12

REPORT NUMBER SIX

CHEMOTHERAPY OF MALARIA

ANNUAL SUMMARY REPORT

DR. LEO PANE

For the period of June 1, 1971 to May 31, 1972

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D. C. 20314

Contract No. DADA 17-70-C-0100

University of Miami
Miami, Florida 33142

DDC AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Six ✓ (9) Annual rept. no. 6, 1 Jun 71-31 May 72,

2. GOVT ACCESSION NO. | 3. RECIPIENT'S CATALOG NUMBER

4. TITLE *(and Subtitle)*: (6) CHEMOTHERAPY OF MALARIA.

5. TYPE OF REPORT & PERIOD COVERED: Annual Report June 1, 1971-May 31, 1972

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s): (10) Dr. Leo Rane

8. CONTRACT OR GRANT NUMBER(s): (15) DADA 17-70-C0100 ✓

9. PERFORMING ORGANIZATION NAME AND ADDRESS: University of Miami ✓ Miami, FL 33142

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Medical Research & Development Command Washington, D. C. 20314

12. REPORT DATE: May 31, 1972 (11) 31 May 72

13. NUMBER OF PAGES: 16

14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*

15. SECURITY CLASS. *(of this report)*: (12) 18 p.

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

P. berghei blood-induced malaria infection in mice - based on mortality.

P. gallinaceum sporozoite-induced malaria infection - Aedes aegypti - chicks - based on mortality.

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Primary screen - quantitative evaluation of potential antimalarial activity.

Primary screen - to provide quantitative assessments of prophylactic values.

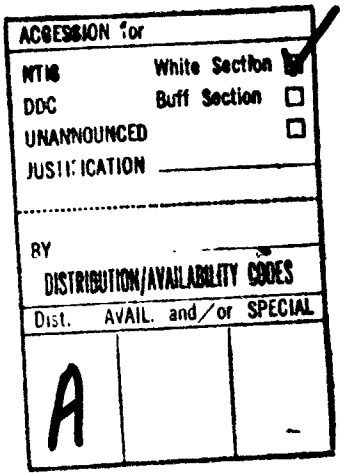
387505

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.



177,844 compounds were screened for antimalarial activity in mice infected with Plasmodium berghei in the period from December, 1961 to June, 1970, covered by Contract No. DA-49-193-MD-2218.

Table I summarizes the number of compounds tested and the number of mice used under the original contract.

This program is continuing under Contract No. DADA 17-70-C-0100, activated on June 1, 1970.

18,108 compounds were tested for antimalarial activity from June 1, 1970 through May 31, 1971. 14,874 compounds were tested for antimalarial activity from June 1, 1971, through May 31, 1972.

Table II records the number of compounds tested and the number of mice used from June 1, 1971, through May 31, 1972.

All compounds tested were obtained from the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research and included:

(1) compounds structurally related to chemicals of known value as antimalarial agents;
(2) compounds structurally unrelated to compounds known to have antimalarial activity;
(3) structural analogues of compounds found active in our test system and representing several novel chemical groups.

Our own breeding colony of ICR/HA Swiss mice continues to supply the animals used in our tests.

We have continued to use the original test system which was designed specifically to give relatively fast but reliable evaluations from standpoints of antimalarial effect and host toxicity.

This test is based on the responses to candidate compounds by Plasmodium berghei malaria in mice as expressed in comparisons of the maximum survival time of treated malaria-infected animals and the survival time of untreated malaria-infected controls.

TABLE I

SUMMARY OF SCREENING LEVELS

DECEMBER, 1961 - MAY, 1970

	NUMBER OF COMPOUNDS	NUMBER OF MICE
DECEMBER, 1961 - MAY, 1964	6,915	250,000*
JUNE, 1964 - MAY, 1965	13,114	215,715
JUNE, 1965 - MAY, 1966	22,731	350,449
JUNE, 1966 - MAY, 1967	34,093	531,200
JUNE, 1967 - MAY, 1968	40,465	636,525
JUNE, 1968 - MAY, 1969	38,150	603,225
JUNE, 1969 - MAY, 1970	22,376	411,270
TOTAL	177,844	2,998,384

*Estimate covers mice used in development of test and screening.

