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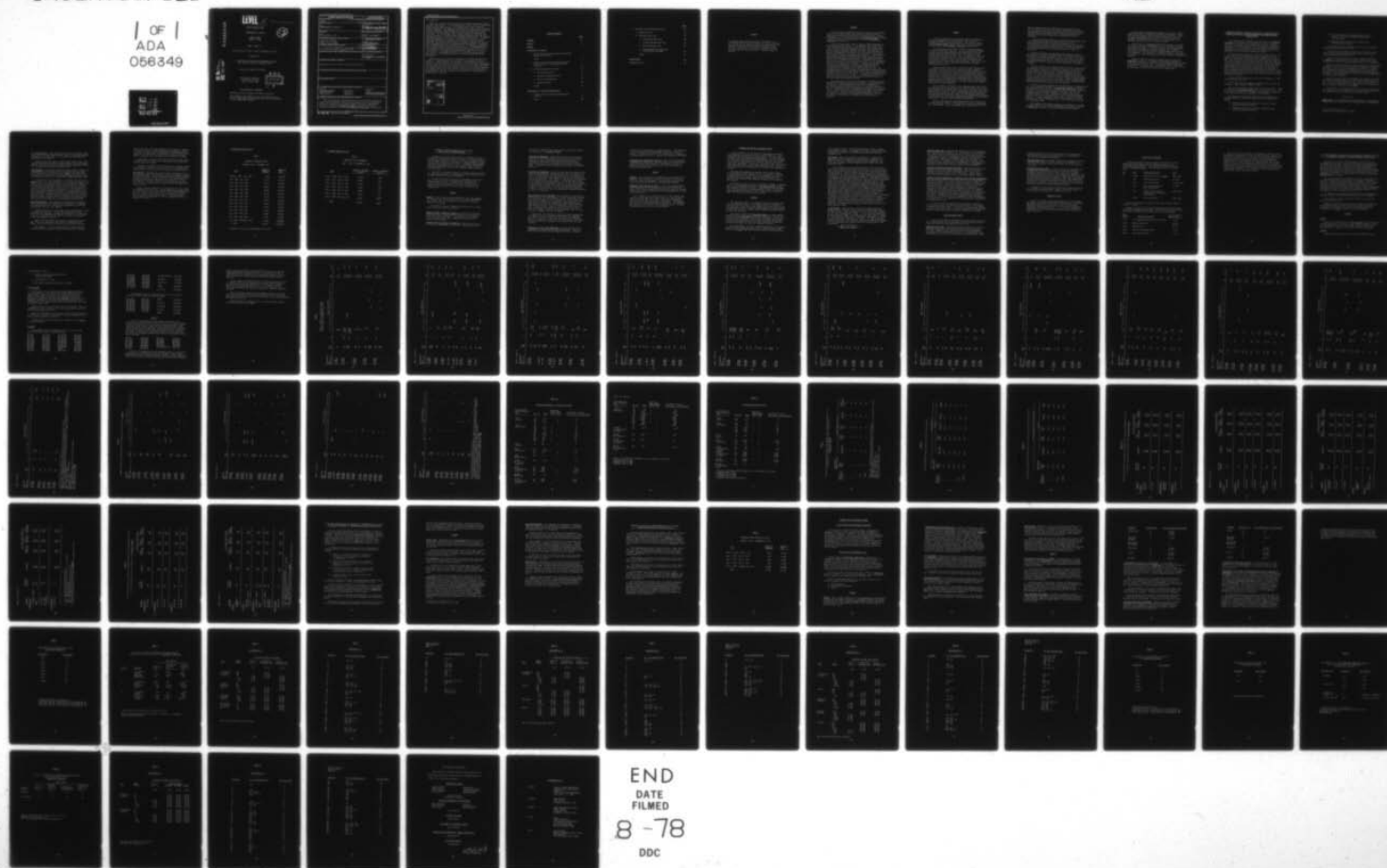
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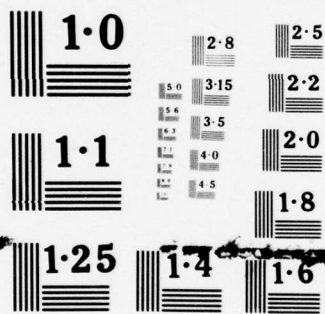
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CHEMOTHERAPY OF MALARIA

ANNUAL REPORT
February, 1978

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ARBA L. AGER, JR.

For the period of June 1, 1976, to September 30, 1977

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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Rane Research Laboratory
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The first primary drug screen in malaria assessed compounds for blood schizonticidal activity. 7,114 compounds were tested, with 1,124 exhibiting blood schizonticidal activity. The second primary screen in malaria assessed compounds for prophylactic antimalarial activity against sporozoite induced infections. 831 compounds were tested for prophylactic antimalarial activity and 405 had curative effects while 149 were more active than primaquine. In the secondary drug screening program, 55 compounds were found to be more active than quinine when administered via the oral and subcutaneous routes. The most active compound was WR 226,337. 36 compounds were tested against one or more of the 6 drug-resistant lines. A line moderately resistant to mefloquine was developed. A special dietary experiment and two tests to determine if synergistic suppressive activity occurred between WR 225,329 + pyrimethamine and WR 225,329 + trimethoprim were completed against the drug-sensitive line. No enhanced suppressive activity occurred between these drug combinations. 29 compounds and two drug combinations were tested for repository antimalarial activity for a 17 day period. 12 of these compounds exhibited repository activity for the 17 days. 11 compounds were tested for repository activity of 90 days with only two retaining activity for this period (WR 102,796 and WR 158,122).

The primary test in African trypanosomiasis included evaluating compounds for trypanosomicidal activity against a drug-sensitive line of parasites. 4,235 compounds were tested for trypanosomicidal activity and 396 were found to be active. The secondary test with African trypanosomiasis involved developing three major drug-resistant lines; a suramin-resistant, a stilbamidine-resistant, and a berenil-resistant line. Selected compounds were tested against the first two resistant lines for cross resistance determinations. One line resistant to a combination of stilbamidine and WR 163,577 was developed.

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FOREWORD

In conducting the research described in this Report, the investigator adhered to the principles set forth in the Guide for Care and Use of Laboratory Animals as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute for Laboratory Animal Resources, National Research Council - National Academy of Sciences.

ABSTRACT

The investigations undertaken during this report period included two primary and one secondary drug screening program in malaria, along with a primary and secondary drug screening program in African trypanosomiasis. The malarial system used Plasmodium berghei infected mice while the African trypanosomiasis system used Trypanosoma rhodesiense infected mice.

The first primary drug screen in malaria assessed compounds for blood schizonticidal activity. 7,114 compounds were tested, with 1,124 exhibiting blood schizonticidal activity. The second primary screen in malaria assessed compounds for prophylactic antimalarial activity against sporozoite induced infections. 831 compounds were tested for prophylactic antimalarial activity and 405 had curative effects while 149 were more active than primaquine. In the secondary drug screening program, 55 compounds were found to be more active than quinine when administered via the oral and subcutaneous routes. The most active compound was WR 226,337. 36 compounds were tested against one or more of the 6 drug-resistant lines. A line moderately resistant to mefloquine was developed. A special dietary experiment and two tests to determine if synergistic suppressive activity occurred between WR 225,329 + pyrimethamine and WR 225,329 + trimethoprim were completed against the drug-sensitive line. No enhanced suppressive activity occurred between these drug combinations. 29 compounds and two drug combinations were tested for repository anti-malarial activity for a 17 day period. 12 of these compounds exhibited repository activity for the 17 days. 11 compounds were tested for repository activity of 90 days with only two retaining activity for this period (WR 102,796 and WR 158,122).

The primary test in African trypanosomiasis included evaluating compounds for trypanosomicidal activity against a drug-sensitive line of parasites. 4,235 compounds were tested for trypanosomicidal activity and 396 were found to be active. The secondary test with African trypanosomiasis involved developing three major drug-resistant lines: a suramin-resistant, a stilbamidine-resistant, and a berenil-resistant line. Selected compounds were tested against the first two resistant lines for cross resistance determinations. One line resistant to a combination of stilbamidine and WR 163,577 was developed.

SUMMARY

Primary and secondary drug screening programs in malaria and African trypanosomiasis for the period of June 1, 1976, to September 30, 1977, are described herein. The malarial system used Plasmodium berghei infected mice while the African trypanosomiasis system used Trypanosoma rhodesiense infected mice.

