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Antimalarial Activities of Various 9-Phenanthrenemethanols with Special Attention to WR-122,455 and WR-171,669†

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Pilot appraisals of the activities of 16 specially selected 9-phenanthrenemethanols against acute infections with *Plasmodium falciparum* in owl monkeys showed that all were more active than the reference compound, WR-33,063. WR-122,455, the most active derivative, and WR-171,669, ranked sixth, were selected for study in human volunteers. To assist this undertaking, appraisals of both compounds in owl monkeys infected with various strains of *P. falciparum* were expanded. These assessments showed: (i) that WR-122,455 was four times as active as chloroquine against infections with chloroquine-sensitive strains and that WR-171,669 equalled chloroquine in activity; (ii) that these compounds were fully active against infections with strains resistant to chloroquine, pyrimethamine, or quinine, or to all three standard drugs; (iii) that the activity of WR-122,455 was a function of total dose, single doses being as effective as the same amounts delivered in three or seven daily fractions; and (iv) that a single dose of WR-122,455 conferred extended, although only partial, protection against challenges with trophozoites. Complementary experiments in rhesus monkeys inoculated with sporozoites of *P. cynomolgi* showed that the activity of WR-122,455 was limited to blood schizonts and did not extend to early or late tissue schizonts. These evaluations were compatible with the results of preliminary studies of the activities of WR-122,455 and WR-171,669 in human volunteers.

The 9-phenanthrenemethanols are one of three classes of aminoalcohols examined in depth in the organized search for new agents effective against infections with strains of *Plasmodium falciparum* resistant to known antimalarial drugs, especially chloroquine (one of the major goals of the current Malaria Research Program, sponsored by the U.S. Army Medical Research and Development Command, organized and coordinated by the Walter Reed Army Institute of Research [WRAIR]). Approximately 275 of these phenanthrene derivatives were acquired between 1963 and 1975 (personal communication, T. R. Sweeney, WRAIR, Washington, D.C.) and were submitted to the Rane Laboratory, University of Miami, Miami, Florida, for evaluation in mice infected with *Plasmodium berghei* (18). A significant number exhibited life-prolonging and/or curative properties in this rodent malaria model. Sixteen of the most promising derivatives, together with a reference compound, WR-33,063 (designated SN-13,465 when prepared for the World War II Malaria Chemotherapy Program [41]), were submitted to our laboratory for pilot appraisals of activities against infections with chloroquine-resistant and pyrimethamine-resistant strains of *P. falciparum* in owl monkeys. These examinations showed that, as a class, the 9-phenanthrenemethanols were fully effective in the face of resistance to the above drugs and identified six derivatives which, as measured by curative doses, were at least as active as chloroquine against infections with a chloroquine-susceptible strain of *P. falciparum*.

The current report records the results of the pilot evaluations referred to above. It also presents the results of broader assessments of the antimalarial properties of two members of the pilot group, WR-122,455 and WR-171,669, the most active of the 9-phenanthrenemethanols with, respectively, 2-piperidyl and alkylaminoalkyl substituents on the 9-methanol (see Table 1 for structures). These assessments were undertaken to support and guide projected trials of WR-122,455 and WR-171,669 in human volunteers. They were aimed at strengthening the pilot appraisals, determining the influence of the dosage regimen on the activities of both compounds, and ascertaining whether WR-122,455 had tissue schizonticidal as well as blood schizonticidal activity.

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MATERIALS AND METHODS

Infections with *P. falciparum* in owl monkeys. (i) General. The majority of the experimental procedures used in the current study were identical with those employed and described in detail in investigations already published. These reports should be referred to for descriptions of: (a) the methods for acquiring, importing, conditioning, caging, and feeding owl monkeys, ascertaining their freedom from naturally acquired filarial or malarial infections, and maintaining and handling them during the various phases of drug evaluation (25, 35); (b) the patient origins of the Vietnam Oak Knoll, Vietnam Monterey, Malayan Camp-CH/Q, Uganda Palo Alto, and Vietnam Smith strains of *P. falciparum* employed in the current study, the backgrounds of these strains prior to use in this laboratory, their adaptation to growth in owl monkeys with intact spleens and routine maintenance in such subjects, and their capacities to produce progressive disease in untreated monkeys (26); (c) the responses of standardized infections with the above strains to treatment with chloroquine, pyrimethamine, and quinine (25, 27) and the procedures utilized for initiating infections, following parasitic events in treated monkeys, and classifying therapeutic responses (25, 27, 35); (d) the design of pilot evaluations of the activities of new test compounds, the dimensions of information derived therefrom, and the reliability of such appraisals (25, 28); and (e) the progressive changes in use of individual strains for assessing activities of new test compounds and the reasons therefor (29).

(ii) Monkeys. Five hundred thirty-two subadult owl monkeys (*Aotus trivirgatus griseimembra*) of northern Colombian origin were used in the investigations summarized in this report. This total was made up of essentially equal numbers of males and females, ranging from 800 to 1,050 g in weight at time of assignment to various experiments. Two hundred eighty-nine were committed to pilot studies and 206 to expanded evaluations of the activities of WR-122,455 and WR-171,669. The remaining 37 monkeys served either as untreated or standard-drug-treated (chloroquine or pyrimethamine) controls.

(iii) Test agents and their administration. The structure of each of the 17 9-phenanthrenemethanol derivatives accorded pilot appraisals, the identity of the laboratory that reported the original synthesis, and the source of the preparation used in current studies have been set forth in Table 1. All compounds were supplied as monohydrochloride salts. Doses delivered to both owl monkeys and rhesus monkeys were calculated as base equivalents.

All of the phenanthrenemethanol hydrochlorides used in the current studies had limited water solubilities, necessitating administration of the agents as aqueous suspensions. These were routinely prepared as follows. An amount of the test compound, 5% in excess of the total base equivalent required for treatment of all subjects under study, was placed in a smooth glass mortar of 2-ounce (ca. 59 ml) capacity, wetted with Tween 80 (0.05 ml for each 50.0 mg of compound), and ground with a smooth glass pestle until an even mixture of test material and dispersing agent was obtained. The resulting paste was ground thoroughly with a small volume of distilled water (1.0 ml per 50.0 mg of compound) and diluted with the same under continual grinding until a final concentration of 1.0 to 20.0 mg of test compound per ml was attained. The quantities of this suspension required for treatment of individual monkeys were measured into Erlenmeyer flasks, diluted to 10.0-ml volumes with distilled water, and delivered to assignees via stomach tube within 20 min of preparation, using a technique detailed elsewhere (25, 27, 35). Suspensions were stable during this time interval.

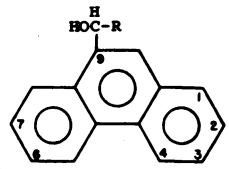
(iv) Expanded evaluations of the activities of WR-122,455 and WR-171,669. Interest in WR-122,455 grew out of two pilot assessments of the activities of eight specially selected agents undertaken in August and September 1969 (28), one against infections with the chloroquine-resistant, relatively pyrimethamine-susceptible Vietnam Monterey strain, the other against infections with the chloroquine-susceptible, pyrimethamine-resistant Uganda Palo Alto strain. The group of test compounds also included another phenanthrenemethanol, WR-33,063 (see Table 1), then undergoing study in human volunteers (2). The results of these evaluations showed that WR-122,455 was equally active against infections with both of the above strains, that it was fully 40 times as active as WR-33,063, and that it was at least as active as chloroquine against infections with the 4-aminoquinoline-susceptible Uganda Palo Alto strain. The remarkable activity of WR-122,455 was fully confirmed in March and April, 1970, in pilot assessments against infections with the chloroquine-resistant, fully pyrimethamine-susceptible Vietnam Oak Knoll strain and the chloroquine-susceptible, pyrimethamine-resistant Malayan Camp-CH/Q strain, which for reasons related elsewhere (29) had replaced the Monterey and Palo Alto strains in our program.

The above findings, coupled with the favorable results of the ongoing evaluation of WR-33,063 (2), led those responsible for clinical studies to conclude that WR-122,455 merited examination in human volunteers. Additional studies of this phenanthrenemethanol in owl monkeys infected with various strains of *P. falciparum* were undertaken to support this examination and assist with its design. These included a pilot assessment of the activity of WR-122,455 against infections with the multidrug-resistant Vietnam Smith strain and a side-by-side comparison of the efficacies of single-dose and three- and seven-consecutive-daily-dose oral treatment regimens against infections with the Vietnam Oak Knoll and Malayan Camp-CH/Q strains. Procedures described previously (29) were utilized in the latter studies.

The duration of protection against infection achieved with a single 35.0-mg/kg dose of WR-122,455 was also investigated. This study was stimulated by the observations: (a) that tritium-labeled WR-122,455 was extensively localized in various organs and tissues of the rat and rhesus monkey and (b) that either the parent compound and/or metabolites were excreted at a relatively constant rate in the urine and feces of each of three owl monkeys for at least 20 days after admin-

TABLE 1

CHEMICAL STRUCTURES AND SOURCES OF 9-PHENANTHRENEMETHANOLS EVALUATED FOR ACTIVITIES AGAINST INFECTIONS WITH VARIOUS STRAINS OF *PLASMODIUM FALCIPARUM*



Compound WR-No.*	Substituents on Position of Phenanthrene Nucleus						R on 9-methanol
	1	2	3	4	6	7	
33,063** (13)[a]	-	-	-	-	Br	-	$-CH_2N[(CH_2)_6CH_3]_2$
149,809 (15)[b]	-	Br	-	-	Br	-	
181,018 (15)[b]	-	CF_3	-	CF_3	-	-	$-CH_2N[(CH_2)_2CH_3]_2$
131,834 (14)[b]	-	Cl	-	-	CF_3	-	
122,455† (16)[b]	-	-	CF_3	-	CF_3	-	
165,355† (17)[c]	-	-	CF_3	-	CF_3	-	
143,803 (16)[b]	-	-	CF_3	-	CF_3	-	$-CH_2N[(CH_2)_3CH_3]_2$
175,412 (8)[d]	-	-	CF_3	-	CF_3	-	$-(CH_2)_2NH(CH_2)_3CH_3$
165,533 (8)[d]	-	-	CF_3	-	CF_3	-	$-(CH_2)_2N[(CH_2)_3CH_3]_2$
146,459 (14)[b]	Cl	-	Cl	-	CF_3	-	
173,060 (X)[e]	Cl	-	Cl	-	CF_3	-	$-CH_2NH(CH_2)_3CH_3$
185,020 (X)[e]	Cl	-	Cl	-	CF_3	-	$-CH_2N[(CH_2)_2CH_3]_2$
150,726 (14)[b]	Cl	-	Cl	-	CF_3	-	$-CH_2N[(CH_2)_3CH_3]_2$
190,420 (X)[e]	Cl	-	Cl	-	CF_3	-	$-CH_2-$ 
171,669 (8)[c, d]	Cl	-	Cl	-	CF_3	-	$-(CH_2)_2N[(CH_2)_3CH_3]_2$
148,763 (14)[b]	-	Cl	Cl	-	CF_3	-	
178,979 (15)[b]	-	CF_3	-	CF_3	Cl	Cl	$-CH_2N[(CH_2)_2CH_3]_2$

*Item in parentheses = literature reference to synthesis. (X) = synthesis accomplished by Ash-Stevens, Inc., Detroit, Michigan, not published. Letter superscript refers to source of compound evaluated: a = E. May, National Institutes of Health, Bethesda, Maryland; b = Germantown Laboratories, Philadelphia, Pennsylvania; c = Cordova Chemical Co., Inc., Sacramento, California; d = Stanford Research Institute, Palo Alto, California; e = Ash-Stevens, Inc., Detroit, Michigan.