TABLE II

MONTHLY SCREENING LEVELS

JUNE 1, 1970 - MAY 31, 1971

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE	2,130	35,925
JULY	1,059	19,140
AUGUST	692	14,310
SEPTEMBER	884	19,140
OCTOBER	1,073	19,140
NOVEMBER	1,264	23,925
DECEMBER	1,484	25,125
JANUARY	1,385	23,910
FEBRUARY	1,328	23,760
MARCH	2,330	39,045
APRIL	2,650	45,330
MAY	1,823	33,390
TOTAL	18,108	322,140

TABLE III

MONTHLY SCREENING LEVELS

JUNE 1, 1971 - MAY 31, 1972

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
JUNE	1,557	27,390
JULY	1,129	26,250
AUGUST	1,194	21,480
SEPTEMBER	1,069	19,080
OCTOBER	1,447	23,850
NOVEMBER	811	14,310
DECEMBER	795	14,310
JANUARY	1,035	19,080
FEBRUARY	1,215	20,265
MARCH	1,745	28,590
APRIL	1,439	23,820
MAY	1,438	23,820
TOTAL	14,874	262,245

Using young ICR/HA Swiss mice and a standard inoculum of Plasmodium berghei, it has been possible to produce a consistently uniform disease that is fatal to 100% of untreated animals within 6 to 8 days.

Since an established disease is less responsive to treatment than a disease in the early stages of development, treatment is withheld deliberately until a high degree of parasitemia has become evident.

Test compounds were administered parenterally in a single dose on the third day post-infection by which time a 10-15% parasitemia has developed.

To be classified as active, a compound must suppress the disease and produce an unquestionably significant increase, 100% or more, in the life span of the treated animals over that of the untreated controls.

The severity of the challenges set up in our test system enhances the reliability of our evaluations and the antimalarial potential of the compounds selected for intensive preclinical studies.

METHOD

ANIMAL HOSTS. The total supply of animals needed to screen candidate compounds was obtained from our own breeding colony of ICR/HA Swiss mice. Test animals weigh from 15 to 18 grams, weight variations in any given experimental or control group being carefully limited to 2-3 grams. In any given test all animals are of a single sex and approximately the same age.

Animals on test are housed in metal topped plastic cages, fed a standard laboratory diet and given water ad lib.

TEST PROCEDURE. Test animals receive an intraperitoneal injection of 0.5 ml. of a 1:100 dilution of heparinized heart's blood with a minimum of 90% parasitized cells, drawn from donor mice infected one week earlier with Plasmodium berghei. The donor strain is maintained by weekly passages in separate groups of mice inoculated with 0.5 ml. of a 1:500 dilution of heparinized heart's blood.

In order to check factors such as changes in the infectivity of our Plasmodium berghei strain or in the susceptibility of the host or to detect technical errors, a group of infected animals treated with pyrimethamine at dose levels known to produce definite increases in survival time is included in every experiment as a positive control.

DRUG ADMINISTRATION. Test compounds are dissolved or suspended in peanut oil before they are administered.

Treatment consists of a single dose given subcutaneously 3 days post-infection. At the time of treatment, a 10-15% parasitemia has developed. Although the disease is well established, it has not yet caused sufficient debility to affect an evaluation of the test compound's toxicity.

Deaths that occur before the 6th day, when untreated controls begin to die, are regarded as the result of a compound's toxic effects and not as the result of action by the infecting parasite.

In each experiment the compound on test is administered in graded doses. Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice.

If an active drug is toxic for the host, the toxicity of this compound may become a limiting factor to changes in dose levels.

Treated animals alive at the end of 60 days are considered as cured.