The first primary drug screen in malaria assessed compounds for blood schizonticidal activity. This test consisted of infecting the mice with asexual parasites and administering one subcutaneous dose of drug three days later. The results of drug activity were based upon survival time of treated mice in relation to infected, untreated controls. A drug was considered active if treated mice survived at least twice as long as untreated mice. Mice surviving for 60 days were considered cured. There were 7,114 compounds tested in this system with 1,124 exhibiting blood schizonticidal activity.

A second primary drug screen in malaria assessed compounds for prophylactic antimalarial activity against sporozoite induced infections. In this test mice were given one subcutaneous injection of drug and four hours later received an intraperitoneal injection of sporozoites. Prophylactic activity was determined by monitoring mortality daily with drug activity based only upon curative effects. Mice alive for a 30 day period were considered cured. There were 831 compounds tested for prophylactic antimalarial activity with 405 compounds exhibiting curative effects. At least 149 of these compounds were more active than primaquine.

In one secondary antimalarial test, compounds were tested against the drug-sensitive P-line and one or more drug-resistant lines. Mice were inoculated with asexual parasites on day 0, followed by oral drug administration on days 3, 4 and 5. Blood smears were made on the 6th day and the percentage of cells parasitized and percent suppression of parasitemia were determined. There were 74 compounds tested against the drug-sensitive line with 63 exhibiting suppressive activity greater than quinine. The most active compound was WR 226,337.

There were 36 compounds administered against one or more of the six drug-resistant lines. A line moderately resistant to mefloquine was also developed. Selected compounds were tested against this line

and the completely resistant mefloquine line by giving drug on days 3, 4 and 5 or days 5, 6 and 7 after inoculation of parasites. The rationale for this delayed administration of drugs was to allow the parasitemia to increase so that a more accurate statistical analysis of the percent suppression of parasitemia could be determined.

A special experiment examined the effects of various foods given after starvation of mice on the suppressive action of the thioquinazoline, WR 158,122, against the drug-sensitive line. In the starved mice receiving a glucose solution the percent suppression of parasitemia was significantly increased. Corn oil and similax (a liquid baby food) also increased the suppressive effect of WR 158,122.

Two tests to detect the synergistic suppressive activity of WR 225,329 + pyrimethamine and WR 225,329 + trimethoprim against the drug-sensitive line were completed. All three of the 1:1, 2:1 and 4:1 mixtures of WR 225,329 + pyrimethamine, respectively, were found to produce antagonistic effects. The two mixtures of WR 225,329 + trimethoprim (1:25 and 1:50, respectively) were found to be additive in nature.

The last aspect of the secondary program in malaria involved testing compounds for repository antimalarial activity. Mice were given one subcutaneous injection of drug and challenged either 3, 10 or 17 days later with infected blood. Selected compounds retaining activity for 17 days were further tested for periods of 30, 60 and 90 days. A total of 29 compounds and two drug combinations were tested for repository activity of up to 17 days. Twelve of these compounds retained repository activity for this period. Eleven compounds were examined for repository activity of 90 days with only two (WR 102,796 and WR 158,122) retaining activity for this period.

A primary screening procedure for the evaluation of trypanosomicidal activity of candidate compounds in Trypanosoma rhodesiense infected mice evaluated a total of 4,235 compounds, 396 of which were recognized as active. In this procedure, groups of mice were infected with trypomastigotes and treated immediately thereafter with one subcutaneous injection of drug. Assessment of activity was made by comparing survival time of treated mice to that of infected, untreated controls. An active compound was one in which treated mice live at least twice as long as untreated animals. Mice surviving for 30 days were considered cured.

The secondary drug screening program in trypanosomiasis included a special repository test and the development and testing of lines of T. rhodesiense resistant to selected trypanosomicidal compounds.

A special test to determine the repository activity of three compounds (ZG 76354, AH 55296 and BG 00521) was completed. Treated mice were challenged at various time intervals and survival time was monitored to determine the duration of repository activity. ZG 76354 and AH 55296 retained activity for seven months, while BG 00521 remained active for at least ten months.

Three lines of T. rhodesiense moderately resistant to suramin, stilbamidine and berenil, respectively, were developed by repeated drug pressure in vivo. The suramin-resistant line displayed a 108-fold degree of resistance to suramin, whereas the stilbamidine-resistant line displayed as high as a 260-fold degree of resistance to stilbamidine. The suramin and stilbamidine-resistant lines were also tested for cross resistance against other selected trypanosomicidal compounds.

A special study was designed to develop and test lines of T. rhodesiense resistant to stilbamidine and WR 163,577 (BG 00521) alone and in combination. Resistance to each compound alone developed at approximately the same rate. In comparing rates of acquisition of resistance, it appeared that the development of resistance was not hindered when the drugs were administered in combination.

A SCREENING PROCEDURE FOR ASSESSING THE BLOOD SCHIZONTICIDAL ANTI-
MALARIAL ACTIVITY OF CANDIDATE COMPOUNDS IN PLASMODIUM BERGHEI
INFECTED MICE

The recognition of chloroquine-resistant strains of Plasmodium falciparum in South America and Southeast Asia first posed what is now a critical problem in the chemotherapy of malaria. Parasite resistance to 4-aminoquinolines (e.g., chloroquine and amodiaquine), antifolates (e.g., pyrimethamine) and other standard antimalarial compounds such as quinine has caused an increased concern for the development of safe alternative therapeutic agents.

The World Health Organization currently estimates that over 100 million cases of malaria worldwide require treatment each year. Recently, chloroquine-resistant parasites have been noted in Africa, where over one million children die from malaria yearly. Reports from India, Pakistan, and Sri Lanka indicate a significant resurgence of malaria in that part of the world, with India alone experiencing a rate of approximately 25,000 new cases per day. The current widespread endemicity of malaria and its potential for recurrence in malaria-free zones, the emergence of populations of parasites in Central and South America, Asia and Africa that are resistant to the major available antimalarial agents, and a decrease in vector control programs emphasize the need for continued mass screening of candidate antimalarial compounds.

A total of 263,771 compounds were tested from December 1, 1961, through September 30, 1977.

Table I summarizes the compounds tested and the mice used from December 1, 1961, through September 30, 1977.

The test system designed specifically for this operation is based on blood-induced Plasmodium berghei malaria infections in mice. It is a relatively simple and fast procedure. Assessments of antimalarial effect and host toxicity are reproducible and reliable.

All compounds evaluated were obtained from the Department of Medicinal Chemistry at the Walter Reed 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;
- (4) compounds known to have activity against other infectious disease agents.

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

Drug activity was assessed by comparing the maximum survival time of treated malaria-infected animals to the survival time of untreated malaria-infected controls.

Using five and six week old mice and a standard inoculum of P. berghei, it has been possible to produce a consistently uniform disease fatal to 100% of untreated animals within six to seven 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 fairly high degree of parasitemia is evident. Test compounds are administered subcutaneously in a single dose on the third day post-infection at which time a 10-15% parasitemia has developed. A similar procedure is followed for the oral administration of selected active compounds.

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. To be considered curative, treated animals must remain alive for 50 days after infection with P. berghei.

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.

M E T H O D *

ANIMAL HOSTS. The total supply of animals needed to screen candidate compounds has been obtained from our breeding colony of ICR/HA Swiss

*Designed and developed by Dr. Leo Rane.

mice (Mus musculus). Test animals weigh from 18 to 20 grams, weight variations in any given experimental or control group being carefully limited to two to three grams. In any given test all animals are approximately the same age.

Animals on test are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib. Once the infected mice are given the drug they are placed in a room maintained at 84° F (+ 2° F) and a relative humidity of 66% (+ 2%).

TEST PROCEDURE. Test animals receive an intraperitoneal injection of approximately 6.8×10^5 parasitized erythrocytes drawn from donor mice infected four days earlier with P. berghei. The donor strain is maintained by passing every four days in separate groups of mice inoculated with 0.5 cc of a 1:50 dilution of heparinized heart blood.

To check factors such as changes in the infectivity of our P. berghei strain or in the susceptibility of the host, one group of mice which serves as the negative control is infected but not treated. In order to determine the effect a drug exerts on a malaria infection two parameters are measured; the first is an increase in survival time, and the second concerns curative action. For comparative purposes one standard compound, pyrimethamine, is administered at one level (120 mg/kg) to a group of 20 mice. Pyrimethamine serves as a positive control, producing definite increases in survival time and curative effects. Another function of the positive control involves monitoring three procedures; the drug weighing, the preparation of drug solutions and suspensions, and the administration of drugs.

