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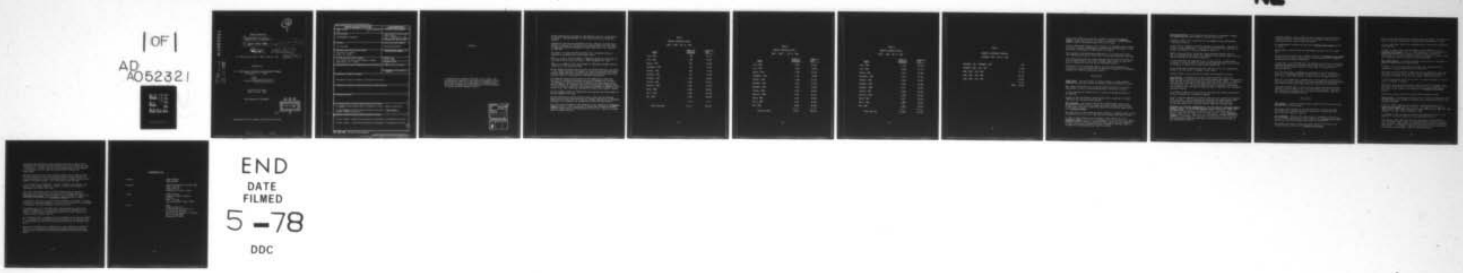

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19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

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.

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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.

34,093 compounds were screened for antimalarial activity in the period from June 1, 1966 through May 31, 1967 under Contract No. DA-49-193-MD-2218.

Although our contractural commitment was the screening of 27,500 compounds, in keeping with the policy set by Walter Reed, the final number of compounds tested has exceeded the number specified in our contract by about 25%.

This year, as in each preceding contract year, increased testing is graphically represented by a sharp upward curve.

Tables 1, 2 and 3 list the number of compounds tested and the number of mice used month by month from June 1, 1964 through May 31, 1967.

Table 4 is a summary of the total number of compounds screened from the inception of this program to date.

We had reached and gone beyond the point of optimum efficiency and safety in our present work area. To improve our working conditions and thereby ensure a higher degree of effeciency and safety, the United States Army provided funds for a new facility, now under construction.

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 supplied the large number of animals needed in our tests.

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

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

TABLE I

MONTHLY SCREENING LEVELS

JUNE 1, 1964 - MAY 31, 1965

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
June, 1964	763	15,111
July, 1964	758	12,810
August, 1964	593	10,306
September, 1964	521	8,543
October, 1964	558	9,146
November, 1964	612	9,788
December, 1964	1,279	20,249
January, 1965	1,634	25,013
February, 1965	1,399	21,228
March, 1965	1,999	30,831
April, 1965	1,378	23,188
May, 1965	1,620	29,502
TOTAL FOR YEAR	13,114	215,715

TABLE 2

MONTHLY SCREENING LEVELS

JUNE 1, 1965 - MAY 31, 1966

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
June, 1965	1,545	25,633
July, 1965	1,297	19,873
August, 1965	1,349	20,645
September, 1965	1,192	18,208
October, 1965	1,539	23,515
November, 1965	1,667	25,525
December, 1965	1,740	26,650
January, 1966	2,384	36,503
February, 1966	2,197	33,015
March, 1966	2,613	39,987
April, 1966	2,241	34,395
May, 1966	2,967	46,500
TOTAL FOR YEAR	22,731	350,449

TABLE 3

MONTHLY SCREENING LEVELS

JUNE 1, 1966 - MAY 31, 1967

MONTH	NUMBER OF COMPOUNDS	NUMBER OF MICE
June, 1966	2,314	36,220
July, 1966	2,686	41,175
August, 1966	2,871	44,825
September, 1966	2,216	34,420
October, 1966	2,644	41,325
November, 1966	2,670	42,285
December, 1966	2,712	42,055
January, 1967	3,048	47,325
February, 1967	3,838	59,970
March, 1967	3,215	49,545
April, 1967	2,886	45,510
May, 1967	2,993	46,545
TOTAL FOR YEAR	34,093	531,200

TABLE 4

NUMBER OF COMPOUNDS SCREENED

DECEMBER, 1961 - MAY 31, 1967

DECEMBER, 1961 - NOVEMBER, 1962	250
DECEMBER, 1962 - MAY, 1964	6,665
JUNE, 1964 - MAY, 1965	13,114
JUNE, 1965 - MAY, 1966	22,731
JUNE, 1966 - MAY, 1967	34,093
TOTAL -	76,853

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

Since an established disease is less sensitive to treatment than a disease in the early stages of development, treatment has been deliberately withheld until a high degree of parasitemia is 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 test compound must suppress the disease and give an unquestionably significant increase (100% or more) to the life-span of treated mice over that of the untreated controls.