DRUG ACTIVITY. Acceptance of a drug as being sufficiently active for detailed studies is predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED). A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die. The minimum effective dose is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

An increase of 100% in survival time is considered the minimum significantly effective response for a candidate compound.

Clearly inactive compounds are rejected after one test, borderline compounds after two tests. Active compounds are subjected to a dose-response curve so that the spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) may be established.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST PLASMODIUM BERGHEI IN MICE

Of the 18,108 compounds tested from June 1, 1970, through May 31, 1971, 805 demonstrated a degree of antimalarial activity sufficient

to produce at least 100% increases in the survival time of treated Plasmodium berghei infected mice.

Of the 14,874 compounds tested from June 1, 1971, through May 31, 1972, approximately 593 demonstrated a degree of antimalarial activity sufficient to produce at least 100% increases in the survival time of treated Plasmodium berghei infected mice.

Supplementary procedures, using different hosts and parasites and performing reliably either as confirmatory tests or as other primary screens, are desirable adjuncts to any screening program.

We have developed a simple but dependable supplementary test with Plasmodium gallinaceum malaria in chicks.

39,530 compounds were screened for antimalarial activity in chicks infected with Plasmodium gallinaceum in the period from January, 1965, to June, 1970, covered by Contract No. DA-49-193-MD-2218.

Table IV summarizes the number of compounds tested and the number of chicks used under the original contract.

This program is continuing under Contract No. DADA-17-70-C-0100, activated June 1, 1970.

36,625 compounds were tested for antimalarial activity from June 1, 1970, through May 31, 1971.

Table V records the number of compounds tested and the number of chicks used from June 1, 1970, through May 31, 1971.

1,651 compounds were tested for antimalarial activity from June 1, 1971, through May 31, 1972.

Table VI records the number of compounds tested and the number of chicks used from June 1, 1971 through May 31, 1972.

Using 9-12 days old chicks and a standard inoculum of Plasmodium gallinaceum, we have been able to produce a consistently uniform disease that is fatal to 100% of untreated controls within 72-96 hours.

In this test, as in our mouse test, the antimalarial activity of candidate compounds is assessed by comparing the maximum survival time of treated malaria-infected chicks and the survival time of untreated malaria-infected controls.

As in the mouse test, a compound has been considered active if it has produced increases of at least 100% in the survival time of treated

chicks over the survival time of untreated controls.

Again as in the mouse test, acceptance of a test compound's antimalarial activity was further predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED).

A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die. A minimum effective dose is defined as the minimum dose increasing the life span of treated animals 100% over the life span of untreated controls.

METHOD

TEST ANIMALS. This test is done with 9-12 day old white leghorn cockerels.

Birds of fairly uniform stock, purchased from local hatcheries, are delivered to the laboratory when 1 day old and then maintained under standard conditions, including a non-medicated diet, until they are ready for testing.

TEST PROCEDURE. Chicks on test are given an intrajugular injection of 0.2 ml. of heparinized heart's blood infected with Plasmodium gallinaceum and having a minimum of 80-90% parasitized red blood cells.

The parasitized blood is drawn by cardiac puncture from donor birds that had been infected 72 hours earlier with Plasmodium gallinaceum.

Donor strains are maintained in separate groups of chicks, 14-16 days old, that also receive inoculations of heparinized infected heart's blood.

In every experiment 100% of the untreated controls have died within 72-96 hours post-infection.

In order to check factors such as changes in the infectivity of our Plasmodium gallinaceum strain or in the susceptibility of the host or to detect technical errors, a group of infected birds treated with pyrimethamine at dose levels known to produce definite increases in survival time has been included in every experiment as a positive control.

DRUG ADMINISTRATION. Candidate compounds are dissolved or suspended in peanut oil before they are administered.

In this supplementary test treatment consists of a single dose that is administered either subcutaneously or per os immediately after infection.

Each experiment is done with graded doses of the compound on test, and increases in the dose levels of highly active compounds were generally followed by increases in the survival time of the treated chicks.