DRUG ADMINISTRATION. Test compounds are dissolved or suspended in peanut oil before they are administered subcutaneously. Compounds to be administered orally are mixed in an aqueous solution of 0.5% hydroxyethylcellulose - 0.1% tween-80.

Treatment consists of a single dose given subcutaneously or orally three 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.

Each compound is initially administered in three graded doses diluted four-fold to groups of five mice per dose level. The top

dose is 640, 320 or 160 mg/kg depending on the amount of compound available for testing. Active compounds are subsequently tested at six or nine dose levels, diluted two-fold from the highest dose. Successive six-level tests are performed at respectively lower doses if necessary until the lower limit of activity is reached.

A drug that is toxic for the host at each of the three levels initially tested is retested at six dose levels diluted two-fold, from the lowest toxic dose.

Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice. Treated animals alive at the end of 60 days are considered 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 effective dose (MED) producing a significant effect. An MTD is defined as the highest dose up to 640 mg/kg causing no more than one of five animals to die from drug toxicity. The MED is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

Clearly inactive compounds are rejected after one test; borderline compounds after two tests. Active compounds are characterized by a dose-response curve, which establishes the spread between the MTD and the lower limit of activity by a determination of drug activity in the dose level dilution tests. The total number of active compounds from December 1, 1961, to September 30, 1977, is summarized in Table II.

P. BERGHEI MALARIA IN MICE

TABLE I

SUMMARY OF SCREENING LEVELS
DECEMBER, 1961 - SEPTEMBER, 1977

<u>YEAR</u>	<u>NUMBER OF COMPOUNDS</u>	<u>NUMBER OF MICE</u>
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
June, 1970 - May, 1971	18,108	322,140
June, 1971 - May, 1972	14,874	262,245
June, 1972 - May, 1973	14,276	231,450
June, 1973 - May, 1974	11,035	168,664
June, 1974 - May, 1975	10,604	168,725
June, 1975 - May, 1976	9,916	155,585
June, 1976 - September, 1977	<u>7,114</u>	<u>123,085</u>
TOTAL	263,771	4,430,278

*Includes mice used in the development of the test.

P. BERGHEI MALARIA IN MICE

TABLE II
SUMMARY OF ACTIVE COMPOUNDS
JUNE 1, 1970 - SEPTEMBER 30, 1977

<u>YEAR</u>	<u>NUMBER OF COMPOUNDS TESTED</u>	<u>NUMBER OF COMPOUNDS ACTIVE</u>
June 1, 1970 - May 31, 1971	18,108	805
June 1, 1971 - May 31, 1972	14,874	593
June 1, 1972 - May 31, 1973	14,276	771
June 1, 1973 - May 31, 1974	11,035	394
June 1, 1974 - May 31, 1975	10,604	616
June 1, 1975 - May 31, 1976	9,916	351
June 1, 1976 - Sept. 30, 1977	<u>7,114</u>	<u>1,124</u>
TOTAL	85,927	4,654

SPOROZOITE INDUCED ANTIMALARIAL TEST IN MICE INFECTED WITH PLASMODIUM BERGHEI

Primaquine is the only drug currently used today for causal prophylactic antimalarial activity in humans. This 8-aminoquinoline has two major limitations; the first is its poor therapeutic index, and the second concerns its involvement in causing hemolytic anemia in persons with a deficiency in glucose 6-phosphate dehydrogenase. New active 8-aminoquinolines as well as other groups of chemicals exhibiting prophylactic activity are needed to combat malaria in the world today.

This test is intended to serve as a primary screening procedure for compounds submitted by the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research.

In this test system mice receive a subcutaneous injection of drug four hours prior to an intraperitoneal inoculation of sporozoites and survival is monitored for a 30 day period. A similar procedure is followed for the oral administration of selected active compounds. Mice alive after 30 days are considered cured.

METHODS

ANIMALS. Male or female outbred ICR/HA Swiss mice (Mus musculus) six to seven weeks old and weighing 16 to 17 grams, are used as test animals. They are maintained in groups of five and fed water and feed ad lib.

Mice used as a source of gametocytes (donor mice) are eight weeks of age and weigh 25 to 30 grams.

MOSQUITO COLONY. Anopheles stephensi are reared in an insectary maintained at 80° F and 70% relative humidity, with 14 hours of light and 10 hours of darkness. Larvae are fed a solution of 2.5% liver powder once a day. Emerged adults are fed a 10% glucose solution ad lib.

INFECTED MICE AS A SOURCE OF GAMETOCYTES. Donor mice to be used as a source of gametocytes are injected intraperitoneally with

a dilution of infected heart blood from mice previously infected with sporozoites of Plasmodium berghei.

INFECTION OF MOSQUITOES. Mosquitoes are placed in a room maintained at 70° F and 70% relative humidity prior to the infected blood meal. Donor mice harboring a 5-20% parasitemia are anesthetized with Nembutal and placed on top of the mosquito cages for one hour to allow the mosquitoes to feed on infected blood. The mosquitoes are thereafter maintained on a 10% glucose solution.

ISOLATION OF SPOROZOITES. On the 17th day after the infected blood meal, the mosquitoes are anesthetized with ether, collected in a plastic bag and weighed. Two and one-half ml. of 0.9% saline plus 2.5 ml. of inactivated mouse plasma are injected into the bag containing the mosquitoes. The contents of this bag are then macerated on a cold table with a teflon plunger. Saline and mouse plasma (1:1) are added to the homogeneous mass on the basis of the weight of the mosquitoes and the dilution desired. This uniform suspension is then filtered to remove legs, wings, tissue and exoskeleton fragments of the mosquitoes. The filtered sporozoite suspension is further diluted until there are approximately 250,000 sporozoites per 0.2 ml. of inoculum.

ADMINISTRATION OF TEST COMPOUNDS. Each compound is ground with a mortar and pestle and then suspended in 0.5% hydroxyethylcellulose-0.1% tween-80 to make the desired drug doses. The percent free base of each compound is not determined. Four hours prior to the inoculation of sporozoites, compounds are administered either subcutaneously or orally at three graded doses diluted four-fold (160, 40 and 10 mg/kg). Groups of five mice per dose level are used. Subsequent tests employing successive lower four-fold dilutions are made if mice are cured at 10 mg/kg, until the lower limit of a compound's activity is reached.

Deaths that occur before the seventh 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. A drug that is toxic for the host at each of the three initial dose levels is retested at doses diluted four-fold from 10 mg/kg.

INOCULATION OF MICE WITH SPOROZOITES. Mice are injected intraperitoneally with approximately 250,000 sporozoites. Twenty of these mice are divided into two groups of ten each. One group

receives no drug and serves as a negative control. The other group is treated with WR 181,023 (100 mg/kg) and acts as a positive control. One additional group of five infected mice, serving as a treated negative control, is treated with chloroquine (100 mg/kg).

DETERMINATION OF ANTIMALARIAL ACTIVITY. After the mice have been inoculated with sporozoites, they are placed in a room maintained at 84° F and 66% relative humidity. Antimalarial activity is determined by monitoring mortality daily. Mice alive after 30 days are considered cured.

RESULTS

CONTROLS. Mice inoculated with sporozoites but receiving no drug (negative control group) all routinely die within 7 to 12 days, as do mice receiving chloroquine. Mice serving as positive controls survive for the duration of the experiment (30 days).

COMPOUNDS TESTED AND DRUG ACTIVITY. In the first 178 experiments, 1,684 three-level tests were performed using over 29,810 mice. A total of 831 different compounds were tested at least once and many compounds were tested several times either subcutaneously or orally or via both routes.

For a compound to be considered active it must produce cures (survivors for 30 days) in at least two out of five mice at the highest tolerated drug level tested. There were 99 compounds which were active both subcutaneously and orally. 162 compounds were active only when administered subcutaneously. 144 compounds were active only via the oral route of administration. At least 149 compounds were more active than primaquine.

SECONDARY ANTIMALARIAL SCREENING SYSTEM

Current prospects for the control of human malaria have been complicated by the occurrence of drug-resistant parasites. Such resistance falls into three categories, namely: (1) resistance to antifolate drugs (pyrimethamine, chloroguanide, etc.); (2) resistance to 4-aminoquinolines and acridines (chloroquine, atebine, quinine, etc.); and (3) a combination of (1) and (2), which is referred to as multiple resistance. Collectively, the several types of resistance impair the effectiveness of all major suppressive drugs. Hence, a great need exists for alternative drugs, as well as new combinations of drugs.