**WR-33,063 designated SN-13,465 when first synthesized.

†Diastereoisomers: WR-122,455 = erythro- form; WR-165,355 = threo- form.

istration of single 5.0-mg/kg doses of the labeled compound (personal communication, C. C. Smith, College of Medicine, University of Cincinnati, Cincinnati, Ohio). The duration-of-protection experiment involved work with 48 owl monkeys, 36 receiving a single 35.0-mg/kg dose of WR-122,455 orally, 12 serving as untreated controls. Two subgroups of four monkeys, each consisting of three treated and one untreated subject, were challenged with 5×10^6 trophozoites of the Vietnam Oak Knoll or Malayan Camp-CH/Q strain, 1, 7, 14, 21, 28, or 35 days after the above treatment. Parasitological examinations of blood films were initiated the day after challenge and were repeated daily until parasitemias of 10^2 per 10^4 erythrocytes were attained, or for 90 days when blood films were consistently negative. Infections that reached the above parasite density were treated with various doses of WR-122,455 on a 7-day dosage schedule. Subjects that did not exhibit parasitemias during the 90-day postinoculation period were considered to be fully protected.

The decision to expand studies on WR-171,669 rested in part on the results of the pilot appraisals, but to a larger extent on the interest of those responsible for work with human volunteers in evaluating an active 9-phenanthrenemethanol differing from WR-122,455 with respect to substituents on the phenanthrene nucleus and 9-methanol group, especially the latter. WR-171,669 satisfied these requirements (see Table 1). The expanded studies of this agent pursued in support of the above interest were limited in scope, involving work with but 45 monkeys. They included replication of the original pilot appraisals and side-by-side comparison of the efficacies of single-dose and seven-consecutive-daily-dose regimens against infections with the Vietnam Oak Knoll strain.

Infections with *P. cynomolgi* in rhesus monkeys. The prophylactic and radical curative properties of WR-122,455 were examined in rhesus monkeys (*Macaca mulatta*) inoculated with sporozoites of the *B* strain of *Plasmodium cynomolgi*. Ten subadult monkeys, all males, weighing between 4.0 and 4.7 kg at time of inoculation, were committed to these explorations. All 10 were included in the prophylactic component of the study; 6 also served in the radical curative component.

The prophylactic properties of WR-122,455 were examined in two essentially identical experiments. In one, five monkeys were challenged intravenously with 1.3×10^6 sporozoites derived from well-infected *Anopheles freeborni*; in the second, five monkeys were similarly inoculated with 1.5×10^6 sporozoites. In each experiment, one monkey served as an untreated control, one subject received daily doses of 1.0 mg of primaquine per kg of body weight, and three received WR-122,455 in daily doses of 1.25, 5.0, or 20.0 mg/kg. Doses of primaquine and WR-122,455 were delivered the day prior to sporozoite challenge, 2 h before inoculation, and once daily thereafter for 7 days. Parasitological studies were initiated on day 7 postinoculation.

The six recipients of WR-122,455 in the above experiments were also used to assess the radical curative properties of this agent. When parasitemias of 20 to 60 per 10^4 erythrocytes were attained, treatment with this phenanthrenemethanol was initiated at doses of 1.25, 5.0, or 20.0 mg per kg, once daily for 7 consecutive days. Persisting or recurring infections were treated with chloroquine, 5.0 mg of base per kg of body weight, daily for 7 days. As shown previously (33, 34), this procedure makes it possible to distinguish between recrudescences and relapses, and thereby to identify agents with limited blood schizonticidal activity and significant action on tissue schizonts.

The procedures utilized in the above studies were identical with those detailed in comparable chemotherapeutic evaluations (32–34).

RESULTS

Pilot evaluations. (i) Structural characteristics of test compounds. The 17 9-phenanthrenemethanol derivatives submitted for pilot appraisals have been arranged in Table 1 so as to emphasize similarities and differences in substitution on the phenanthrene nucleus. As this table shows, WR-33,063, the reference compound with a bromo- group at position 6, was the only mono-substituted derivative. Eight of the remaining 16 were di-substituted, 7 were tri-substituted, and 1 was tetra-substituted. Six of the eight di-substituted compounds carried trifluoromethyl substituents: in five, at positions 3 and 6; in the sixth agent (WR-181,018), at positions 2 and 4. Six of the seven tri-substituted derivatives had chloro- groups at positions 1 and 3 and a trifluoromethyl group at position 7; the seventh compound (WR-148,763), with a trifluoromethyl group at position 6, had chloro- substituents at positions 2 and 3. It is noteworthy that 16 of the 17 derivatives were 6-substituted, and that 12 of these 16 were also 3-substituted.

Ten of the 17 compounds had monoalkylaminoalkyl or dialkylaminoalkyl substituents at the 9-carbinol (Table 1). The remaining seven had 2-piperidyl substituents; two of these, WR-122,455 and WR-165,355, were diastereoisomers.

(ii) Activities of test compounds. The results of the pilot evaluations have been detailed in Table 2 and summarized and analyzed in Table 3. The latter table records the estimates of the total course doses (seven times the daily doses) of each test compound required to cure 90% of infections with the respective strains of *P. falciparum* (CD_{90}'s). These CD_{90}'s were calculated by the method of Litchfield and Wilcoxon (12). Table 3 also records the CD_{90}'s of the various agents against infections with *P. berghei*, calculated by the same procedure, from data acquired in the Rane Laboratory and made available for current use through the efforts and courtesy of D. E. Davidson and co-workers, WRAIR.

As the data in Table 3 show, the various compounds differed strikingly in activity; CD_{90}'s ranged from approximately 25.0 mg/kg for the

TABLE 2. *Pilot assessments of the activities of various 9-phenanthrenemethanols against infections with chloroquine-resistant and pyrimethamine-resistant strains of P. falciparum*

Compound WR- no.	Strain[a]	Daily dose[b] (mg of base per kg of body wt)	Response to treatment (no. of monkeys)[c]			
			Effect on parasitemia			Cured
			None	Suppressed	Clearance with recrudescence	
33,063	VnOK	50.0	3 (R)			
		100.0			4 (P)	1 (P)
		200.0			3 (P)	1 (R)
	VnM	12.5		1 (P)	1 (P)	
		50.0		1 (R)	2 (P)	1 (R)
		200.0			1 (P)	1 (P)
	MC-CH/Q	50.0	3 (P_2, R)		2 (R)	
		100.0	4 (P_3, R)	1 (P)	2 (P, R)	
		200.0	3 (P_2, R)			1 (P)
	UPA	3.125	2 (P)			
		12.5	2 (P)			
		50.0	2 (P)			
		200.0		1 (P)	1 (P)	
149,809	MC-CH/Q	1.25	2 (P)			
		5.0	1 (P)		3 (P, R_2)	
		20.0			1 (R)[d]	2 (P)
181,018	VnOK	2.5		1 (P)	1 (P)	
		5.0			2 (P)	
		10.0			1 (R)	3 (P_2, R)
		20.0				1 (R)
		40.0				2 (P)
	VnS	2.5	1 (P)	1 (P)		
		5.0		2 (R)		
		10.0		1 (R)	2 (P)	1 (R)
		20.0			1 (R)	2 (R)
		40.0				3 (P_2, R)
131,834	MC-CH/Q	1.25	1 (P)		1 (P)	
		5.0			1 (R)[d]	3 (P_2, R)
		20.0				2 (P)
122,455	VnOK	1.25			3 (P)	
		2.5			2 (P, R)	4 (P_2, R_2)
		5.0				3 (P)
	VnM	0.3125	1 (R)	1 (R)		
		1.25		2 (P)		
		5.0				3 (P_2, R)
		20.0				5 (P_2, R_3)
	MC-CH/Q	1.25		2 (P)	1 (P)	
		2.5			2 (P)	4 (P, R_3)
		5.0				3 (P)
	UPA	0.3125	1 (P)	1 (P)		
		1.25		2 (P)		
		5.0				3 (P_2, R)
		20.0				5 (P_2, R_3)
	VnS	1.25	1 (P)	1 (P)		
		2.5			2 (P, R)	2 (P, R)
		5.0				4 (P_2, R_2)
165,355	VnOK	1.25			2 (P)	1 (P)
		2.5			1 (R)	4 (P_3, R)
		5.0				4 (P_3, R)
		10.0				3 (P)