If an active drug was toxic for the host, its toxicity became a limiting factor to changes in dosages.

Deaths that occurred within 48 hours after infection and treatment were considered as deaths due to the toxic effects of a test compound, not as the result of the infection introduced by the Plasmodium gallinaceum parasite.

Chicks with survival periods of 30 days are recorded as cured.

DRUG ACTIVITY. In the chick test, as in the mouse test, an increase of 100% in survival time has been considered as the minimum significantly effective response to the antimalarial activity of a compound.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST PLASMODIUM GALLINACEUM MALARIA IN CHICKS. Of the 36,625 compounds tested in chicks from June 1, 1970, to May 31, 1971, 1,483 demonstrated a degree of activity that produced a minimum of 100% increase in the survival time of Plasmodium gallinaceum infected chicks.

Of the 1,651 compounds tested in chicks from June 1, 1971, to May 31, 1972, 85 demonstrated a degree of activity that produced a minimum of 100% increase in the survival time of Plasmodium gallinaceum infected chicks.

The test was phased out at the end of this period.

TABLE IV

SUMMARY OF SCREENING LEVELS

JANUARY, 1965 - MAY, 1970

	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JANUARY, 1965 - MAY, 1965	375	5,715
JUNE, 1965 - MAY, 1966	2,400	31,935
JUNE, 1966 - SEPTEMBER, 1966*	1,002	17,220
SEPTEMBER, 1967 - MAY, 1968	2,982	24,855
JUNE, 1968 - MAY, 1969	6,722	64,625
JUNE, 1969 - MAY, 1970	26,049	166,765
TOTAL	39,530	311,115

*In September of 1966 an outbreak of an avian infectious disease involving entire flocks made it impossible to get the healthy birds that we required, and the chick test was temporarily dropped.

TABLE V

MONTHLY SCREENING LEVELS

JUNE 1, 1970 - MAY 31, 1971

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JUNE	4,164	22,165
JULY	3,758	20,380
AUGUST	3,841	20,515
SEPTEMBER	4,081	22,340
OCTOBER	4,112	22,130
NOVEMBER	3,563	19,000
DECEMBER	3,458	17,385
JANUARY	3,039	16,195
FEBRUARY	2,336	13,380
MARCH	3,131	16,705
APRIL	882	5,005
MAY	260	2,060
TOTAL	36,625	197,260

TABLE VI

MONTHLY SCREENING LEVELS

JUNE 1, 1971 - MAY 31, 1972

MONTH	NUMBER OF COMPOUNDS	NUMBER OF CHICKS
JUNE	342	2,585
JULY	301	2,075
AUGUST	306	2,060
SEPTEMBER	151	1,590
OCTOBER	36	335
NOVEMBER	105	1,095
DECEMBER	142	1,860
JANUARY	76	815
FEBRUARY	119	2,275
MARCH	58	615
APRIL	--	--
MAY	15	225
TOTAL	1,651*	15,730

*Avian blood test phased out May 31, 1972.

It is generally recognized that a screening procedure with sporozoite-induced Plasmodium gallinaceum malaria in chicks would be effective both as a primary screen of therapeutic and/or prophylactic activity or as a confirmatory test.

The development of such a procedure was undertaken chiefly to assess prophylactic values of candidate compounds.

In its initial phase, under Contract No. DA-49-193-MD-2218, the progress of our study was completely dependent on weekly shipments of frozen infected material prepared by an outside and distant supplier.

Within a period of twelve months studies with approximately 12,000 chicks and 200 compounds indicated that our dependence on this routine limited our investigations and thwarted our efforts to design a sporozoite-induced avian test approaching the degree of uniformity and reproducibility of our blood-induced mouse test and our blood-induced chick test. We were convinced that our studies would continue to be hampered until we were able to prepare the necessary infected material on our own premises.

Under Contract No. DADA 17-70-0100, activated June 1, 1970, we were authorized to set up a mosquito-rearing facility to provide the Aedes aegypti that we would require.