New candidate compounds are emerging from a primary blood schizonticidal screening program, and it is particularly important to determine quite early which of the new candidates are likely to be useful against the various types of drug-resistant malaria. Experience has indicated that plasmodia of animals can be used for this purpose.

The specific aims of this test system were to conduct a sequential battery of chemotherapeutic studies in Plasmodium berghei infected mice on active compounds (discrete or open) emerging from the Department of Defense-sponsored screening programs in order to determine which substances were worthy of further consideration as potential agents for dealing with drug-resistant malaria.

METHODS

The techniques used in this secondary drug testing program fell into two categories, namely: (1) studies designed to determine if a new agent was likely to be useful against the various types of drug-resistant malaria; and (2) general chemotherapeutic characterization of selected new agents to suggest optimal methods of use and specific purposes they may serve.

The testing was done with Plasmodium berghei in outbred ICR/HA female Swiss mice (Mus musculus) weighing 20-25 grams. Briefly, this testing entailed procedures for the direct assessment of the effects of drugs on the parasitemia. Various gross tolerance observations were also recorded which served as guides indicating the usefulness of the new test agents as drugs for treatment of malaria.

More specifically, activities included elucidation of the apparent mode of action of agents by testing them in parallel against drug sensitive P. berghei (KBG-173) and various drug-resistant derivatives

of this malaria strain. The 6 drug-resistant derivatives included a chloroquine-resistant, a cycloguanil-resistant, a dapsone-resistant, a mefloquine-resistant, a pyrimethamine-resistant and a quinine-resistant line.

TEST DESIGN. When a new compound is obtained it is subjected to a battery of testing procedures, the extent of which depends on its degree of activity in suppressing murine malaria infections. The first test procedure is a 6-day suppressive test against the drug-sensitive P-line.

If the compound is active against the P-line then a 6-day test against one or more drug-resistant lines follows. In this basic 6-day test, mice are divided into groups of 7 and inoculated with parasites intraperitoneally. Drugs are administered twice a day, usually orally, in a volume of 10 ml/kg on the 3rd, 4th and 5th days after inoculation of parasites. All drugs are mixed in aqueous 0.5% hydroxyethylcellulose-0.1% tween-80 and ultrasonicated when necessary. Drug doses are prepared using 100% of the free base of each drug. One group of 10 infected mice receives the vehicle alone and serves as a negative control. Thin blood films and final group weights are taken on the 6th day after inoculation of parasites. Microscope examination of Giemsa-stained blood smears is made to determine the percentage of cells parasitized. Raw data are evaluated with the aid of a computer which calculates the percent weight change of mice, percent of cells parasitized, percent suppression of parasitemias, and significance values for the suppression of parasitemias. Significance values are based on a calculation of the percent suppression of parasitemia which is determined by comparing the parasitemia of each treated mouse with the mean parasitemia of the negative controls. Drug tolerance is reflected by the percent weight change and the proportion of mice that survive treatment. Toxicity is attributed to drug action when a -14% or greater weight change occurs or when one or more mice die before the blood smears are taken.

P-LINE TESTING. Each new drug is tested first against the drug-sensitive P-line, usually via both the oral and subcutaneous routes of administration. The drug dosages for the first test are normally 64, 16, 4 and 1 mg/kg/day for 3 days. If less than a 90% suppression of the parasitemia (SD_{90}) is obtained with the lower dose of 1 mg/kg/day then testing at lower doses is performed. Chloroquine is tested as a reference against the P-line at levels of 2, 3 and 4 mg/kg/day. A quinine index (Q) is calculated by comparing the SD_{90} value obtained from the chloroquine dose response curve and the SD_{90} value of the new compound:

$$Q = \frac{SD_{90} \text{ of chloroquine}}{SD_{90} \text{ of new compound}} \times 30$$

DRUG-RESISTANT LINES. Compounds that suppress the P-line parasitemia by at least 90% with 64 mg/kg or less are subjected to testing against one or more of the six drug-resistant lines. These lines include a chloroquine-resistant, a cycloguanil-resistant, a dapsone-resistant, a mefloquine-resistant, a pyrimethamine-resistant and a quinine-resistant line. The amount of testing against the resistant lines depends upon the structure of each new compound as it relates to the structure of known antimalarials. A maximum dose of 256 mg/kg/day is administered orally along with doses of 64, 16 and 4 mg/kg/day.

ESTIMATES OF POTENCY AND CROSS RESISTANCE. Doses required for a given degree of effect, such as 90% suppression or SD₉₀, are estimated graphically from plots made on log-probit paper. The ratios of the SD₉₀ (or whatever other level of effect, e.g., SD₇₀ or SD₅₀) is used to delineate the degree of cross resistance (Tables I and II).

SYNERGISTIC AND/OR ANTAGONISTIC SUPPRESSIVE TEST WITH DRUG COMBINATIONS. When two drugs are administered at the same time to an established infection of malaria one of three things can result with regard to the ensuing parasitemia: an additive suppressive effect; a greater than additive suppressive effect (potentiation or synergism); or a less than additive suppressive effect (antagonism). A synergistic suppressive effect appears to be most pronounced when the compounds involved have related but different modes of action. For example, sulfonamides and pyrimethamine inhibit the metabolism of the parasites at different sequential steps along the same biochemical pathway of folic acid. Sulfonamides block para-aminobenzoic acid from being incorporated into folic acid while pyrimethamine inhibits dihydrofolic acid reductase which is responsible for the conversion of dihydrofolic to tetrahydrofolic acid.

In order to test for synergistic or antagonistic suppressive activity the two drugs are administered either alone or as a mixture by gavage twice daily on days 3, 4 and 5 after the mice were infected via the intraperitoneal route. The effects determined from parasitemia counts of blood smears made one day after completion of treatment.

DRUG-RESISTANT STUDIES

A total of 74 different compounds were tested against the P-line. 36 of these compounds were tested against one or more drug-resistant lines, while 38 were tested against only the P-line.

DRUG-SENSITIVE P-LINE. 55 compounds were active both orally and subcutaneously against the P-line and were more active than quinine. Of 5 active compounds administered both orally and subcutaneously, 3 were more active than quinine only orally, 1 was more active only subcutaneously, and 1 was of the same activity by both routes. Eight

compounds administered only orally were more active than quinine. Four compounds were not active either orally or subcutaneously. The most active compound was WR 226,337.

DRUG-RESISTANT LINES. The number of dose level dilution tests used with each drug-resistant line are indicated in parentheses: A-line (10), C-line (23), M-line (15), S-line (13), T-line (23) and U-line (12).

MEFLOQUINE-RESISTANT LINES. A series of tests were performed with the moderately mefloquine-resistant B-line and the completely mefloquine-resistant A-line as to their sensitivity to several different standard antimalarials and to several new compounds. Drugs were administered on the 3rd, 4th and 5th and/or on the 5th, 6th and 7th days after inoculation of parasites. Blood smears were taken on the day following the last drug administration. The rationale for delaying the drug administration until the 5th day was to allow the parasitemia to increase in the negative control so a more reliable statistical analysis of the data could be attained.

It appears from the data that delaying drug administration for two additional days does allow for better statistical analysis of the suppression of parasitemia. (Tables III and IV.)

SYNERGISTIC TESTS

Two tests to detect synergistic suppressive activity between WR 225,329 + pyrimethamine (WR 2,978) and WR 225,329 + trimethoprim (WR 5,949) against the drug-sensitive P-line were performed. Antagonistic effects were noted with 1:1, 2:1 and 4:1 mixtures of WR 225,329 + WR 2,978, respectively. Two mixtures of WR 225,329 + WR 5,949 (1:25 and 1:50, respectively) were found to exhibit only additive effects.

SPECIAL DIET EXPERIMENT

A special experiment was done to examine the effects of various foods given after starvation on the suppressive action of the thioquinazoline WR 158,122 in mice infected with the drug-sensitive P-line of Plasmodium berghei. The experimental design is outlined below.

<u>Day</u>	<u>Time</u>	<u>Procedure with mice</u>	<u>Diet</u>
0	10 AM	Infected mice with <u>P. berghei</u>	Mouse chow
2	7 PM	Removed mouse chow	No food
3	7 AM	Gave various foods	Various foods
3	9 AM	Gave drug (WR 158,122) then removed food	No food
3	9 PM	Placed all mice back on mouse chow until completion of experiment	Mouse chow
6	10 AM	Took blood smears	Mouse chow

The test plan showing the groups of mice given different foods is outlined in Table V.