TABLE 2—*Continued*

Compound WR- no.	Strain[a]	Daily dose[b] (mg of base per kg of body wt)	Response to treatment (no. of monkeys)[c]			
			Effect on parasitemia			Cured
			None	Suppressed	Clearance with recrudescence	
	MC-CH/Q	1.25		3 (P)		
		2.5		2 (R)	1 (R)	3 (P)
		5.0				4 (P_3, R)
		10.0				3 (P)
143,803	VnOK	2.5	1 (P)	1 (P)	1 (P)[d]	
		5.0			2 (R)	
		10.0		1 (R)	2 (P)	1 (P)
		20.0			1 (R)	1 (R)
		40.0			1 (R)	3 (P)
	MC-CH/Q	2.5	1 (P)	2 (P)		
		5.0		1 (R)	2 (R)	
		10.0		2 (P)		2 (R)
		20.0		1 (R)	1 (R)[d]	
		40.0			4 (P_3, R)	
		80.0			1 (R)	2 (R)
175,412	VnOK	5.0	3 (P)			
		10.0		1 (R)	1 (R)	1 (R)
		20.0		1 (R)	1 (P)	3 (P_2, R)
		40.0			1 (R)	1 (R)
		80.0				3 (P)
	MC-CH/Q	5.0	1 (P)	2 (P)		
		10.0		2 (R)	1 (R)	
		20.0			2 (P, R)	3 (P_2, R)
		80.0				3 (P)
165,533	VnOK	5.0			3 (P)	
		10.0			1 (R)	2 (R)
		20.0			1 (P)	3 (P_2, R)
		80.0				3 (P)
	MC-CH/Q	5.0	1 (P)	2 (P)		
		10.0		1 (R)	1 (R)	
		20.0				5 (P_3, R_2)
		80.0				1 (P)
146,459	VnOK	1.25	2 (P)			
		5.0				4 (P_2, R_2)
		20.0				2 (P)
	MC-CH/Q	1.25	2 (P)			
		5.0	1 (P)			3 (P, R_2)
		20.0				2 (P)
173,060	VnOK	1.25	2 (P)			
		5.0	3 (P, R_2)	1 (P)		
		20.0		2 (P)		
	MC-CH/Q	1.25	2 (P)			
		5.0	3 (P_2, R)	1 (R)		
		20.0		2 (P)		
		80.0				2 (R)
185,020	VnOK	2.5	1 (P)	1 (P)		
		10.0		1 (P)	1 (P)	
		40.0				2 (P)
	MC-CH/Q	2.5	1 (P)	1 (P)		
		10.0		3 (P_2, R)		
		40.0				2 (P)

TABLE 2—*Continued*

Compound WR- no.	Strain[a]	Daily dose[b] (mg of base per kg of body wt)	Response to treatment (no. of monkeys)[c]			
			Effect on parasitemia			Cured
			None	Suppressed	Clearance with recrudescence	
150,726	VnOK	1.25	2 (P)			
		5.0	1 (P)	1 (P)		
		20.0		1 (R)		3 (P_2, R)
	MC-CH/Q	1.25	2 (P)			
		5.0	2 (R)	2 (P)		
		10.0	2 (R)			
		20.0			3 (P_2, R)	
190,420	VnOK	1.25	3 (P)			
		5.0	1 (P)		2 (P)	3 (P_2, R)
		20.0			1 (R)	4 (P_3, R)
		40.0				1 (R)
	VnS	1.25	3 (P)			
		5.0	3 (P)			
		20.0			2 (P)	1 (P)
		40.0				3 (R)
171,669	VnOK	2.5			3 (P)	
		5.0			3 (P)	
		10.0			1 (P)	1 (P)
		20.0				3 (P)
	MC-CH/Q	1.25	2 (P)			
		5.0			3 (P, R_2)	1 (P)
		20.0				2 (P)
148,763	VnOK	2.5				3 (P)
		10.0				3 (P)
		40.0				3 (P)
	MC-CH/Q	2.5	1 (P)			2 (P)
		10.0				3 (P)
		40.0				2 (P)
178,979	VnOK	2.5			1 (P)	1 (P)
		5.0				1 (R)
		10.0			1 (P)[d]	1 (P)
		40.0			2 (P)[d]	
	MC-CH/Q	2.5	1 (P)		1 (P)	
		5.0		2 (R)		
		10.0			1 (P)[d]	2 (P, R)
		40.0				2 (P)

[a] VnOK and VnM, Chloroquine-resistant Vietnam Oak Knoll and Vietnam Monterey strains. MC-CH/Q and UPA, Pyrimethamine-resistant Malayan Camp-CH/Q and Uganda Palo Alto strains. VnS, Chloroquine-resistant, pyrimethamine-resistant Vietnam Smith strain.

[b] Dose administered once daily for 7 consecutive days.

[c] Letters in parentheses refer to treatment status of monkey. P, Initial treatment; R, retreatment. Subscripts indicate numbers of subjects in each category.

[d] Death from intercurrent disease prior to completion of follow-up period; no recrudescence prior to death.

two most active agents, WR-122,455 and WR-165,355 (the diastereoisomers), to in excess of 1,400.0 mg/kg for the least active agent, the reference compound WR-33,063. The data also show that 13 of the test compounds were essentially equally active against infections with chloroquine-resistant, pyrimethamine-susceptible and chloroquine-susceptible, pyrimethamine-resistant strains of *P. falciparum*. The positions of three compounds with respect to this issue are not clear; WR-149,809 and WR-131,834 were evaluated against infections with but a single strain; the appraisal of the activity of WR-178,-979 against infections with the chloroquine-re-

TABLE 3

THE ACTIVITIES OF VARIOUS 9-PHENANTHRENEMETHANOLS AGAINST INFECTIONS WITH STRAINS OF PLASMODIUM FALCIPARUM WITH DIVERSE LEVELS OF SUSCEPTIBILITY TO CHLOROQUINE AND PYRIMETHAMINE CONTRASTED WITH THE ACTIVITIES OF THESE AGENTS AGAINST INFECTIONS WITH PLASMODIUM BERGHEI

Compound WR- No.	Approximate CD_{90} - Total Dose Mg Base per Kg Body Weight*					
	Infections with Strains of P. falciparum**					Infections with P. berghei†
	VnOK	VnM	MC-CH/Q	UPA	VnS	
33,063	>1400.0	>1400.0	>1400.0	>1400.0	-	>640.0
149,809	-	-	105.0	-	-	50.0
181,018	100.0	-	-	-	200.0	30.0
131,834	-	-	65.0	-	-	90.0
122,455‡	25.0	25.0	25.0	25.0	25.0	120.0
165,355‡	23.0	-	27.0	-	-	32.0
143,803	350.0	-	700.0	-	-	100.0
175,412	350.0	-	280.0	-	-	10.0
165,533	175.0	-	125.0	-	-	50.0
146,459	27.0	-	50.0	-	-	54.0
173,060	>140.0	-	430.0	-	-	20.0
185,020	220.0	-	220.0	-	-	110.0
150,726	190.0	-	>140.0	-	-	80.0
190,420	140.0	-	-	-	210.0	>300.0
171,669	105.0	-	90.0	-	-	30.0
148,763	<18.0	-	50.0	-	-	80.0
178,979	?	-	105.0	-	-	130.0

*The respective agents were administered orally, once daily for seven days, to monkeys infected with P. falciparum, subcutaneously, in single doses to mice infected with P. berghei.

**Cf footnote, Table 2, for susceptibility of respective strains to chloroquine and pyrimethamine.

†The data on which these calculations were based were acquired by the late Leo Rane and Dora Rane of the Rane Laboratory, University of Miami, Florida, and were made available to us through the courtesy of Ltc. D.E. Davidson, Walter Reed Army Institute of Research.

‡WR-122,455 and WR-165,353 are diastereoisomers.

sistant Oak Knoll strain was compromised by intercurrent disease. The 17th compound, WR-148,763, appeared to be significantly more active against infections with the chloroquine-resistant Oak Knoll strain than against infections with the pyrimethamine-resistant Malayan Camp-CH/Q strain. Confirmation of this aberrant finding would be desirable before concluding that the activity of this compound is either enhanced by chloroquine resistance or prejudiced by pyrimethamine resistance.

Six of the 17 phenanthrenemethanol derivatives compared very favorably to chloroquine with respect to the dose required to cure infections with the 4-aminoquinoline-susceptible Malayan Camp-CH/Q strain. Chloroquine has a CD_{90} of 91.0 mg/kg of body weight against infections with this strain (27). The CD_{90}'s of WR-122,455, WR-165,355, WR-146,459, WR-148,763, WR-131,834, and WR-171,669 ranged from 25.0 to 90.0 mg/kg.

Although a full analysis of structure-activity relationships is beyond the scope of this report, it is worth noting that the five most active compounds in the pilot group were 2-piperidyl-substituted carbinols. The sixth most active compound, WR-171,669, was an alkylaminoalkyl-substituted 9-phenanthrenemethanol.

Since all test compounds other than the reference agent, WR-33,063, were selected for examination against *P. falciparum* infections in owl monkeys on the basis of activity exhibited in mice infected with *P. berghei*, it is pertinent to compare the results of evaluations in the two systems. Casual inspection of the relevant data in Table 3 indicates that many of the test compounds had markedly different activities in these models. Especially noteworthy were the differences encountered with WR-175,412 and WR-173,060, which were the most active of the 17 derivatives against infections with *P. berghei*, but were far down the list of "actives" against *P. falciparum*, and with the diastereoisomers, WR-122,455 and WR-165,355, which on a milligram-per-kilogram basis were essentially equally active in the human plasmodium-owl monkey model, but had markedly different activities in mice infected with *P. berghei*. These and other divergent results had a very significant effect on the rankings of the various test compounds in the respective model systems (see Table 4). WR-165,355 was the only agent among the five most active against infections with *P. falciparum* that was also included among the five most active against infections with *P. berghei* (Table 4). The remaining top-ranked compounds in the owl monkey model rated 8th, 10th, 11th, and 14th in the rodent model. The substituent on the 9-carbinol appeared to be the critical determinant of relative activity in the respective systems. Each of the five most active agents in the owl monkey model had a 2-piperidyl group attached directly to the methanol, whereas four of the five most active in the *P. berghei*-mouse model had alkylaminoalkyl substituents (cf. Tables 1 and 4). It is of interest that the reference com-

TABLE 4. *Rankings of various 9-phenanthrenemethanols against infections with diverse strains of P. falciparum in owl monkeys as compared with rankings against infections with P. berghei in mice*

Ranking	*P. falciparum*		*P. berghei*	
	Compound WR- no.	CD_{90}[a]	Compound WR- no.	CD_{90}[a]
1	165,355[b]	23.0–27.0	175,412	10.0
2	122,455[b]	25.0	173,060	20.0
3	148,763	<18.0–50.0	181,018	30.0
4	146,459	27.0–50.0	171,669	30.0
5	131,834	65.0	165,355[b]	32.0
6	171,669	90.0–105.0	149,809	50.0
7	178,979	?105.0	165,533	50.0
8	149,809	105.0	146,459	54.0
9	165,533	125.0–175.0	150,726	80.0
10	181,018	100.0–200.0	148,763	80.0
11	190,420	140.0–210.0	131,834	90.0
12	150,726	>140.0–190.0	143,803	100.0
13	185,020	220.0	185,020	110.0
14	175,412	280.0–350.0	122,455[b]	120.0
15	173,060	>140.0–430.0	178,979	130.0
16	143,803	350.0–700.0	190,420	>300.0
17	33,063	>1400.0	33,063	>640.0

[a] Total dose, milligrams of base per kilogram of body weight, required for cure of 90% of infections; administered orally in equal fractions, once daily for 7 days, to owl monkeys, subcutaneously in a single dose to mice.

