the course of parasitemia or fever (Class I). On the other hand, mean concentrations of 5.0 mg. per liter or higher consistently produced a permanent interruption of the infection (Class III). Plasma concentrations between 2.0 and 5.0 mg. per liter resulted in

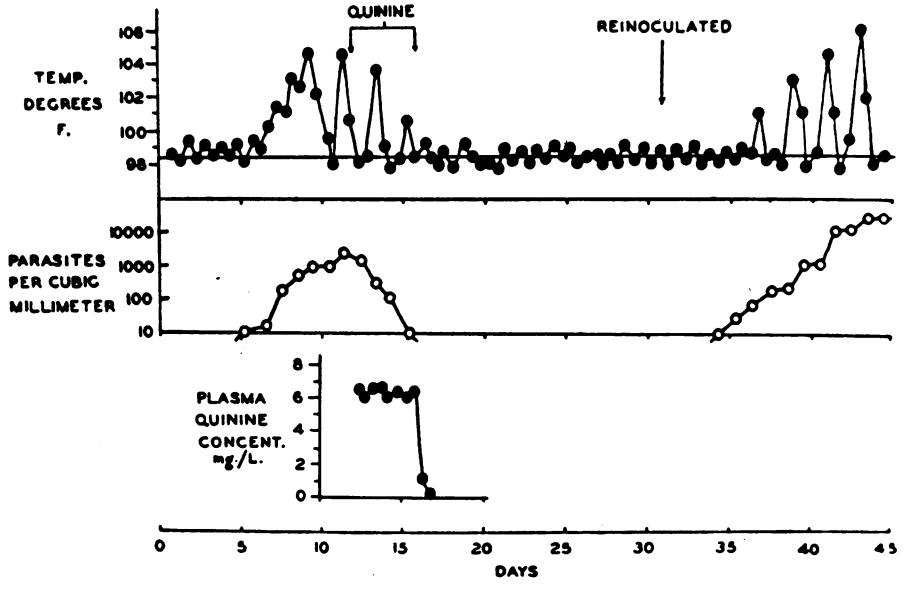

FIG. 3. CLASS III RESULT—COMPLETE PERMANENT EFFECT, POSITIVE REINOCULATION

TABLE I

The relationship between dosage and plasma concentration of quinine and therapeutic effect in four-day tests against blood-induced McCoy vivax malaria

1942 series

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Dia	0.30	6.2			x
Pol	0.30	5.8			x
Mes	0.30	5.1			x
Fat	0.20	4.3			x
Bai	0.30	3.9		x	
Lyn	0.17	3.7		x	
Def	0.24	3.0		x	
Gol	0.10	2.5		x	
Mac	0.10	2.3		x	
Sic	0.10	2.2	x		
Sha	0.10	1.8	x		
Car	0.10	1.4	x		

Class II effects, temporary suppression or interruption of parasitemia and fever.

A series of similar observations was obtained in 18 subjects one and a half years later (Table II). The same critical plasma quinine concentrations were found to divide the three classes of therapeutic effect. These data indicate that the susceptibility to quinine is a stable characteristic of the infective organism.

TABLE II

The relationship between dosage and plasma concentration of quinine and therapeutic effect in four-day tests against blood-induced McCoy vivax malaria

1943 series

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Irr	0.30	8.9			x
Gue	0.30	8.5			x
Klo	0.47	6.5			x
Gre	0.47	6.1			x
Set	0.30	6.1			x
Bal	0.38	5.0			x
Pin	0.30	5.0		x	
Ker	0.39	4.1			x
Mau	0.30	3.5		x	
Tar	0.30	3.4		x	
Hop	0.36	3.0		x	
Tsu	0.10	2.9		x	
Mor	0.10	2.9		x	
Don	0.30	2.9		x	
And	0.43	2.9		x	
Cic	0.30	2.8		x	
Hun	0.10	1.9	x		
Jam	0.10	1.2	x		

The relationship between mean plasma quinine concentration, maintained for four days, and the therapeutic result is sufficiently consistent to permit the definition of a critical plasma drug concentration above which permanent interruption of the erythrocytic phase may be expected. The minimal quinine concentration capable of producing a detectable suppressive action may also be defined from data obtained with this testing procedure.