Groups 1-5 served as non-starved infected controls receiving mouse chow ad lib throughout the experiment. The amount of each type of food given orally to groups 6-30 at 7 AM on day 3 is outlined below:

<u>Mouse Group #</u>	<u>Description of food</u>	<u>Amt. of food per mouse orally</u>
6-10	0.5% hydroxyethylcellulose-0.1% tween-80	1.0 cc
11-15	Regular mouse chow	<u>ad lib</u>
16-20	100% corn oil	0.5 cc
21-25	Similax (liquid baby food)	1.0 cc
26-30	50% glucose solution	1.0 cc

The results of this experiment are tabulated in Tables VI and VII. Based upon the parasitemias in Table VI and the percent suppression of parasitemias in Table VII it can be seen that after the infected mice had been starved for 12 hours the glucose solution enhanced the suppressive activity of WR 158,122 at each of the four dose levels (0.0625, 0.25, 1.0 and 4.0 mg/kg) to a greater degree than the other foods. Corn oil and Similax were much more effective in aiding the suppressive effect of the thioquinazoline than mouse chow and HEC-tween. In conclusion, it appears that the suppressive action of WR 158,122 is enhanced when infected mice are starved for 12 hours and then given food two hours before drug administration.

A SCREENING PROCEDURE FOR ASSESSING THE REPOSITORY ANTIMALARIAL ACTIVITY OF CANDIDATE COMPOUNDS IN PLASMODIUM BERGHEI INFECTED MICE

An effective, reliable screening program is essential for the development of single dose antimalarial drugs that are protective for prolonged periods of time. In ten years of research and development only a few drugs have shown promise as repository antimalarials (acedapsone and cycloguanil in particular). These have found limited use in the field, due to such factors as the ease with which resistance to some of the compounds is induced, the variable drug sensitivity of Plasmodium species, and the local discomfort that may be produced upon administration of the drug.

The screening program described herein permits a determination of the repository activity of large numbers of compounds in different vehicles. The experimental design for such a program is based on tests of several standard antimalarials and new drugs found active in this laboratory's primary antimalarial screening tests, mixed in up to three different vehicles and administered to mice. Mice are subsequently challenged at various time intervals with Plasmodium berghei infected erythrocytes to test for repository activity.

All compounds evaluated have been obtained from the Department of Medicinal Chemistry at the Walter Reed Army Institute of Research.

Animals used in the tests have been supplied by our breeding colony of ICR/HA Swiss mice.

Compounds displaying curative activity through the seventeen day challenge are further evaluated for long term repository activity by extending the length of time between drug administration and challenge with P. berghei to periods of up to three months.

METHODS

ANIMALS

Male or female ICR/HA Swiss mice (Mus musculus) six to seven weeks old and weighing 19 to 21 grams are used. They are placed in a room maintained at 75° F (+ 2° F) and a relative humidity of 66% (+ 2%). Mice are housed in groups of five and fed water and feed ad lib.

VEHICLES

Compounds tested for repository action are suspended in up to

three different vehicles:

- 1) aqueous - 0.5% hydroxyethylcellulose - 0.1% tween-80 (HEC);
- 2) refined peanut oil (PO);
- 3) 40% benzyl benzoate and 60% castor oil (BBC).

TEST PROCEDURE

On day zero, mice are given a single subcutaneous injection of the drug suspension. Negative controls receive injections of the vehicle alone. On days +3, +10 or +17, treated and control subgroups are challenged in parallel with approximately 5.0×10^5 parasitized erythrocytes obtained from P. berghei infected donor mice. Compounds displaying activity when administered 17 days prior to challenge are further tested using a similar procedure with challenges on days +30, +60 or +90.

Negative controls all die 6-9 days after the challenge. Mice are challenged only once. Mortality over a four week period is used as an index of drug repository activity.

Deaths occurring before the 6th day post-infection are considered to be a result of a compound's toxic effects. Mice are examined daily for local cutaneous reactions to the drug.

Treated animals alive four weeks after infection with P. berghei are considered cured.

COMPOUNDS

The compounds tested for repository activity of up to 17 days and the drug levels used are summarized below:

WR 448	160 mg/kg	WR 142,490	640 mg/kg
WR 1,543	400 mg/kg	WR 158,122	80 mg/kg
WR 1,544	100 mg/kg	WR 159,412	400 mg/kg
WR 2,976	256 mg/kg	WR 180,409	160 mg/kg
WR 2,978	100 mg/kg	WR 180,872	80 mg/kg
WR 4,629	320 mg/kg	WR 219,774	40 mg/kg
WR 5,473	200 mg/kg	WR 226,337	40 mg/kg
WR 6,012	200 mg/kg	WR 228,258	40 mg/kg
WR 6,527	400 mg/kg	DADDS	200 mg/kg
WR 7,557	80 mg/kg	PAM 1,392	256 mg/kg

WR 12,921	200 mg/kg	Sulfadimethoxine	100 mg/kg
WR 30,090	640 mg/kg		
WR 33,063	400 mg/kg	WR 7,557	40 mg/kg
WR 38,839	400 mg/kg	+	+
WR 49,808	200 mg/kg	WR 158,122	40 mg/kg
WR 87,781	400 mg/kg		
WR 102,796	256 mg/kg	DADDS	200 mg/kg
WR 122,455	200 mg/kg	+	+
		WR 5,473	200 mg/kg

The compounds tested for repository activity of up to 90 days and the drug levels used are summarized below:

WR 5,473	320 mg/kg	DADDS	320 mg/kg
WR 49,808	320 mg/kg		
WR 102,796	400 mg/kg	WR 5,473	320 mg/kg
WR 122,455	400 mg/kg	+	+
WR 158,122	640 mg/kg	WR 49,808	320 mg/kg
WR 159,412	640 mg/kg		
WR 226,337	160 mg/kg	WR 5,473	320 mg/kg
WR 228,258	40 mg/kg	+	+
		DADDS	320 mg/kg

RESULTS

A total of 31 compounds and drug combinations mixed in three vehicles were tested for repository activity of up to 17 days. Eighteen of these displayed activity in at least one vehicle through the 3-day challenge; 15 were active through the 10-day challenge; and 12 were active through the 17-day challenge. The curative effects of compounds exhibiting repository activity when administered to mice 3, 10 and 17 days prior to challenge are summarized by chemical type in Table VIII. The remaining 13 compounds demonstrating little or no repository activity are listed below:

WR 448	160 mg/kg	WR 7,557	80 mg/kg
WR 1,544	100 mg/kg	WR 33,063	400 mg/kg
WR 2,976	256 mg/kg	WR 38,839	400 mg/kg
WR 2,978	100 mg/kg	WR 87,781	400 mg/kg
WR 4,629	320 mg/kg	PAM 1,392	256 mg/kg
WR 6,012	200 mg/kg	Sulfadimethoxine	100 mg/kg
WR 6,527	400 mg/kg		

A total of 11 compounds and drug combinations mixed in two vehicles were tested for repository activity of up to 90 days. Ten of these displayed activity in at least one vehicle through the 30-day challenge; 8 were active through the 60-day challenge; and 5 were active through the

90-day challenge (WR 158,122 and WR 102,796 in particular). One compound demonstrated no repository activity. The curative effects of compounds exhibiting repository activity when administered to mice 30, 60 or 90 days prior to challenge are summarized by chemical type in Table IX.

Results suggest that HEC and P0 are the vehicles of choice for use in repository testing. HEC appears to be the most effective vehicle, as indicated by the 90 day challenge data. The lipophilic vehicle BBC may be eliminated from future tests, as it does not offer any advantages over HEC and P0 relative to enhancing a compound's repository activity.

Several compounds produced a local cutaneous reaction in the mice, characterized by redness of the area surrounding the site of injection. Those that caused sores include WR 1,543, WR 12,921, WR 122,455, WR 226,337, WR 142,490 and PAM 1,392.

Deaths attributed to compound toxicity occurred following administration of WR 159,412 at 640 mg/kg.