[b] WR-122,455 and WR-165,355 are diastereoisomers.

pound, WR-33,063, was clearly the least active of the 17 phenanthrenemethanols in both the *P. falciparum*-owl monkey and *P. berghei*-mouse models.

Expanded evaluations of the activities of WR-122,455 and WR-171,669 against infections with *P. falciparum*. (i) Activities of various oral dosage regimens. The results of the expanded evaluations of the activity of WR-122,455 against infections with the Vietnam Oak Knoll and Malayan Camp-CH/Q strains, including explorations of the effectiveness of various oral dosage regimens, have been summarized in Table 5. The data pertaining to the efficacy of the 7-day treatment regimen are in excellent agreement with the results of pilot assessments (cf. columns 2 and 4, Table 3). The data in Table 5 also show that in terms of total dose required to attain a common end point (CD_{90}), single doses and three and seven fractional doses administered on consecutive days were equally effective against infections with the chloroquine-resistant Vietnam Oak Knoll strain. Three- and seven-fractional-daily-dose regimens were also equally effective against infections with the pyrimethamine-resistant Malayan Camp-CH/Q strain. The single-dose regimen appeared to be slightly less effective against infections with this strain than divided dose schedules.

As noted previously, the expanded studies on WR-171,669 were quite limited in scope. They sufficed to confirm the results of pilot appraisals of the activity of a 7-day treatment course with this agent against infections with the Vietnam Oak Knoll and Malayan Camp-CH/Q strains (cf. CD_{90} data, Table 6 and Table 3, columns 2 and 4) and to provide an indication of the relative efficacies of single- and repeated-dose treatment schedules. The latter suggested that WR-171,669 was significantly less active against infections with the Oak Knoll strain when administered in single doses than when delivered in a divided dose regimen over 7 consecutive days. This inferior performance in the single-dose schedule

TABLE 5

THE ACTIVITIES OF WR-122,455, ADMINISTERED ORALLY IN VARIOUS DOSAGE SCHEDULES, AGAINST INFECTIONS WITH THE CHLOROQUINE-RESISTANT VIETNAM OAK KNOLL STRAIN AND THE PYRIMETHAMINE-RESISTANT MALAYAN CAMP-CH/Q STRAIN OF PLASMODIUM FALCIPARUM

Dosage Regimen			Responses to Treatment - No.**				Mean Day (Range) From		Approx. CD_{90} (Total) Mg/Kg
			Effect on Parasitemia						
Individual Dose Mg/Kg	No. of Doses*	Total Dose Mg/Kg	None	Suppressed	Clearance with Recrudescence	Cured	Initial Dose to Parasite Clearance†	Last Dose to Recrudescence	
Infections with Vietnam Oak Knoll Strain - P. falciparum									
8.75	1	8.75	-	-	3(P)	-	9.0(8-11)	21.7(20-23)	
17.5	1	17.5	-	1(P)	1(P)	6(P_3, R_3)	6.1(3-9)	17.0	28.0
35.0	1	35.0	-	-	2(P)	15(P_{14}, R)	7.0(4-10)	14.0(12, 16)	
2.92	3	8.75	-	-	3(P)	-	12.7(12-13)	20.0(19-22)	
5.83	3	17.5	-	-	1(P)	5(P_2, R_3)	6.7(5-9)	17.0	25.0
11.67	3	35.0	-	-	-	4(P_3, R)	7.5(5-9)	-	
1.25	7	8.75	1(P)	3(P)	7(P)	-	10.4(8-12)	17.9(8-19)	
2.5	7	17.5	-	-	2(P)	6(P_3, R_3)	7.0(3-11)	14.0	25.0
5.0	7	35.0	-	-	-	20(P_{14}, R_6)	8.0(6-11)	-	
Infections with Malayan Camp-CH/Q Strain - P. falciparum									
8.75	1	8.75	-	1(P)	2(P)	-	10.5(8, 13)	21.5(21, 22)	
17.5	1	17.5	-	1(R)	5(P_3, R_2)	1(R)	6.7(3-12)	23.2(16-36)	44.0
35.0	1	35.0	-	-	2(P)	13(P_{12}, R)	6.9(3-9)	28.0(20, 36)	
2.92	3	8.75	-	1(P)	2(P)	-	8.0	23.0(13, 33)	
5.83	3	17.5	-	2(P)	-	3(P, R_2)	7.0(6-8)	-	27.0
11.67	3	35.0	-	-	-	7(P_3, R_4)	5.9(3-8)	-	
1.25	7	8.75	-	8(P)	2(P)	-	13.0	17.5(15, 20)	
2.5	7	17.5	-	5(P_2, R_3)	2(P, R)	5(P, R_4)	9.1(3-14)	22.0(16, 28)	27.0
5.0	7	35.0	-	-	-	13(P_8, R_5)	8.4(5-12)	-	

*Repetitive doses administered once daily on successive days.

**Letters in parentheses refer to treatment status of monkeys. P = initial treatment; R = retreatment. Subscripts indicate numbers of subjects in each category.

†Days from first dose of drug to first of a series of five negative thick blood films on consecutive days. A single number without range implies identical clearance times for all subjects.

TABLE 6

THE ACTIVITIES OF WR-171,669, ADMINISTERED ORALLY, AGAINST INFECTIONS WITH THE CHLOROQUINE-RESISTANT VIETNAM OAK KNOLL STRAIN AND THE PYRIMETHAMINE-RESISTANT MALAYAN CAMP-CH/Q STRAIN OF *PLASMODIUM FALCIPARUM*

Dosage Regimen			Responses to Treatment - No.**				Mean Day (Range) From		Approx. CD_{90} (Total) Mg/Kg
			Effect on Parasitemia						
Individual Dose Mg/Kg	No. of Doses*	Total Dose Mg/Kg	None	Suppressed	Clearance with Recrudescence	Cured	Initial Dose to Parasite Clearance†	Last Dose to Recrudescence	
Infections with Vietnam Oak Knoll Strain - *P. falciparum*									
35.0	1	35.0	-	2(P)	1(P)	-	5.0	19.0	?
70.0	1	70.0	-	-	3(P)	-	5.0	14.7(10-17)	>140.0
140.0	1	140.0	-	-	4(P, R_3)	2(P)	5.5(5-6)	16.0(10-24)	
2.5	7	17.5	1(P)	4(P)	1(P)	-	7.0	12.0	110.0
5.0	7	35.0	-	5(P_3, R_2)	7(P_6, R)	2(R)	6.1(5-8)	11.1(5-14)	
10.0	7	70.0	-	-	8(P_4, R_4)	12(P_4, R_8)	5.8(4-7)	15.2(9-27)	
20.0	7	140.0	-	1(R)	-	13(P_9, R_4)	5.7(3-9)	-	
Infections with Malayan Camp-CH/Q Strain - *P. falciparum*									
1.25	7	8.75	2(P)	-	-	-	-	-	90.0
2.5	7	17.5	-	3(R)	1(R)	-	6.0	16.0	
5.0	7	35.0	-	-	5(P, R_4)	3(P, R_2)	6.0(5-8)	11.5(8-18)	
10.0	7	70.0	-	-	1(R)	3(R)	5.3(4-6)	19.0	
20.0	7	140.0	-	-	-	6(P_2, R_4)	5.5(3-8)	-	

*Repetitive doses administered once daily on successive days.

**Letters in parentheses refer to treatment status of monkeys. P = initial treatment; R = retreatment. Subscripts indicate numbers of subjects in each category.

†Days from first dose of drug to first of a series of five negative thick blood films on consecutive days. A single number without range implies identical clearance times for all subjects.

could have been due to the need to deliver doses of WR-171, 669 (140.0 mg/kg) that exceeded the capacity of the gastrointestinal tract of the owl monkey to absorb this poorly water-soluble agent.

(ii) Duration of protection accorded by a single dose of WR-122,455. As shown in Table 7, protection accorded by a single dose of 35.0 mg of WR-122,455 against challenge with blood schizonts of the Vietnam Oak Knoll and Malayan Camp-CH/Q strains was of limited duration. All six monkeys inoculated 24 h after dosage were fully protected; only three of six and one of six were refractory to challenge 7 and 14 days later. All monkeys were susceptible to challenge with the Vietnam Oak Knoll strain 21, 28, and 35 days after dosage and to challenge with the Malayan Camp-CH/Q strain 14, 21, 28, and 35 days after dosage.

Although single doses of WR-122,455 provided full protection for only a brief period, they did affect the rate at which the resulting infection evolved. Thus, there were delays in the onset of patency (appearance of parasites on thick blood films) in 8 of the 12 monkeys susceptible to challenge with the Vietnam Oak Knoll strain and in 6 of the 14 monkeys susceptible to challenge with the Malayan Camp-CH/Q strain (Table 7). In three of the monkeys inoculated with the former strain and one with the latter, the intervals between onset of patency and the times when parasitemias of 100 per 10^4 erythrocytes were attained were significantly longer than in the untreated controls (Table 7). These events are compatible with the demonstrated persistence of WR-122,455 in organs and tissues of owl monkeys and the slow excretion of parent compound and/or metabolites referred to previously. In some subjects, the amounts that persisted must have been large enough to inhibit parasite multiplication and invasion of the peripheral blood, but not sufficiently large to eliminate blood schizonts from deep vascular sites where the larger asexual parasites of *P. falciparum* are often sequestered (26, 40). In others, infections evolved within the same time framework as in the untreated controls. These differences point to significant subject-to-subject variation in localization and/or metabolism of WR-122,455.