The correlation between the daily oral dose of quinine and the therapeutic effect is not as close

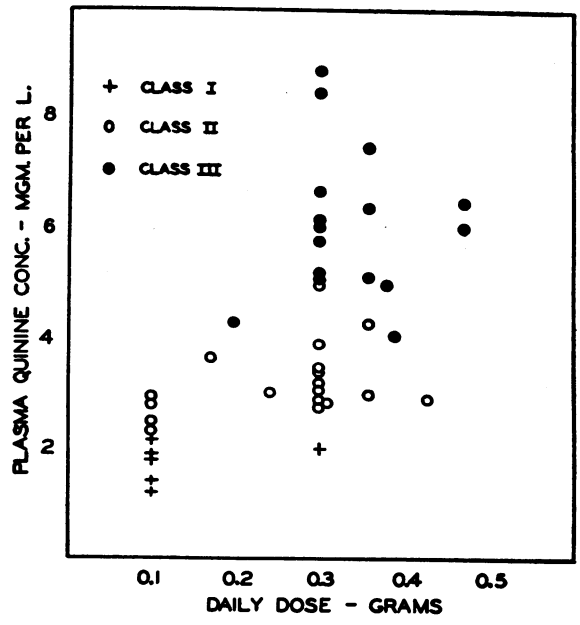

FIG. 4. RELATION BETWEEN DAILY ORAL DOSE AND MEAN PLASMA QUININE LEVELS AND EFFECT IN FOUR-DAY TESTS AGAINST BLOOD-INDUCED VIVAX (McCOY STRAIN) MALARIA

as that between mean plasma quinine level and effect (Figure 4). This is presumably the result of the considerable variation in plasma quinine concentrations achieved by different individuals receiving the same dosage regimen. It may be noted, in Figure 4, that all three classes of therapeutic effect were obtained with total daily doses of 0.3 gram, the response being dependent upon the plasma quinine concentration achieved. It is apparent that a quantitative appraisal of the antimalarial activity of quinine is more readily obtained on the basis of the mean plasma quinine

concentration than on the basis of oral dosage.

The relationship between the class of therapeutic effect and the mean plasma drug concentration is independent of the parasite density at the beginning of therapy (Table III). Thus, a given drug concentration produces the same therapeutic effect whether the initial parasite count is low or high in otherwise comparable individuals.

MOSQUITO-INDUCED MCCOY STRAIN VIVAX MALARIA

Several investigators have expressed the belief that blood-induced malaria is more susceptible to therapy than is naturally-acquired or sporozoite-induced malaria (6). Consequently, it was important to determine whether the erythrocytic phase of a given strain of *P. vivax* has the same susceptibility to chemotherapeutic agents when established by the injection of infected blood as when derived from the underlying tissue phase of the mosquito-induced infection. Were this true, the suppressive action of an antimalarial agent, as tested against blood-induced vivax malaria, would be a correct measure of its suppressive activity in the mosquito-induced disease.

TABLE III

Distribution of 109 consecutive subjects by the parasite count at the start of therapy and by the therapeutic effect obtained

Parasite count at start of therapy	Number of subjects with therapeutic effect			
	Class I	Class II	Class III	Total
per cu. mm.				
1–100	8	11	8	27
101–1000	10	15	10	35
1001–10,000	11	18	8	37
>10,000	1	6	3	10
Total	30	50	29	109

The selection of patients, the technique for testing for antimalarial activity, and the criteria for interpreting results were the same for sporozoite-induced malaria as was described for the blood-induced infection, except for the mode of infection and the time of observation before reinoculation. Malaria was induced by the bites of *A. quadrimaculatus* mosquitoes infected with the McCoy strain of *P. vivax*.[5] This strain had been transmitted by mosquitoes for several years prior to this study. Mosquitoes were applied between four and 14 days after becoming gland positive. Subsequent to biting, the salivary glands of the mosquitoes were removed by dissection and examined for the presence of sporozoites. The density of gland infections was recorded on the basis of 1 to 4 plus. The inoculum indicated in Table IV represents the summation of the infection-densities of the mosquitoes biting each patient. Reinoculation was performed after an observation period of 21 days rather than the 14-day period utilized in the blood-induced infections. Infected blood from patients with mosquito-induced McCoy vivax malaria was used for the reinoculations.

TABLE IV

The relationship between dosage and plasma concentration of quinine and therapeutic effect in four-day tests against mosquito-induced McCoy vivax malaria

Patient	Inoculum*	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect			Interval between inoculation and first true relapse
				I	II	III	
		grams (base)	*mg./L*				*days*
Fad	5+	1.44	13.1			x	
Bla	2+	1.44	10.7			x	
Dav	10+	1.44	9.8			x	247
Kas	4+	1.44	9.3			x	244
O'B	3+	1.44	8.6			x	276
Sch	11+	0.36	7.5			x	
Lon	15+	0.30	6.7			x	260
Dot	7+	0.36	6.4			x	
Koz	9+	0.30	5.2			x	261
Sfm	7+	0.36	5.1			x	
Sma	8+	0.36	4.3		x		234
Hol	15+	0.30	3.2		x		264
Pol	15+	0.30	3.1		x		
Kin	14+	0.30	2.9		x		
Bue	11+	0.30	2.0	x			167

* Summation of individual mosquito infection densities.