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

M E T H O D

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 of 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 times is included in every experiment as a positive control.

DRUG ADMINISTRATION. Test compounds are dissolved or suspended in peanut oil before they are administered to infected test animals.

Treatment consists of a single dose of the compound 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 times 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 MALARIA IN MICE. Of the 34,093 compounds tested since June 1, 1966, over 336 demonstrated a degree of antimalarial activity sufficient to produce at least 100% increases in the survival times of treated Plasmodium berghei infected mice. These are: (1) sulfonamides, sulfones and related compounds; (2) sydnones; (3) pteridines; (4) antibiotics; (5) quinolines; (6) heterogeneous chemicals unrelated to structures previously shown to have antimalarial activity.

A second procedure, using a different host and parasite and performing reliably either as a confirmatory test or as another primary screen, is a desirable adjunct to any screening program.

Our supplementary procedure was done with Plasmodium gallinaceum malaria in chicks.

We developed a new and simple but dependable procedure for this second test.

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

In this test, as in the mouse test, the antimalarial activity of candidate compounds was assessed by comparing the maximum survival times of treated malaria-infected chicks with the survival times of untreated malaria-infected controls.

As in the mouse test, a compound was considered to be active against malaria if it produced increases in the survival times of treated chicks that were at least 100% over the survival times 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. 9-12 day old white Leghorn cockerels of uniform stock were obtained from a single breeder.

The animals were delivered to the laboratory when 1 day old and then maintained under standard conditions, including a non-medicated diet, until they were ready for testing.

TEST PROCEDURE. Chicks on test were given an intravenous 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 was drawn by cardiac puncture from donor birds infected 72 hours earlier with Plasmodium gallinaceum.

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

In every experiment 100% of the untreated controls 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 animals treated with chloroquine at dose levels producing definite increases in survival times was included as a positive control in every experiment.

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

Treatment in the chick consisted of a single dose administered subcutaneously or per os immediately after infection.

Each experiment was 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 times 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 were recorded as cured.

DRUG ACTIVITY. An increase of 100% in survival time was recognized as the minimum significantly effective response to the antimalarial activity of a candidate compound.

A total of 3,877 compounds was tested for antimalarial activity in Plasmodium gallinaceum malaria in chicks: 375 compounds from January, 1965 to June, 1965; 2,500 compounds from June, 1965 to June, 1966, and 1,002 compounds from June, 1966 to September, 1966.

In September, 1966, we began to notice disturbing peculiarities in the behavior of our positive controls and discontinued the test.

From January, 1965, when the chick test became part of our operation, until September, 1966, except for occasional minor variations, the antimalarial activity demonstrated in positive control groups by chloroquine was constant. In September, 1966, the antimalarial activity manifested by the same drug was greatly reduced, almost negligible.

Chloroquine was replaced by other accepted antimalarial agents and by compounds that had been found to be highly active in our mouse test and/or chick test. In every case the result was the same; the high degree of antimalarial activity previously established no longer was demonstrable.

Believing that our chicks might have been taken from an infected flock and that contaminants in the test chicks might be responsible for the altered antimalarial values, we obtained chicks of several species from a number of different sources. Still the results were the same.

An investigation was immediately started to determine the cause or the causes of the strange reactions. However, we have no satisfactory explanation to offer at this time.

Until the investigations that we have started reveal satisfactory explanations for the cause or the causes of the changed reactions of Plasmodium gallinaceum infected chicks to known antimalarial agents, our avian tests with be done with Plasmodium lophurae malaria in ducks.

In developing this test, we shall keep the methods of procedure, including treatment by candidate compounds, as nearly like as possible to the methods of procedure, including treatment, of our chick test.

In the duck test, as in the mouse test, the antimalarial activity of candidate compounds will be assessed by comparing the maximum survival times of treated malaria-infected ducks with the survival times of untreated malaria-infected controls.

As in the mouse test, a compound will be considered active against malaria if it suppresses the disease and produces increases in the survival times of treated ducks at least 100% over the survival times of untreated controls.

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

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