TABLE I

Summary of Data on Compounds Tested
June 1, 1976 - September 30, 1977

WR Compound No./ Bottle No.	Exp. No.	Q ¹	P	SD ₉₀ (mg/kg/day) ²						R _X ³	T.I. ⁴
				A	C	M	S	T	U		
25,979 AH 78744	141	1	78 98							P.O. S.C.	> 1
107,596 BG 72401	103	N.D. ⁵	> 64 ^{a*} > 64 ^a		> 256 ^a					P.O. S.C. P.O. P.O. S.C.	N.D. N.D.
148,799 BG 89219	129	2	34 9.8							P.O. S.C. P.O. S.C.	> 5
154,923 BH 14020	138	43	1.9 1.5							P.O. S.C. P.O. P.O.	> 33
155,004 BH 13158	138	35	2.3 2.5							P.O. S.C. P.O. P.O.	> 27
	139 140					3.8	6.6	3.8			
					< 1		10	9			

Table I (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	Q	SD ₉₀ (mg/kg/day)							R _x	T.I.
			P	A	C	M	S	T	U		
157,358 BG 59024	106 108	7	12		> 256 ^a					P.O. P.O.	> 21
165,356 ZM 86060	90	11	6.6 13							P.O. S.C.	> 2
177,602 BG 58518	91	28	3.3 5.9							P.O. S.C.	> 19
BE 77728	91	26	3.8 5.8							P.O. S.C.	> 2
BG 58518	94			> 256 ^a	90 ^{b@}				> 256 ^a		
BG 58518	95					2.5		2.7		P.O.	
181,613 BG 62110	91	27	3.5 9.2							P.O. S.C. P.O.	> 57
	93			> 200 ^a	> 200				> 200 ^a		
194,965 BE 13813	92	22	3.5 5.8							P.O. S.C.	> 4
BG 56327	92	25	3.2 5.8							P.O. S.C. P.O.	20
	94 95			256 ^a	5		3.1	4.2	> 256 ^a		

Table 1 (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	SD ₉₀ (mg/kg/day)							R _x	T.I.
		Q	P	A	C	M	S	T		
225,329 BG 71030	100	140	0.6 < 0.25						P.O. S.C.	> 106
	101			<1	1.6				P.O.	
	102					2.8		6.8	P.O.	
BG 80529	109	28	2.9						P.O.	
BG 71030	131	23	3.9						P.O.	
	144		3.3						P.O.	
	148		0.78						P.O.	
225,448 BG 98352	127	142	0.57						P.O.	> 28
BH 35761	154	> 288	< 0.25 < 0.25						P.O. S.C.	> 64
225,449 BG 94925	126	108	0.75 2.2						P.O. S.C. P.O. P.O.	> 21
	137					0.71		0.66		
	139						2.3			
226,337 BG 79026	109	> 81	<1 <1						P.O. S.C. P.O. P.O. S.C.	13
	110									
	118	270	0.3 0.13	1.7		2.3	1	1.4		
226,768 BG 47364	105	3	32 35						P.O. S.C.	> 2

Table 1 (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	SD ₉₀ (mg/kg/day)									
		Q	P	A	C	M	S	T	U	R _x	T.I.
226,970 BG 56023	105	27	3							P.O. S.C. P.O.	>21
	140		3.3			< 0.25		0.4			
228,258 BG 59793	92	104	0.75 0.74							P.O. S.C. P.O.	>266
	93			>200 ^a	>200 ^a				>200 ^a		
BG 85640	125	115	0.7 1.1	66 ^b	190 ^b				>256 ^a	P.O. S.C.	>365
228,340 BG 60741	91	48	2							P.O. S.C. P.O. P.O.	>128
	101		3.2	>256 ^a	<4				45 ^a		
	106						1.7	1.5			
	110										
228,399 BG 60830	103	2	44 >64 ^a							P.O. S.C. P.O.	1
	106							44			
228,400 BG 60821	103	2	45 50							P.O. S.C.	>1
228,402 BG 60750	100	2	46 >64 ^a							P.O. S.C. P.O.	>2
	102					42					

Table I (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	Q	SD ₉₀ (mg/kg/day)								R _x	T.I.
			P	A	C	M	S	T	U			
228,404 BG 60867	98	N.D.	> 64 ^a > 64 ^a								P.O. S.C.	N.D.
	106	N.D.	> 128 ^a > 128 ^a								P.O. S.C.	N.D.
228,405 BG 60803	106	N.D.	< 128 ^a > 128 ^a								P.O. S.C.	> 1
	100	2	47 54								P.O. S.C.	1
228,407 BG 60787	102								44			
	96	2	49 46								P.O. S.C.	> 5
228,410 BG 60849	97										P.O. S.C.	
	102			58	42	110		42		> 128 ^a	P.O. S.C.	
228,974 BG 67268	96	5	16 25.5								P.O. S.C.	> 16
	97										P.O. S.C.	
228,977 BG 66921	102				20 ^b	20		32		> 256 ^a	P.O. S.C.	> 4
	105			< 4 ^b	> 16						P.O. S.C.	
	96	5	14.4 27.5								P.O. S.C.	

Table 1 (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	Q	SD ₉₀ (mg/kg/day)							R _x	T.I.
			P	A	C	M	S	T	U		
228,979 BG 66850	99	116	0.67 0.84							P.O. S.C. P.O.	>382
	105			>256 ^a							
BH 08326	130	98	0.86 1.3							P.O. S.C. P.O.	>297
	136				34 ^a				>256 ^a		
228,984 BG 66887	99	2	44 45							P.O. S.C. P.O.	1
	104				88						
228,985 BG 66878	99	2	45 62							P.O. S.C. P.O.	>5
	104				145						
229,049 BG 85319	116	6	12.5 12							P.O. S.C. P.O.	1
	119			14	13						
229,090 BG 85686	116	2	41 11							P.O. S.C. P.O. P.O.	>1
	119 124			55 ^b	44				38		
229,403 BG 71002	110	3	30 33							P.O. S.C.	>1

Table I (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	Q	SD ₉₀ (mg/kg/day)							R _x	T.I.
			P	A	C	M	S	T	U		
229,404 BG 71011	110	1.5	52 29							P.O. S.C.	> 1
229,555 BG 72429	112	10	8.4							P.O.	7
229,561 BG 72901	107	7	11.8 14.5							P.O. S.C. P.O.	1
	108				14.5 ^b						
229,601 BG 74941	112 130	N.D. 8	>4 ^a 10.5							P.O. P.O.	6
229,605 BG 74969	107	2	45 98							P.O. S.C. P.O.	> 5
	108				130						
229,606 BG 74932	107	4 ^b	14.5 ^b 14 ^a							P.O. S.C. P.O.	> 17
	108				145 ^a						
229,607 BG 74950	112 129	N.D. 8	>4 ^a 11							P.O. P.O.	> 5
230,083 BG 78985	126	6	12.3 > 16 ^a							P.O. S.C.	> 20

Table 1 (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	Q	SD ₉₀ (mg/kg/day)							R _x	T.I.
			P	A	C	M	S	T	U		
230,083 BG 78985	136				38				> 256 ^a	P.O.	
230,084 BG 78976	126	7	11.5 > 16 ^a							P.O. S.C. P.O.	> 22
	136				35				> 256 ^a		
230,190 BG 85373	116	3	27 11.5							P.O. S.C.	> 9
	119			205	225			11		P.O. P.O. P.O.	
	124						15				
	139										
230,222 BG 81071	111	N.D.	> 64 ^a > 64 ^a							P.O. S.C.	N.D.
	129	N.D.	> 256 ^a > 256 ^a							P.O. S.C.	N.D.
230,385 BG 81491	117	7	11 26							P.O. S.C. P.O.	> 5
	124				11			11.7			
230,386 BG 81624	127	N.D.	> 64 ^a 3.5							P.O. S.C.	N.D.
230,390 BG 81517	112	2	48 46							P.O. S.C.	> 1

Table 1 (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	Q	P	SD ₉₀ (mg/kg/day)						R _x	T.I.
				A	C	M	S	T	U		
230,397 BG 81544	117	7	11.5 25.5							P.O. S.C. P.O.	2
	124				12			12			
231,030 BG 89077	134	83	0.98 1.7							P.O. S.C.	>16
231,133 BG 89139	120	30	2.7 3.0							P.O. S.C.	>23
231,134 BG 89157	129	30	2.8 9							P.O. S.C.	>22
231,135 BG 89200	134	25	3.3 6.2							P.O. S.C.	>19
231,158 BG 89148	150	> 84	<1 <1							P.O. S.C.	>64
231,159 BG 89273	120	34	2.4 2.8							P.O. S.C.	>106
	124				2.3						
231,160 BG 89120	120	115	0.7 3.6							P.O. S.C.	>22

Table 1 (cont'd.)