The relationship between plasma quinine concentration and therapeutic effect in sporozoite-induced McCoy vivax malaria was examined in 15 susceptible individuals (Table IV). The plasma quinine level which, when maintained for four days, results in Class III effects, was 5.0 mg. per liter. Quinine concentrations between 3.0 and 5.0 mg. per liter produced Class II effects, while there was no effect in one patient who had a mean plasma quinine concentration of 2.0 mg. per liter. Reinoculation after a parasite-free period of 21 days was invariably successful.[6] The short time (two to nine days) between reinoculation and parasitemia and clinical malaria supports the

[5] The infections were originally established on this Service by infected mosquitoes kindly furnished by Doctor Robert B. Watson.

[6] Contrary to experience with avian malarias, it is possible to produce an acute attack by the inoculation of erythrocytic parasites in patients who have persisting infections unaccompanied by demonstrable parasitemia.

belief that these recurrences were the result of the reinoculation and not true relapses of the original mosquito-induced malaria. The time of occurrence of subsequent true relapse is indicated in the last column of Table IV.

It was shown in the preceding section that the critical plasma quinine concentration for Class III effects is 5 mg. per liter for blood-induced McCoy vivax malaria in the standard therapeutic test. The mosquito-induced infection, when tested in a similar fashion, was found to yield Class III effects at essentially the same plasma quinine level. This level, which permanently interrupts the blood-induced infection, does not prevent the later relapses which are characteristic of mosquito-induced infections. These results are in accord with the belief that, unlike the blood-induced infection, mosquito-induced malaria is characterized by a persisting tissue phase which is not materially affected by quinine and which is capable of producing relapses.

The suppressive antimalarial activity of quinine thus would appear to be identical in blood- and mosquito-induced infections. It is logical to assume that a similar situation obtains in the case of other suppressive drugs.

SOUTH PACIFIC (CHESSON STRAIN) VIVAX MALARIA

Blood-induced infections

It has been shown that the erythrocytic phases of both blood- and sporozoite-induced vivax malaria of the same strain (McCoy) have the same susceptibilities to quinine. However, there is reason to believe that the erythrocytic phases of malaria due to other strains of *P. vivax* have different susceptibilities to chemotherapeutic agents (7). Therefore, the suppressive antimalarial effect of various plasma quinine concentrations against another strain of *P. vivax* was studied. This strain (Chesson), obtained from a soldier who contracted malaria in New Guinea in 1944, produces an infection characterized by frequent, repeated relapses which occur as early as one week after the termination of a full course of quinine therapy (8, 9).

Except for the strain of parasite used and the time of reinoculation, the routine of the therapeutic test was the same as that described for the blood-induced McCoy strain vivax infections.

TABLE V

The relationship between dosage and plasma concentration of quinine and therapeutic effect in four-day tests against blood-induced Chesson vivax malaria

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Lyn	1.50	12.8		x	
Ruf	1.50	12.5		x	
War	1.50	11.9			x
Hop	1.50	11.2			x
Wil	1.50	10.4			x
McP	0.36	5.5		x	
Kup	0.36	5.1		x	

The effect of four days of quinine therapy against blood-induced Chesson vivax malaria was studied in seven patients (Table V). It is apparent that the highest plasma quinine concentrations which can be achieved without undue discomfort to the patient do not consistently result in Class III, or "permanent," effects. Furthermore, two patients with mean plasma quinine concentrations of 5.5 and 5.1 mg. per liter, respectively, had only the slightest detectable effects. Plasma quinine levels of this magnitude, when maintained for four days, invariably result in Class III effects with the McCoy strain of *P. vivax*. Therefore, the duration of therapy was extended to six days and the effect of quinine examined in 10 patients (Table VI). Under these conditions, it was found that the critical plasma

TABLE VI

The relationship between dosage and plasma concentration of quinine and therapeutic effect in six-day tests against blood-induced Chesson vivax malaria

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Des	1.50	14.6			x
Sei	1.50	12.8			x
Ger	1.90	12.6			x
McL	1.90	11.8			x
Wen	0.70	11.5			x
Hur	1.50	8.6			x
Meh	1.90	8.0			x
Anz	0.30	6.6		x	
Oak	0.70	6.0		x	
Per	0.70	3.6		x	

quinine concentration for Class III effects falls in the range of 7 to 8 mg. per liter.