The data set forth in Table 7, although limited, indicate that protection accorded against challenge with the Malayan Camp-CH/Q strain was in all respects less than that against challenge with the Vietnam Oak Knoll strain. This observation, together with that on the effectiveness of the single-dose regimen against developed infections with these two strains (see CD_{90} data, Table 5), suggests that the blood schizonts of the Malayan Camp-CH/Q strain are slightly but significantly less susceptible to WR-122,455 than the schizonts of the Vietnam Oak Knoll strain.

All 11 infections that evolved after challenge

TABLE 7. *Duration of protection accorded by a single dose of WR-122,455 against challenge with trophozoites of the Vietnam Oak Knoll or Malayan Camp-CH/Q strains of P. falciparum*

Challenge strain	Days from R_x to inoculation[a]	No. of monkeys infected[b]/no. inoculated	Days from inoculation to patency[c]	Days from patency to parasitemia ca. 10^2 per 10^4 erythrocytes
Vietnam Oak Knoll	1	0/3		
	7	1/3	34	14
	14	2/3	23, 29	9, 22
	21	2/2[d]	8, 20	7, 9
	28	3/3	3, 6, 15	4, 6, 8
	35	3/3	2, 3, 18	6, 7, 13
	No R_x	6/6	1, 1, 1, 1, 2, 2	5, 6, 7, 7, 7, 7
Malayan Camp-CH/Q	1	0/3		
	7	2/3	2, 27	6, 39
	14	3/3	4, 4, 15	5, 8, 8
	21	3/3	3, 4, 11	5, 7, 8
	28	3/3	2, 2, 2	5, 6, 7
	35	3/3	1, 2, 2	5, 6, 6
	No R_x	6/6	1, 1, 1, 1, 1, 2	4, 4, 5, 5, 5, 7

[a] All monkeys received a single dose of WR-122,455, 35.0 mg/kg of body weight, orally on the same day. Subgroups of three treated monkeys, plus an untreated control, were challenged with the respective strains 1, 7, 14, etc., days later.

[b] Number of monkeys exhibiting parasitemia within 90 days after inoculation.

[c] Days from challenge to the first day of an uninterrupted series of positive thick blood films.

[d] One of the three monkeys in this group died of pneumonia 3 days after inoculation.

with the Vietnam Oak Knoll strain and all 14 resulting from challenge with the Malayan Camp-CH/Q strain were treated with 5.0 mg of WR-122,455 per kg daily for 7 days, when parasitemias reached intensities of 100 per 10^4 erythrocytes. This dosage was curative in every instance.

(iii) Incidental observations. The data set forth in Table 5, column 8, show that the time between administration of the first dose of WR-122,455 and clearance of parasitemia in infections with either the Vietnam Oak Knoll or Malayan Camp-CH/Q strain varied substantially (from 3 to 14 days) and that, overall, this variation could not be related to either dose or dosage regimen. Variability is reduced somewhat if the results attained in previously untreated and previously treated infections are evaluated separately. Thus, the time to parasite clearance in original treatment cases ranged from 6 to 14 days (mean 9.3 ± 1.9 days). The time to clearance in recrudescent infections, shorter than the above because of the contribution of immunity acquired during a previously treated attack, ranged from 3 to 7 days (mean 4.8 ± 1.5 days). In either setting, the time required for WR-122,455 to effect parasite clearance in initial treatment cases was significantly longer than the time required when chloroquine and quinine are used to treat comparable infections with the chloroquine-quinine-susceptible Malayan Camp-CH/Q, Uganda Palo Alto, and Cambodian I strains (27), or when mefloquine is employed against infections with either these strains or the chloroquine-resistant Oak Knoll, Monterey, and Smith strains, all of Vietnam origin (29).

Detailed daily examinations of blood films, a routine of the experimental procedures employed in the current investigation (28), provided information on the impacts of WR-122,455 on parasite multiplication and morphology that is relevant to the slow clearance of parasitemia noted above. These examinations in subjects with previously untreated infections showed that, irrespective of dose delivered or dosage regimen, there was a lag of 48 to 72 h between administration of the first dose of WR-122,455 and a significant decrease in parasite numbers. Parasitemias remained at or above pretreatment levels during this period and then declined steadily and slowly to reach negative-thin-film, positive-thick-film status (ca. < 1 parasite per 10^4 erythrocytes) 6 or 7 days after the initial dose. In most subjects, alterations in parasite morphology were first noted on the day of the initial decrease in parasitemia; in a few cases, on the day before. This was perplexing, since, to avoid an increase in parasite numbers during the lag period, multiplication had to be inhibited. When morphological changes did appear, they were limited to the early ring stages. Initially, only a small fraction of these forms (not more than 10%) were affected; however, the proportion increased with time until essentially every parasite visualized was abnormal. In relatively regular sequence, morphological abnormalities progressed from appearance of a vacuole in the center of the chromatin dot and swelling and thinning of the latter to form a quoitlike structure, to spreading of the azurophilic ring band inwardly and outwardly to yield a pale blue-staining, coarsely granular, irregularly contoured form, to fragmentation of the chromatin mass with random distribution of pleomorphic particles in the azurophilic structure, and finally to coalescence of all elements into a darkly pigmented, formless body with wisps of pale blue-staining material extending from the periphery. In most subjects, this sequence of morphological changes was completed in 4 to 5 days; in some, it occurred over 7 or 8 days.

From a qualitative viewpoint, the sequence of morphological changes evoked by WR-171,669 was identical to that produced by WR-122,455; however, derangements in parasite structure were often noted 24 h after administration of the first dose, never later than 48 h, and were usually completed 72 to 96 h later. Parasitemias decreased significantly as soon as there were alterations in the morphology of the rings. As shown in Table 6, this resulted in clearance of parasitemia within 5 to 9 days of the first dose of WR-171,669 (mean 6.1 ± 1.1 days) in previously untreated infections, a time to clearance approximately 2 days less than that associated with administration of WR-122,455. It should be stressed that this temporal difference was encountered when therapeutically equivalent doses of the two compounds were delivered.

The ease with which resistance to an active member of a new compound class emerges is always an important concern, fortunately one susceptible to examination by a variety of approaches. As has been reported elsewhere (28, 30, 31), studies in rhesus monkeys infected with *P. cynomolgi* and in owl monkeys infected with *P. falciparum* and *Plasmodium vivax* have shown that failure to control parasitemia, on retreatment of a persisting or recrudescing infection with doses of an agent equal to or greater than those that are routinely curative when applied to previously untreated infections, is a sensitive and invariably reliable indicator of the emergence of parasites resistant to the test compound. Inspection of the data in Tables 5 and 6,

as well as those in the relevant segments of Table 2, shows that in only one instance was a treatment failure encountered when doses of WR-122,455 or WR-171,669, which cured previously untreated infections regularly, were used in retreating previous treatment failures. Unfortunately, this exceptional infection was retreated and cured with a drug of another class before resistance or susceptibility of the parasite could be documented. Assuming that the experience with the five strains of *P. falciparum* employed in the current investigation has broad applicability, it seems unlikely that resistance to either WR-122,455 or WR-171,669 will develop rapidly. This position derives further support from: (a) observations on 14 other active phenanthrenemethanol derivatives, summarized in Table 2, where doses of each agent effective against untreated infections were uniformly curative when applied to previous treatment failures on lower doses of the same compound; and (b) the results of the duration-of-protection study (Table 7) that showed that infections previously exposed to subcurative doses of WR-122,455 were fully susceptible to treatment with 5.0-mg/kg daily doses of this compound.

Tissue schizonticidal activities of WR-122,455 as exhibited in infections with sporozoites of *P. cynomolgi*. The data summarized in Fig. 1 indicate that WR-122,455 was without effect on development of the pre-erythrocytic forms of *P. cynomolgi* and therefore lacks prophylactic activity. Recipients of this compound in daily doses of 1.25, 5.0, or 20.0 mg/kg throughout the incubation period developed patent infections on day 8 after sporozoite challenge, as did the untreated controls. As would be anticipated from results of previous studies (34), daily doses of 1.0 mg of primaquine per kg of body weight provided complete protection against sporozoite challenge (Fig. 1D).

Although none of the doses of WR-122,455 used in the prophylactic studies affected maturation of primary tissue schizonts, the two larger doses did affect development of erythrocytic parasites for a significant period after infections became patent. Parasite numbers were held at low levels for 7 and 14 to 15 days after onset of patency in recipients of 5.0- and 20.0-mg/kg doses (Fig. 1B and C). At the end of this lag period, parasitemias increased at a rate comparable to that in the untreated controls. This sequence of events indicates that WR-122,455 could not cope completely with the outpouring of erythrocyte-infective forms from tissue schizonts, but shows that the amount of the compound present after end of dosage sufficed to block development of these forms and normal evolution of parasitemia. This phenomenon would be expected from what is known of the persistence of WR-122,455 in tissues and circulating blood, referred to earlier, assuming that this compound has significant activity against the blood schizonts of *P. cynomolgi*.

Data summarized in Fig. 2 show that WR-122,455 has significant activity against the blood schizonts of *P. cynomolgi*, but is devoid of activity against persisting or secondary tissue schizonts; i.e., the compound has suppressive, but not radical curative properties. Administration of daily doses of 1.25 mg/kg effected a modest reduction in parasitemia. Parasite clearance was achieved with doses of 5.0 or 20.0 mg/kg, but infections relapsed 7 to 29 days after delivery of the last of these doses. Retreatment of these relapses or persisting infections with chloroquine, 5.0 mg/kg daily for 7 days, resulted in prompt clearance of parasitemias in all cases, followed by relapses within 4 to 11 days. The brevity of the intervals between the end of chloroquine treatment and relapse is presumptive evidence that previous treatment with WR-122,455 neither reduced the burden of tissue schizonts nor affected their viability.