The sporozoite-induced avian malaria test that we have developed, like our blood-induced mouse test and our blood-induced chick test, is based on mortality, not on morbidity.

Modifications in methods of rearing and handling non-infected and infected mosquitoes were required to achieve this end.

These modifications, highly controlled, involved:

(a) nutritional requirements of larvae and pupae;
(b) more satisfactory methods of feeding and watering adult mosquitoesprior to and following blood-meals;
(c) types of blood-meals;
(d) methods for preparing a standard inoculum;
(e) avenues of drug administration and regimens.

Our results, obtained from studies with more than 100,000 chicks, indicate that our sporozoite-induced screen is approximating and will meet the standards demonstrated in our blood-induced screens.

Using chicks weighing 53-57 grams and a standard inoculum of sporozoites from Plasmodium gallinaceum-infected Aedes aegypti mosquitoes, we have been able to produce a disease that is fatal to 100% of untreated controls within 7.0 - 10.6 days, an overall average of 8.46 days.

Prophylactic activity is assessed by comparing the maximum survival time of treated sporozoite-infected chicks and the survival time of untreated sporozoite-infected controls.

A compound is considered active if it has produced increases of at least 100% in the survival time of treated chicks over the survival time of untreated controls.

Acceptance of a compound's prophylactic activity is predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED).

METHOD

TEST ANIMAL. White leghorn cockerels weighing 53-57 grams are used in these tests.

The birds, of fairly uniform stock, are purchased from local hatcheries, delivered to the laboratory when 1 day old and then maintained under stardard conditions, including a non-medicated diet, until they are ready for a test.

TEST PROCEDURE. Chicks on test receive an intrajugular injection of 0.5 ml. of Plasmodium gallinaceum-infected Aedes aegypti mosquitoes, ground whole and containing sporozoites.

The parasite strain is maintained in separate groups of chicks bitten by infected mosquitoes.

In order to check factors such as changes in the infectivity of the sporozoites or the Plasmodium gallinaceum strain, changes in the susceptibility of the host or simply to detect technical errors, a group of infected chicks treated with sulfadiazine at dose levels known to produce definite increases in survival time is included as a positive control in every experiment.

DRUG ADMINISTRATION. Treatment consists of a single dose, administered subcutaneously or per os, on the day of infection.

Compounds are dissolved or suspended in peanut oil before they are administered.

Each test is done with graded doses of the candidate compound and increases in the dose levels of highly active compounds have generally been followed by increases in the survival time of the treated chicks.

If an active compound was found to be toxic for the host, its toxicity became a limiting factor in changes of doses.

Deaths that occurred within 5 days after infection and treatment have been considered as deaths due to toxic effects of a test compound, not as the result of the infection introduced by the sporozoites from infected mosquitoes.

Chicks with survival periods of 30 days are recorded as "survivors".

DRUG ACTIVITY. An increase of 100% in survival time has been considered as the minimum significantly effective response to the prophylactic activity of a compound.

COMPOUNDS WITH PROPHYLACTIC ACTIVITY AGAINST PLASMODIUM GALLINACEUM MALARIA IN CHICKS. Routine testing began October 1, 1971. Of the 680 compounds tested from that day through December 31, 1971, 120 demonstrated a degree of activity that produced a minimum of 100% increase in the survival time of sporozoite-induced Plasmodium gallinaceum infections in chicks.

Of 268 compounds tested from January 1, 1972, to May 31, 1972, 9 demonstrated a degree of activity that produced a minimum of 100% increase in the survival time of sporozoite-induced Plasmodium gallinaceum infections in chicks.

DISTRIBUTION LIST

4 copies	HQDA (SGRD-RP) WASH DC 20314
12 copies	Defense Documentation Center (DDC) ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 copy	Dean School of Medicine Uniformed Services, University of the Health Sciences Office of the Secretary of Defense 6917 Arlington Road Bethesda, MD 20014