WR Compound No./ Bottle No.	Exp. No.	Q	SD ₉₀ (mg/kg/day)						R _x	T.I.
			P	A	C	M	S	T		
231,350 BG 94630	127	8	10 14						P.O. S.C.	1
231,530 BG 94916	127	81	1 1.3						P.O. S.C. P.O.	64
	136				< 4			< 4		
231,533 BG 94952	135	11	7.4 9						P.O. S.C.	2
	137					4.8		4.8		
231,623 BG 94836	123	28	2.9 11						P.O. S.C.	> 22
231,624 BG 94827	122	28	2.9 2.7						P.O. S.C.	> 5
231,628 BG 94818	122	33	2.5 2.8						P.O. S.C.	> 29
	123	29	2.8 2.8						P.O. S.C.	
232,708 BH 07776	130	29	2.9 6.6						P.O. S.C.	> 22
232,745 BH 07801	130	2	44 10 ^b						P.O. S.C.	> 1

Table 1 (cont'd.)

Compound No./ Bottle No.	Exp. No.	SD ₉₀ (mg/kg/day)							R _x	T.I.
		Q	P	A	C	M	S	T		
232,750 BH 07758	134	N.D.	> 64 ^a						P.O.	> 1
	141	> 1	> 64 ^a < 64 < 64						S.C. P.O. S.C.	
232,956 BH 08773	131	> 90	< 1						P.O.	> 128
	135	162	< 1 0.5 0.64						S.C. P.O. S.C.	
	137					0.87		0.87	P.O.	
	139						1.9		P.O.	
233,078 BG 08764	131	> 90	< 1						P.O.	50
	135	253	< 1 0.32 0.56						S.C. P.O. S.C.	
	137					0.53		0.45	P.O.	
	139						0.94		P.O.	
233,124 BG 09118	131	8	12 27						P.O. S.C.	> 5
	141	N.D.	> 64 ^a > 64 ^a						P.O. S.C.	N.D.
233,325 BH 10657	149	26	3.1 5.8						P.O. S.C.	> 20

Table 1 (cont'd.)

Compound No./ Bottle No.	Exp. No.	SD ₉₀ (mg/kg/day)								R _x	T.I.
		Q	P	A	C	M	S	T	U		
233,335 BH 10648	149	29	2.8 2.9							P.O. S.C.	> 22
233,342 BH 10700	147	9	9							P.O.	> 7
233,343 BH 10719	147	8	10							P.O.	> 6
233,344 BH 10693	147	7	11							P.O.	> 5
233,348 BH 10595	149	26	3.1 2.3							P.O. S.C.	> 20

¹Quinine Index = potency relative to quinine against sensitive parasites (P-line).

²Amount of drug to suppress 90% of the parasites for the following lines: P = drug-sensitive; A = Mefloquine-resistant; C = Chloroquine-resistant; M = Pyrimethamine-resistant; S = Dapsone-resistant; T = Cycloguanil-resistant; and U = Quinine-resistant.

³P.O. = oral; S.C. = subcutaneous.

⁴Therapeutic Index

⁵N.D. = Not determined due to lack of activity of compound.

*a = comparison point at SD₅₀.

@b = comparison point at SD₇₀.

TABLE II

Degrees of cross resistance with the six drug-resistant lines of *P. berghei*.

Compound No./ Bottle No.	Exp. No.	Cross resistance ¹					
		A	C	M	S	T	U
107,596 BH 72401	104		N.D.				
154,923 BH 14020	139 140			2	3	2	
155,004 BH 13158	139 140			0	6	3	
157,358 BG 59024	108		> 34 ^a				
177,602 BG 58518	94 95	> 111	54 ^b		0	0	> 111
181,613 BG 62110		> 118 ^a	> 57				> 118 ^a
194,965 BG 56327	94 95	> 82	0		0	0	
225,329 BG 71030	101 102	0	2.6	4.6		11	0
225,449 BG 94925	137 139			0	3	0	

Table II (cont'd.)

Compound No./ Bottle No.	Exp. No.	Cross-resistance						
		A	C	M	S	T	U	
226,337 BG 79026	110 118	5		7	0		4	
226,970 BG 56023	140			0		0		
228,258 BG 59793	93	> 56 ^a	> 56 ^a				> 56 ^a	
BG 85640	125	137 ^b	395 ^b				> 691 ^a	
228,340 BG 60741	101 106 110	> 256 ^a	< 2		0	0	45 ^a	
228,399 BG 60830	106					0		
228,402 BG 60750	102			0				
228,407 BG 60787	102					0		
228,410 BG 60849	97 102		0	0	2	0	> 2	
228,974 BG 67268	97 102 105	2 ^b	< 2 ^b	0	0	0	< 10	

Table II (cont'd.)

Compound No./ Bottle No.	Exp. No.	Cross-resistance					
		A	C	M	S	T	U
228,979 BG 66850	105	> 382 ^a					
BH 08326	136		68 ^a				> 512 ^a
228,984 BG 66887	104		2				
228,985 BG 66878	104		3				
229,049 BG 85319	119	0	0				
229,090 BG 85686	119 124	2	0			0	
229,561 BG 72901	108		1.8 ^b				
229,605 BG 74969	108		3				
229,606 BG 74932	108		13 ^a				
230,083 BG 78985	136		3				> 32 ^a
230,084 BG 78976	136		3				> 37 ^a

Table II (cont'd.)

Compound No./ Bottle No.	Exp. No.	Cross-resistance					
		A	C	M	S	T	U
230,190 BG 85373	115 124 139	16	8		0	0	
230,385 BG 81491	124		0			0	
230,397 BG 81544	124		0			0	
231,159 BG 89273	124		0				
231,530 BG 94916	136		0				0
231,533 BG 94952	137			0		0	
232,956 BH 08773	135 139			0	3	0	
233,078 BG 08764	137 139			0	2	0	

¹ Cross resistance value obtained by comparisons at SD₉₀ with the following drug-resistant lines:
A = Mefloquine-resistant; C = Chloroquine-resistant; M = Pyrimethamine-resistant; S = Dapsone-resistant; T = Cycloguanil-resistant; and U = Quinine-resistant.

^a Cross resistance value obtained by comparisons at SD₅₀.

^b Cross resistance value obtained by comparisons at SD₇₀.

TABLE III

Mefloquine Moderately - Resistant Line (B)

WR Compound No. Name or Chemical Type	Exp. No.	SD ₉₀	Days Drug Administered		Fold Degree of Cross Resistance* with Mefloquine ¹
			3,4,5	5,6,7	
1,543 (Atebrin)	113	9.6		x	2
	132	14		x	3
	146	12.5		x	3
1,544 (Chloroquine)	78	3	x		0
	82	3.9	x		0
	88	3.7	x		0
	98	3.7	x		0
	111	3.2	x		0
	122	> 8 ^a	x		> 4 ^a
	122	4.4 ^b		x	2 ^b
	123	70	x		26
	128	90	x		33
	138	< 4	x		0
2,976 (Quinine)	132	240		x	3
2,977 (Amodiaquine)	111	9.4	x		3
	113	6.7 ^a		x	3 ^a
	132	< 4		x	0
4,835 (Amopyroquine)	146	12		x	2
30,090 (A quinoline- methanol)	146	4.4 ^b		x	4 ^b
33,063 (A phenanthrene- methanol)	128	150 ^a	x		18 ^a
	132	28 ^b		x	2.5 ^b
	146	> 256 ^b		x	> 25.6 ^b
49,808 (Menoctone)	146	7.4		x	0
122,455 (A phenanthrene- methanol)	113	> 256 ^a		x	> 116 ^a
	123	42 ^b	x		15 ^b

Table III (cont'd.)

WR Compound No. Name or Chemical Type	Exp. No.	SD ₉₀	Days Drug Administered		Fold Degree of Cross Resistance* with Mefloquine
			3,4,5	5,6,7	
142,490 (Mefloquine)	68	3.9	x		0
	78	45 ^b	x		16 ^b
	98	>100 ^b	x		>35 ^b
	111	10.8 ^b	x		3.5 ^b
	113	>256 ^a		x	>116 ^a
	122	>100 ^a	x		>45 ^a
	122	>100 ^a		x	>45 ^a
	123	20 ^a	x		9 ^a
	142	150 ^a	x		68 ^a
	142	>256 ^a		x	>116 ^a
171,669 (A phenanthrene- methanol)	146	10.7 ^b		x	10.7 ^b
181,203 (An anthracene)	146	180 ^b		x	66 ^b
226,663 (A quinoline- methanol)	88	>100	x		>14
228,979 (An amodiaquine type)	146	16 ^b		x	26 ^b

*Except with mefloquine (142,490) which is the degree of resistance.

^l Compounds done at SD₉₀.

^a Compounds done at SD₅₀.

^b Compounds done at SD₇₀.