DISCUSSION

Early interest in 9-phenanthrenemethanols. Since current interest in the 9-phenanthrenemethanols rests on extensive experimental animal and more limited clinical explorations of this and related groups of phenanthrenemethanols pursued during the World War II Malaria Chemotherapy Program, the major features of these earlier studies merit some attention. The initial investigations, undertaken at the experimental animal level in the Division of Physiology, National Institute of Health, made use of a large pool of 2-, 3-, and 9-phenanthrenemethanol derivatives that had been prepared and studied as potential morphine substitutes in a drug addiction program (37). This pool was supplemented by specially synthesized derivatives, bringing to 198 the number of compounds that were compared with quinine for activity against the blood schizonts of *Plasmodium gallinaceum* and *Plasmodium lophurae* in chickens and ducks, respectively. This total included 26 2-phenanthrenemethanols, 66 3-phenanthrenemethanols, and 106 9-phenanthrenemethanols. Of the latter group, 55 were 1,2,3,4-tetrahydro derivatives. Seventy-four of the 198 exhibited activity against infections with *P. gallinaceum*, ranging from one-third to four times that of quinine (4). Although the proportions of actives among the three major classes were similar, the therapeutic indexes of the 9-phenan-

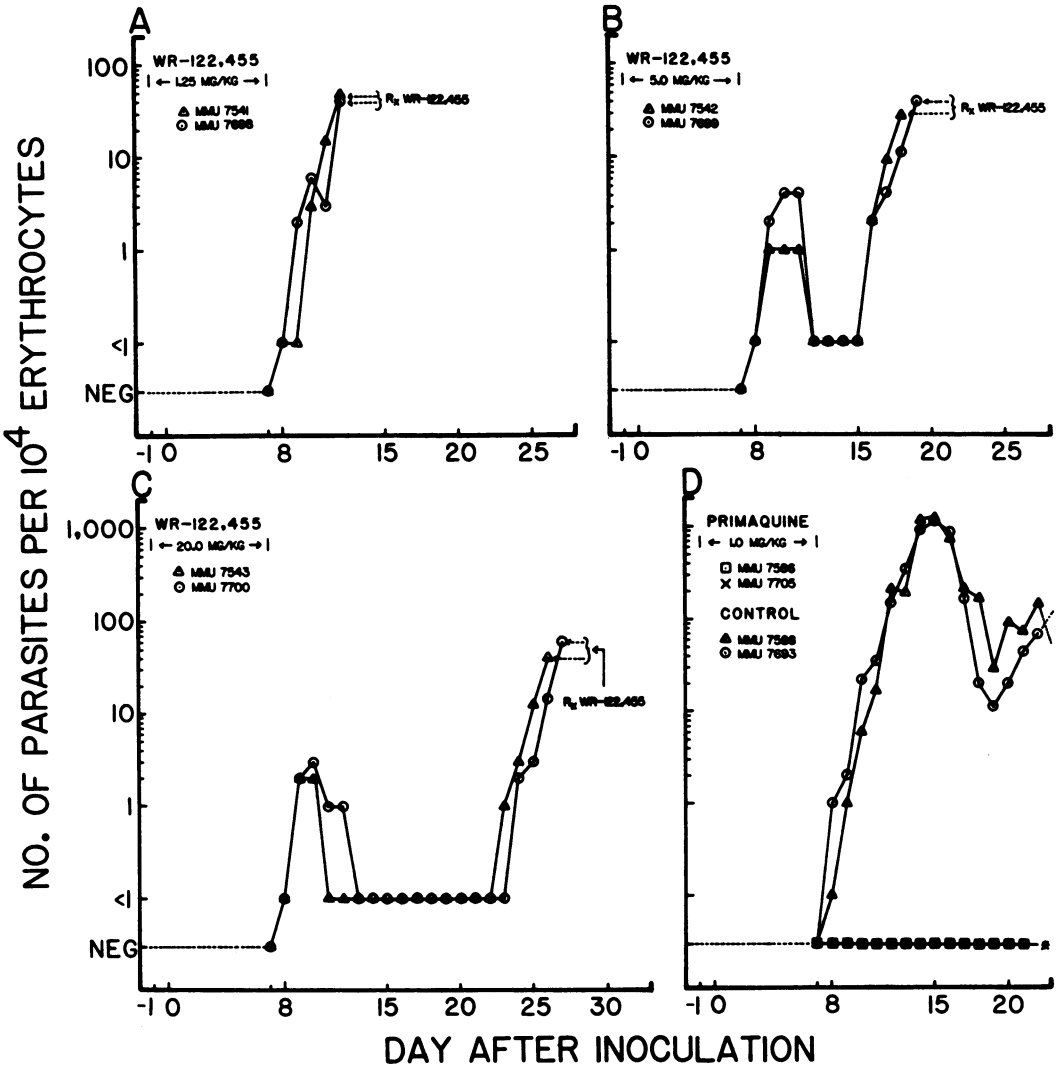


FIG. 1. *Preliminary evaluation of the prophylactic activity of WR-122,455 as exhibited in rhesus monkeys challenged with sporozoites of the B strain of P. cynomolgi.*

threnemethanols were, with rare exceptions, greater than those of isomeric compounds with aminoalcohol substituents in positions 2 or 3 (6), a finding that accounts for the selective focus on 9-methanol derivatives.

Four of the 9-phenanthrenemethanols that exhibited greatest promise in the avian test systems were examined for activity against *P. vivax* infections in human volunteers (3, 7, 9, 23). The group included SN-1796 [α-(diamylaminomethyl)-1,2,3,4-tetrahydro-9-phenanthrenemethanol], SN-5241 [α-(dinonylaminomethyl)-1,2,3,4-tetrahydro-9-phenanthrenemethanol], SN-8867 [α-(dinonylaminomethyl)-9-phenanthrenemethanol], and SN-9160 [6-chloro-α-(diheptylaminomethyl) - 9 - phenanthrenemethanol]. Each of these compounds effected cure of trophozoite-induced infections when administered in total doses of 4.0 to 12.0 g over periods of 4 to 8 days (3). Neither SN-1796 nor SN-5241, the only compounds examined in volunteers inoculated with sporozoites, exhibited prophylactic activity when delivered in doses up to 2.0 g daily throughout the incubation period, nor did they effect radical cure of established infections when delivered in daily doses of 1.0 to 2.0 g for 6 to 8 days (7, 9, 23). SN-1796, the only 9-phenanthrenemethanol evaluated for activity against trophozoites of *P. falciparum*, was not curative when administered in daily doses of 1.5 to 2.0 g for 6 to 8 days; such regimens did reduce parasitemias to low levels (3, 9).

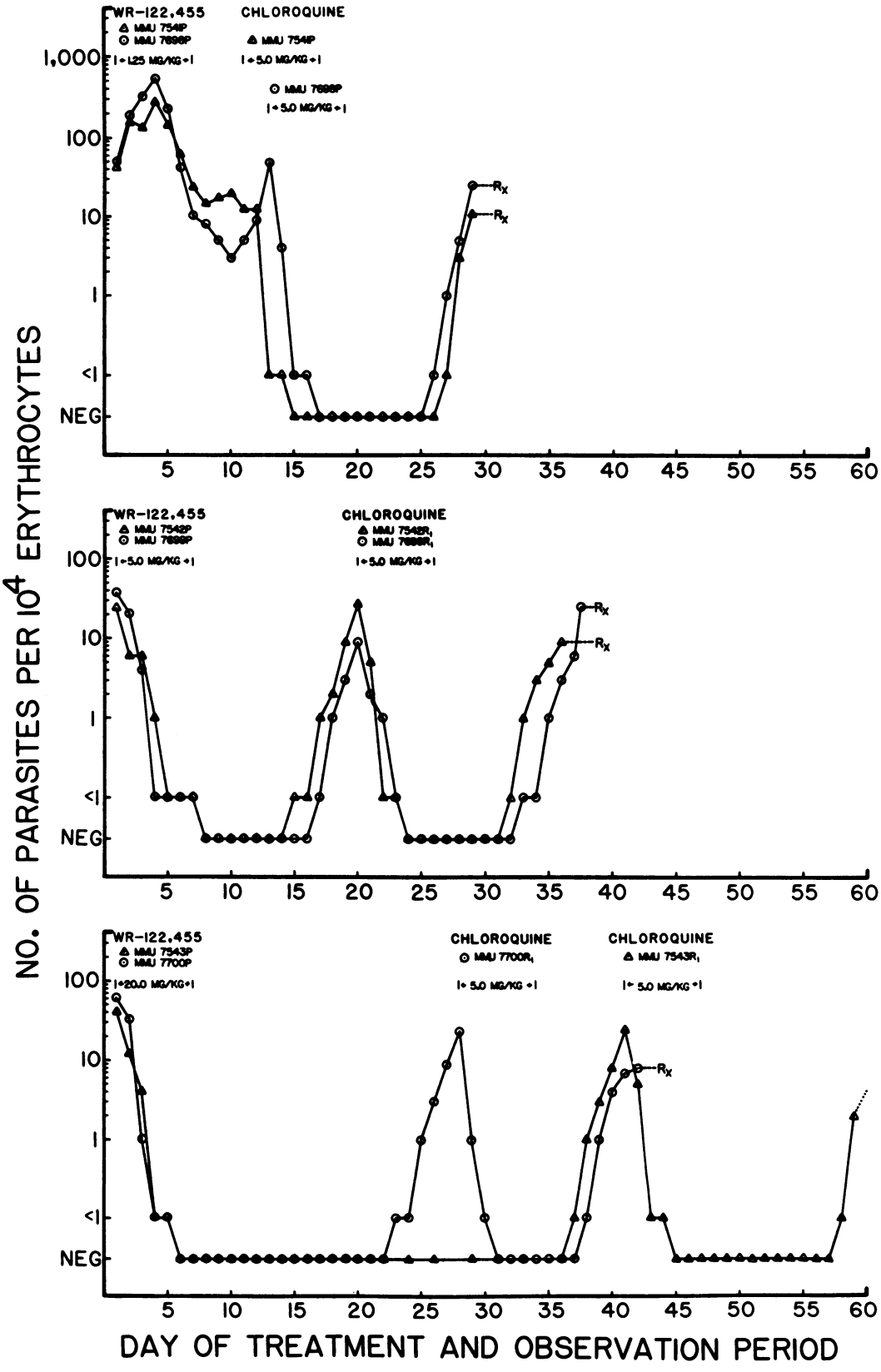


FIG. 2. *Preliminary evaluation of the radical curative activity of WR-122,455 as exhibited in rhesus monkeys with established infections induced by challenge with sporozoites of the B strain of P. cynomolgi.*

Two problems that influenced subsequent interest in the phenanthrenemethanols were encountered during these clinical evaluations of SN-1796 and SN-5241. The lesser of the two was the poor relation between dose and therapeutic efficacy, which appeared to stem from irregular absorption and/or metabolism of these derivatives (3). The more serious problem was the nondose-related occurrence of bradycardia and pilomotor reactions in more than half of the recipients of SN-1796, and dysuria in approximately one-fourth of the volunteers, reflections of significant disturbances in autonomic nervous system activity (3, 9; Malaria Report no. 20, Pharmacological and Toxicological Studies on Compound No. 204, November 2, 1943, to the Board for Coordination of Malarial Studies, National Research Council). These side reactions were less frequent and intense among volunteers treated with SN-5241; however. severe skin rashes in 4 of 28 recipients of this tetrahydrophenanthrene presented another problem. These skin reactions were not a response to exposure to light and are not to be confused with the phototoxicity evoked by certain 4-quinolinemethanol derivatives (21, 27). Bradycardia, pilomotor reactions, and dysuria were not encountered in the 13 recipients of SN-8867 nor in the 12 recipients of SN-9160; 1 of the latter exhibited a skin rash. Although it was less of a problem than with SN-1796 and SN-5241, evaluations of the activities of the above agents were also complicated by erratic absorption (3).