The data presented demonstrate that the erythrocytic phase of Chesson strain vivax is considerably more resistant to the action of quinine than is that of McCoy strain vivax. The complete eradication of the erythrocytic phase requires, on the average, a daily dosage of 2 grams quinine sulfate (equals 1.6 grams quinine base) for a minimum period of six days.

Mosquito-induced infections

The suppressive action of quinine could not be quantified in sporozoite-induced Chesson vivax malaria, since relapses occur during the usual period of observation preceding reinoculation. That the early recurrences of clinical activity following the termination of suppressive therapy are true relapses, and not simple recrudescences due to inadequate therapy, is indicated by the data in Table VII. All of these patients received quinine therapy considerably in excess of that required to eradicate the erythrocytic forms in blood-induced Chesson strain infections. Thus, in dealing with strains which characteristically produce relapses soon after termination of therapy with suppressive drugs, the use of blood-induced malaria is of special value in determining the resistance of the erythrocytic phase to chemotherapy.

DISCUSSION

The data presented indicate that the susceptibility of the erythrocytic phase of the plasmodium to quinine can be quantitatively and reproducibly described. A wide gradation of clinical response has been demonstrated to bear a striking relationship to the mean plasma quinine concentration maintained during the therapeutic period. Of greater importance is the fact that the use of plasma quinine concentrations permits a precise definition of antimalarial activity with relatively few experimental subjects. *A priori,* one may expect a similar situation to obtain in the examination of the suppressive activities of other agents.

TABLE VII

Interval between treatment and relapse in mosquito-induced Chesson vivax malaria treated with quinine

Number of patients	12
Daily dose of quinine	1.5 grams (base)
Duration of therapy	16 days
Interval from end of therapy to appearance of parasitemia	*days*
Primary attack	
Range	5–9
Mean	8
First relapse	
Range	6–13
Mean	9
Second relapse	
Range	6–18
Mean	12

It has been demonstrated that the erythrocytic forms derived from blood- and sporozoite-induced infections possess an equal susceptibility to quinine. This finding implies that the susceptibility of erythrocytic forms to quinine is a stable characteristic of the strain of the plasmodium. The long-accepted belief that blood-induced malaria is unusually susceptible to chemotherapeutic agents would appear to be the result of studies in which inadequate consideration was given to the degree of acquired immunity in experimental subjects, the differentiation of simple recrudescence from true relapse, and possibly inherent differences in various strains of the same species. Observations with a second strain of *P. vivax* (Chesson) reveal that it has a much greater resistance to quinine than the McCoy strain.

Important features of the standard therapeutic test which require some comment are the control exercised over the variable of natural and acquired immunity, the duration of therapy, and selection of the time for reinoculation. In order to exclude immunity as an important determining factor in the experimental results, several measures were taken in the design of the routine testing procedure: (*1*) observations were restricted to individuals presumed to be completely susceptible; (*2*) treatment with the test drug was begun early in the course of the disease, *i.e.,* within five days after the onset of fever; and (*3*) a reinoculation procedure was utilized to test for continuing susceptibility in those persons who showed an apparently permanent therapeutic effect.

A stable plasma concentration of the test drug is maintained for four days in order that the period of therapy may encompass two complete cycles of schizogony. Although four days of therapy may be insufficient to interrupt permanently blood-induced infections due to all strains of *P. vivax,* it permits a wide gradation of thera-

peutic response and provides a basis for the comparative assay of other agents.

Selection of the time for reinoculation was based on the observation that, in blood-induced McCoy vivax, the spontaneous reappearance of parasites almost invariably occurs within 14 days after the last effective plasma drug level, if such a recrudescence is to be expected. The duration of the observation period which is necessary to separate Class II from Class III effects varies from strain to strain in any species of plasmodium as well as from species to species. With drugs that persist in the body, the interval beween the termination of therapy and reinoculation must be extended to 14 days after the plasma drug concentration has reached a level known to have no therapeutic effect.

SUMMARY

1. A standard procedure suitable for the quantitative appraisal of drug activity in blood-induced vivax malaria has been described. The close relationship between plasma quinine concentration and therapeutic response makes possible the quantitative appraisal of drug activity with a limited number of experimental subjects.

2. By means of this procedure, it has been possible to demonstrate that the quinine-susceptibility of the erythrocytic phase of vivax malaria is a stable strain characteristic and that it is independent of the mode by which the malaria is transmitted.

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