Whereas synthesis of phenanthrenemethanols and evaluation of the activities of the products of this effort against avian malarial infections were continued to the end of the World War II Malaria Program, investigations in human volunteers terminated with the examinations of SN-8867 and SN-9160. In part, this was due to the failure to develop a derivative that was strikingly more active than the latter compound in the avian models, but in larger part to commitment of clinical resources, from 1944 on, to appraisals of the blood schizonticidal activities of the 4-aminoquinolines and tissue schzonticidal activities of the 8-aminoquinolines.

Renewed interest in 9-phenanthrenemethanols; attributes of WR-33,063. There were several reasons for the revival of interest in the 9-phenanthrenemethanols in the early stages of the U.S. Army Malaria Research Program. First, they represented one of the few classes of compounds with demonstrated activity against blood schizonts of any human plasmodium. Second, there were many gaps in the structure-activity explorations of the World War II Program, particularly with respect to substituents on various segments of the phenanthrene nucleus, that could be filled via application of newly available chemical technology. Third, clinical data acquired in the earlier program, although not extensive, suggested that the autonomic nervous system toxicity that had reduced interest in this compound class might be less of a problem with conventional phenanthrenemethanols than with tetrahydro derivatives. Since new syntheses required time, attention was directed initially to a group of derivatives recovered from the stocks of compounds examined in the World War II Malaria Chemotherapy Program.

These older phenanthrenemethanols were systematically evaluated in the Rane Laboratory for activity in mice infected with *P. berghei*, utilizing a model system (18) that places emphasis on capacity of test agents to cure established infections rather than to achieve suppression of parasitemia to an arbitrarily selected level as in the avian models (6). WR-33,063, previously designated SN-13,465, the bromo- analog of SN-9160 referred to above, emerged as the most active of the group, exhibiting significant curative activity at a dose that was well tolerated, CD_{50} = 450.0 mg/kg (personal communication, W. E. Rothe and D. P. Jacobus, WRAIR). Interestingly, this compound, developed and examined late in the World War II Program, had also ranked first among the 9-phenanthrenemethanols examined for activity against infections with *P. gallinaceum*, where it was approximately twice as active as SN-9160 (6). Studies in a rodent model (39), somewhat different from that used in the Rane Laboratory, showed that, dose for dose, WR-33,063 was five times as active as quinine and that it was fully active against infections with parasites resistant to pyrimethamine and cycloguanil (the active metabolite of proguanil), but that it was less active against infections with various chloroquine-resistant parasites than against infections with the parent drug-susceptible strain (38). This diminution in efficacy was directly related to the level of chloroquine resistance, but did not parallel it. Thus, there was but a 4-fold reduction in activity of WR-33,063 against infections with a strain of *P. berghei* that exhibited a 40-fold decrease in chloroquine susceptibility. Since the results of in vitro studies indicated that the difference in susceptibility to chloroquine between the moderately resistant Malayan Camp strain of *P. falciparum* and the susceptible Uganda I strain was no more than twofold (personal communication, K. H. Rieckmann, University of New Mexico, Albuquerque), it was suggested that WR-33,063 might well retain a useful level of activity against infections with 4-aminoquinoline-resistant strains of this plasmodium (38).

In the late 1960s, malarial infections that did not respond to therapy with known drugs were a serious burden to the U.S. Armed Services. The urgency of developing a new procedure for managing such infections led those responsible for the clinical investigative components of the Malaria Research Program to recommend that WR-33,063 be prepared for study in human volunteers. Studies in various experimental animals showed that this compound had an acceptable level of toxicity when delivered in either single large or repetitive smaller doses (personal communication, M. H. Heiffer, WRAIR). Unlike SN-1796, it did not affect cardiac rate and output or resistance to pulmonary blood flow in rabbits and dogs (24). Tolerability studies, involving 52 human volunteers, showed that doses of WR-33,063, up to and including 4.6 g daily (1.15 g every 6 h), could be administered for 10 successive days without evoking untoward reactions (2). The pilomotor reactions, bradycardia, dysuria, and skin rashes that led to curtailment of clinical studies during the World War II Malaria Chemotherapy Program were conspicuously absent.

With the above assurance of tolerability, the capacity of WR-33,063 to control infections with various strains of *P. falciparum* was assessed in human volunteers (2). Daily doses of 1.6 g (0.4 g every 6 h) for 6 consecutive days proved to be a highly effective regimen, leading to cure of 6 of 6 infections with trophozoites of the fully drug-susceptible Uganda I strain, 5 of 5 infections with the pyrimethamine-resistant Malayan Camp strain, and 18 of 23 and 5 of 5 infections with the multidrug-resistant Smith and Marks strains, both of Vietnam origin. The same daily dose administered for only 3 days, or smaller doses for longer periods, were distinctly less effective, curing but 6 of 15 infections with the above strains. There was a suggestion in this component of the volunteer study that infections with the highly chloroquine-resistant Smith strain responded less well to these lower doses than infections with the chloroquine-susceptible Uganda I strain and the slightly chloroquine-resistant Malayan Camp strain.

The observations summarized above led promptly to application of WR-33,063 to treatment of hospitalized military personnel who, while serving in Vietnam, had acquired *P. falciparum* infections that had resisted treatment with all currently available antimalarial drugs (5). Administration of daily doses of 1.6 g (0.4 g every 6 h) for 6 days cured 11 of 11 long-standing infections; the same daily doses administered for 10 days cured 23 of 25 infections of somewhat shorter duration. Six patients among the latter group exhibited infections with *P. vivax* during a follow-up period of 30 days, under conditions that precluded reinfection, thereby indicating that WR-33,063 could eliminate the blood schizonts of this plasmodium, but not the tissue schizonts responsible for relapse. There was no evidence of drug toxicity in any of the recipients of this phenanthrenemethanol. Therapeutic responses, remarkably similar to those described above, were subsequently obtained in two groups of Thai patients naturally infected with chloroquine-resistant strains of *P. falciparum* (10, 36).

Despite its impressive activity against infections with chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum*, WR-33,063 exhibited certain deficiencies that made it seem unlikely that this compound could fill the need for a generally useful blood schizonticidal drug. In the first place, administration of this agent in relatively large divided daily doses for 6 to 10 days appeared to be a requisite for cure. Patient compliance and administrative monitoring of such regimens are difficult to obtain outside of a hospital setting and even there are inconvenient. Second, there was evidence that WR-33,063 was poorly and irregularly absorbed in some individuals (5) and that this quality was reflected in the near absence of effect on parasitemias in some treatment failures. The problem of adequacy of absorption would be difficult to manage in any but hospitalized patients maintained under close supervision where measurements of blood levels or urinary output of drug are possible. Because of these deficiencies, it was decided to look beyond WR-33,063 to some of the newly synthesized 9-phenanthrenemethanols for a derivative of potentially greater general utility.

Attributes of WR-122,455 and WR-171,669. As indicated in the body of this report, each of the 16 newly synthesized 9-phenanthrenemethanols accorded pilot appraisal in the *P. falciparum*-owl monkey model exhibited greater activity than WR-33,063. The two most active derivatives, WR-122,455 and WR-165,355 (diastereoisomers), were more than 50 times as active as the reference compound. More importantly, based on their CD_{90} levels, the activities of 6 of the 16 agents compared favorably with that of chloroquine against infections with a strain of *P. falciparum* susceptible to the latter drug. Two of the six derivatives, WR-122,455 and WR-171,669, the first and sixth in order of activity, were selected as possible candidates for study in human volunteers. This selection led to an expansion of earlier evaluations of the activities of both compounds against *P. falciparum* infections in owl monkeys, with particular attention to the influence of the dosage regimen, and to a

limited but critical appraisal of the prophylactic and radical curative properties of WR-122,455 as exhibited in rhesus monkeys infected with *P. cynomolgi*.

The composite assessments of the activities of WR-122,455 in owl monkeys infected with various strains of *P. falciparum* showed that: (i) this compound was equally active against infections with chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum* and indicated that efficacy was not compromised by concomitant resistance to pyrimethamine; (ii) that on the basis of comparative CD_{90}'s, WR-122,455 was from two to four times as active as chloroquine against infections with the chloroquine-susceptible Uganda Palo Alto and Malayan Camp-CH/Q strains; (iii) that the capacity of this phenanthrenemethanol to cure established infections was a function of the total dose administered, single doses being essentially as effective as three or seven fractional doses administered on as many days; (iv) that in keeping with the persistence of WR-122,455 and/or its metabolites in tissues and plasma, a single dose of this agent conferred limited protection against infection for as long as 21 days after dosage, although protection was complete for less than 7 days. The evaluations undertaken in rhesus monkeys inoculated with sporozoites of *P. cynomolgi* showed: (i) that administration of WR-122,455 throughout the incubation period did not delay the onset of parasitemia, although such doses did retard evolution of patent infections; and (ii) that delivery of appropriate doses to monkeys with established infections effected clearance of parasitemia, but that all infections so treated relapsed and continued to do so upon retreatment with chloroquine. These latter findings suggest that WR-122,455 would effectively suppress naturally acquired infections with *P. vivax*, but, because of lack of tissue schizonticidal activity, would not function as either a prophylactic or radical curative drug.

Although the examination of WR-171,669 was quite limited as compared to that of WR-122,455, it sufficed to show: (i) that this compound was essentially equally active against infections with the chloroquine-resistant Vietnam Oak Knoll and chloroquine-susceptible Malayan Camp-CH/Q strains of *P. falciparum*; (ii) that on a comparable dose basis, it was at least 12 times as active as WR-33,063 against infections with the above strains; (iii) that it was between one-third and one-fourth as active as WR-122,455 against infections with both strains; (iv) that its activity against infections with the Malayan Camp-CH/Q strain was equal to that of chloroquine; (v) that single doses were significantly less active against infections with the Vietnam Oak Knoll strain than the same total dose delivered in seven equal fractions on successive days; and (vi) that in a 7-day dosage schedule and at the CD_{90} level, it effected control of parasite development and clearance of parasitemia more promptly than did WR-122,455 in a comparable regimen.

Preclinical pharmacological studies pursued elsewhere (personal communication, M. H. Heiffer, WRAIR) showed that WR-122,455 and WR-171,669 had acceptable levels of acute and subacute toxicity for various experimental animals and that they did not affect cardiac rate or output of mice, rats, and dogs. These studies also showed that each compound was absorbed at a moderately rapid rate and in most cases completely from the gastrointestinal tracts of mice, rats, rhesus monkeys, and owl monkeys and persisted in significant concentrations in blood plasma for days to weeks. Both agents were extensively localized in organs and tissues of the above test subjects, released slowly therefrom, secreted in the bile and probably reabsorbed from the intestinal tract, and eliminated primarily as unchanged drug in the feces over a period of 14 days in rats and more than 21 days in rhesus and owl monkeys.

Studies of the tolerability of WR-122,455 in human volunteers (22) showed that single doses, ranging from 320.0 to 1,280.0 mg, could be administered without evoking untoward reactions; single doses of 1,520.0 mg provoked nausea, diarrhea, and abdominal cramps in two of two volunteers. Daily doses of 480.0 mg (240.0 mg twice daily) for 3 to 6 days evoked mild abdominal cramps and diarrhea in one of eight subjects. Daily doses of 960.0 mg (240.0 mg four times daily) evoked nausea, vomiting, and diarrhea of such severity that delivery of WR-122,455 was halted after 3 days on this regimen. WR-171,669 evoked no untoward reaction in single doses up to 750.0 mg, the largest amount administered at one time. Abdominal cramps and nausea were encountered when total doses of 1,440.0 and 1,920.0 mg (480.0 mg every 8 or 6 h) were administered within a single day, when doses of 1,260.0 mg (420.0 mg every 8 h) were administered daily for 3 days, or when 1,000.00 mg (250.0 mg every 6 h) was administered daily for 6 days. There is a strong suggestion in these data that the toxicities of both phenanthrenemethanols were cumulative. Neither compound evoked disturbances in autonomic nervous system activity, such as were encountered with the 9-tetrahydrophenanthrenes, nor did they induce phototoxicity (22).

Appraisals of the capacities of WR-122,455 and WR-171,669 to control infections with the multidrug-resistant Vietnam Smith strain in hu-

man volunteers, although limited, showed that both compounds had high levels of therapeutic activity (22). Single doses of 720.0, 800.0, or 880.0 mg of WR-122,455 effected temporary clearance of parasitemia in three of three volunteers (with recrudescences delayed for 21, 34, and 38 days, respectively). Daily doses of 480.0 mg (240.0 mg every 12 h) for 3 to 6 days cured infections in all nine recipients. Daily doses of 1,000.0 mg of WR-171,669 (250.0 mg every 6 h) for 3 days cured six of six infections. Thus, WR-122,455 effected cure of infections with the Vietnam Smith strain in a total dose of 1,440.0 mg, WR-171,669 in a total dose of 3,000.0 mg. Although the latter observations suggest that WR-122,455 is more active than WR-171,669, the appraisals were too limited to support such a conclusion. It is clear that each of these derivatives is significantly more active than WR-33,063, the reference compound, against infections with the Smith strain. Again, paucity of data on WR-122,455 and WR-171,669 makes any attempt to quantify this activity differential premature. It is worth noting that the total dose of WR-122,455 that effected cure of infections with a strain of *P. falciparum* highly resistant to chloroquine was not more, and may prove to be significantly less, than the dose of this 4-aminoquinoline that is generally recommended and used for cure of infections with drug-susceptible strains of this plasmodium.

Future of 9-phenanthrenemethanols in malaria therapy. Although the above evaluations of WR-122,455 and WR-171,669 were of limited dimensions (and could not be extended because of curtailment of the human volunteer component of the current Malaria Research Program), they sufficed to show that, with respect to antimalarial activity, tolerability, and other pharmacological characteristics, these compounds are major improvements over the four phenanthrenemethanols evaluated clinically in the World War II Malaria Chemotherapy Program and, except for tolerability, are improvements over WR-33,063. Supported by observations in owl monkeys infected with various strains of *P. falciparum*, the evaluations suggest that either of the newer compounds, but most probably WR-122,455, might meet requirements for a broadly active blood schizonticidal drug.

It is obvious that clinical studies will have to be expanded greatly to determine whether this projected promise can be realized. Although discussion of such investigations would not be appropriate here, identification of studies considered to be of highest priority is supportable. In decreasing order of importance, these would include: (i) a critical comparison of the capacities of 3-day regimens of WR-122,455 and WR-171,669 to cure established infections with the multidrug-resistant Vietnam Smith strain, with due attention to tolerability—a study aimed at selection of one or the other of these compounds for the examinations listed below; (ii) comparison of the efficacies of 1-, 2-, and 3-day dosage regimens, utilizing both single and divided daily doses; (iii) appraisal of the capacity of the optimal dosage regimen, as derived from (ii), to cure infections with a variety of strains of *P. falciparum* and infections with trophozoites of the New Guinea Chesson and some other strain of *P. vivax* of subtropical origin; (iv) determination of the duration of protection accorded by a single drug dose and, as a corollary, the capacity of spaced doses to effect suppressive cure of naturally acquired *P. falciparum* infections and suppression of such infections with *P. vivax*; and (v) examination of the feasibility and efficacy of intravenous administration of water-soluble salts of the selected agent (e.g., WR-122,455 lactate or methanesulfonate [1]) to cover the need for parenteral medication when oral dosage is not possible.

It would be inappropriate to conclude this report without taking cognizance of two observations that some may feel should temper enthusiasm for the future of the phenanthrenemethanols. Both observations have emerged from a study of the activity of WR-122,455 against infections with *P. berghei* in mice (20). The first stemmed from appraisals of the efficacy of WR-122,455 against infections with various drug-resistant strains of *P. berghei* which, among other things, showed that this compound was essentially devoid of activity against infections with a strain 200-fold resistant to chloroquine. Although this finding generated concerns for the future of WR-122,455, there are a number of reasons for discounting its relevance to treatment of infections with chloroquine-resistant strains of *P. falciparum*. In the first place, the results of studies on WR-122,455, WR-171,669, and WR-33,063 recorded in the current report show that these agents are essentially equally active against infections with chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum*. These results in owl monkeys are solidly supported by the evaluations of the activities of WR-33,063 pursued in human volunteers (2) and in naturally infected patients (5, 36), and of WR-122,455 in volunteers (22). Second, WR-33,063 and WR-122,455 inhibit in vitro development of parasites of the drug-susceptible Uganda I strain and the chloroquine-resistant Vietnam Marks strain at essentially the same concentrations (42; personal communication, K. H. Rieckmann, University of New Mexico, Albuquerque), whereas WR-171,669 is equally effective in blocking in vitro incorporation of hy-

poxanthine into parasites of the Uganda I strain and chloroquine-resistant Vietnam Smith strain (R. E. Desjardins, J. D. Haynes, J. D. Chulay, and C. J. Canfield, Fed. Proc. 37:379, 1978). Third, the investigators who observed that WR-122,455 was inactive against infections with a strain of *P. berghei* 200-fold resistant to chloroquine also noted that this phenanthrenemethanol was essentially fully active against infections with a strain that was 5-fold resistant (20). This finding deserves emphasis, for it relates to a parasite with a level of resistance roughly comparable to that encountered in so-called highly chloroquine-resistant strains of *P. falciparum* and to results not complicated by the aberrations in morphology, virulence, multiplication rate, and host cell preference that characterize the 200-fold chloroquine-resistant strain of *P. berghei* (11, 19). As was pointed out in an earlier investigation of cross-resistance to WR-33,063, results acquired with such highly resistant strains of *P. berghei* are interesting, but probably have little relationship to events in infections with strains of plasmodia with clinically relevant levels of chloroquine resistance (38).

The second concern with the long-term usefulness of WR-122,455 stemmed from the demonstration that both the parent drug-susceptible and moderately chloroquine-resistant strains of *P. berghei* acquired resistance to this phenanthrenemethanol when passaged serially through mice treated with a single noncurative dose of the compound on the day of trophozoite challenge (20). Although it is said that resistance to WR-122,455 developed "fairly rapidly" in the susceptible strain and "very rapidly" in the chloroquine-resistant strain (20), the wide fluctuations in response to this compound that occurred during serial transfers make this conclusion untenable. The plotted data show that stabilized responses to WR-122,455, clearly indicative of developed resistance, were attained after 50 serial transfers of the susceptible strain over an 18-month period and after 33 transfers of the resistant strain over a 12-month period. Whereas development of resistance to WR-122,455 is clear, it was hardly a rapid event with either strain. When resistance was finally established, it did not preclude therapeutic benefits when tolerated doses of this compound were administered (20). As pointed out earlier in this report, there was no evidence of emergence of resistance to any of the phenanthrenemethanols evaluated in any component of the current study despite employment of a retreatment procedure designed specifically for early identification of this phenomenon (28). This negative result does not mean that resistance to WR-122,455, WR-171,-669, or other phenanthrenemethanols will not ultimately appear if such agents are utilized widely for long time periods, but suggests that such resistance is not likely to emerge promptly with application of these compounds.

Stepwise evaluation of specially synthesized phenanthrenemethanols in mice infected with *P. berghei* and owl monkeys infected with various strains of *P. falciparum*, complemented by controlled studies of tolerability and activity in human volunteers, led to identification of two derivatives, WR-122,455 and WR-171,669, that appear to have substantial potential as blood schizonticides. If broad clinical studies show that both agents have use-limiting toxicological and/or pharmacological shortcomings, there are at least three other derivatives, only slightly less active than WR-122,455 in the owl monkey model, that merit attention. If none of these agents meets the desiderata of a generally useful antimalarial drug, continued exploration of this compound class would still be warranted. Except for the 4-quinolinemethanols (29) and 4-pyridinemethanols (unpublished data), no other known chemical series can compare with the phenanthrenemethanols with respect to broad-spectrum blood schizonticidal activity.

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