TABLE IV

Mefloquine-Resistant A-Line@

WR Compound No. Name or Chemical Type	Exp. No.	SD ₉₀	Days Drug Administered		Fold Degree of Cross Resistance* with Mefloquine ¹
			3,4,5	5,6,7	
1,543 Atebrin	113	9.4		x	3
	132	28		x	7
1,544 Chloroquine	109	9 ^b	x		4 ^b
	113	3.7		x	0
	121	< 2	x		0
	121	110 ^b		x	50 ^b
	128	< 4	x		0
	132	82		x	30
2,976 Quinine	132	156		x	2
2,977 Amodiaquine	109	> 256 ^b	x		> 116 ^b
	113	15		x	4.8
	132	19		x	6
33,063 A Phenanthrene- methanol	128	28 ^b	x		2.8
	132	150		x	10
122,455 A Phenanthrene- methanol	113	> 256 ^a		x	> 116 ^a
	121	> 256 ^b	x		> 88 ^b
	121	> 256 ^a		x	> 116 ^a
142,490 Mefloquine	111	> 100 ^a	x		> 52 ^a
	113	> 256 ^a		x	> 134 ^a
225,449 An Amodiaquine type	128	16	x		21

* Except with Mefloquine (WR 142,490) which is the degree of resistance.

¹ Compounds done at SD₉₀.^a Compounds done at SD₅₀.^b Compounds done at SD₇₀.

@ Completely resistant to mefloquine.

TABLE V

Test plan for examining the effects of different foods given after starvation on the suppressive action of WR 158,122 in mice infected with Plasmodium berghei

Mg/kg of WR 158,122	Non-starved Groups of Mice		Starved Groups of Mice				
	Mouse Chow ¹	HEC-Tween ²	Mouse Chow ¹	Corn Oil ³	Similax ⁴	Sugar Solution ⁵	
0	1	6	11	16	21	26	
4	2	7	12	17	22	27	
1	3	8	13	18	23	28	
0.25	4	9	14	19	24	29	
0.0625	5	10	15	20	25	30	

¹ Mouse chow = regular Teklad feed we routinely use for all mice.

² HEC-tween = aqueous 0.5% hydroxyethylcellulose-0.1% tween-80. (This is the vehicle in which WR 158,122 was mixed.)

³ Corn oil = 100% pure corn oil.

⁴ Similax = liquid baby food.

⁵ Sugar solution = 50% glucose solution.

TABLE VI

The parasitemias of mice given various foods after starvation.

<u>Mg/kg of WR 158,122</u>	<u>Parasitemia (%)</u>					
	<u>Non-starved</u>			<u>Starved</u>		
	<u>Mouse Chow</u>	<u>HEC-tween</u>	<u>Mouse Chow</u>	<u>Corn Oil</u>	<u>Similax</u>	<u>Sugar</u>
0	78.1	60.8	63.6	48.8	37.1	14.4
4	22.0	9.5	20.3	0.8	1.9	0.2
1	44.6	36.9	30.4	5.7	6.3	0.7
0.25	61.9	47.4	30.6	9.1	4.8	1.7
0.0625	64.3	53.6	29.3	21.0	16.6	3.1

TABLE VII

The percent suppression of parasitemia of mice given various foods after starvation.

<u>Mg/kg of WR 158,122</u>	<u>Percent Suppression of Parasitemia</u>					
	<u>Non-starved</u>			<u>Starved</u>		
	<u>Mouse Chow</u>	<u>HEC-Tween</u>	<u>Mouse Chow</u>	<u>Corn Oil</u>	<u>Similax</u>	<u>Sugar</u>
4	71.8	84.3	68.1	98.3	94.9	98.8
1	42.9	40.4	52.1	88.4	83.2	95.1
0.25	20.8	27.5	51.9	81.4	87.0	88.1
0.0625	22.2	16.5	53.9	56.9	55.4	78.6

TABLE VIII

The curative effects of antimalarial compounds administered subcutaneously to mice
3, 10 and 17 days prior to challenge* with Plasmodium berghei.

Compound	Amt. Drug (mg/kg)	Vehicle**	No. Mice Cured***/Total		
			3-Day Challenge	10-Day Challenge	17-Day Challenge
<u>Anthracene:</u>					
WR 1,543 Atebrin	400	HEC	5/5	0/5	0/5
		PO	3/5	0/3	0/5
		BBC	5/5	0/5	0/5
WR 12,921	200	HEC	3/5	0/5	0/4
		PO	5/5	0/5	0/5
		BBC	1/4	0/5	0/5
<u>Naphthoquinone:</u>					
WR 49,808 Menoctone	200	HEC	2/5	0/5	0/5
		PO	1/5	0/5	0/5
		BBC	1/5	0/5	0/5
<u>Phenanthrene:</u>					
WR 122,455	200	HEC	5/5	5/5	3/5
		PO	5/5	5/5	3/4
		BBC	5/5	1/5	0/4

TABLE VIII (cont'd.)

Compound	Amt. Drug (mg/kg)	Vehicle**	No. Mice Cured***/Total		
			3-Day Challenge	10-Day Challenge	17-Day Challenge
Pyridine:					
WR 180,409	160	HEC	5/5	2/5	0/5
		PO	5/5	3/5	0/5
		BBC	4/4	4/5	0/5
Quinazoline:					
WR 158,122	80	HEC	5/5	5/5	5/5
		PO	3/5	3/5	3/5
		BBC	5/5	5/5	3/5
WR 159,412	400	HEC	5/5	4/5	3/5
		PO	4/5	2/5	2/5
		BBC	5/5	4/5	4/5
WR 180,872	80	HEC	4/5	1/5	1/4
		PO	1/5	1/5	0/5
		BBC	0/5	0/5	0/5
Quinoline:					
WR 30,090	640	HEC	5/5	0/5	0/5
		PO	4/5	1/5	0/5
		BBC	5/5	1/5	0/5
WR 102,796	256	HEC	1/3	4/5	1/4
		PO	2/5	3/5	3/5
		BBC	4/5	2/5	1/5

TABLE VIII (cont'd.)

Compound	Amt. Drug (mg/kg)	Vehicle**	No. Mice Cured***/Total		
			3-Day Challenge	10-Day Challenge	17-Day Challenge
<u>Quinoline (con't.):</u>					
WR 142,490 Mefloquine	640	HEC	5/5	0/5	0/5
		PO	5/5	5/5	0/5
		BBC	5/5	1/5	0/5
WR 219,774	40	HEC	5/5	5/5	5/5
		PO	5/5	4/5	5/5
		BBC	5/5	4/5	5/5
WR 228,258	40	HEC	5/5	5/5	5/5
		PO	5/5	4/5	3/5
		BBC	4/4	4/5	4/5
<u>Sulfone:</u>					
Acedapsone (DADDs)	200	HEC	4/4	4/4	4/4
		PO	5/5	5/5	5/5
		BBC	5/5	5/5	5/5
<u>Triazine:</u>					
WR 5,473 Cycloguanil pamoate	200	HEC	5/5	5/5	3/5
		PO	5/5	4/5	5/5
		BBC	5/5	4/5	4/5

TABLE VIII (cont'd.)

Compound	Amt. Drug (mg/kg)	Vehicle**	No. Mice Cured***/Total		
			3-Day Challenge	10-Day Challenge	17-Day Challenge
Combinations:					
Cycloguanil pamoate	200	HEC	5/5	4/4	4/4
+	+	P0	5/5	5/5	5/5
DADDS	200	BBC	5/5	5/5	5/5
WR 158,122	40	HEC	5/5	5/5	5/5
+	+	P0	5/5	2/5	0/5
WR 7,557@	40	BBC	5/5	3/5	0/5
Sulfadiazine					
Miscellaneous:					
WR 226,337	40	HEC	5/5	4/5	5/5
		P0	5/5	3/5	2/5
		BBC	5/5	5/5	0/5

* 5.0 x 10⁵ parasitized erythrocytes/mouse.

** HEC - aqueous 0.5% hydroxyethylcellulose - 0.1% tween-80.

P0 - refined peanut oil.

BBC - 40% benzyl benzoate and 60% castor oil.

*** Alive four weeks after challenge with P. berghei.

@ WR 7,557 administered alone at 80 mg/kg exhibited no repository activity.

TABLE IX

The curative effects of antimalarial compounds administered subcutaneously to mice
30, 60 and 90 days prior to challenge* with Plasmodium berghei.

Compound	Amt. Drug (mg/kg)	Vehicle**	No. Mice Cured***/Total		
			30-Day Challenge	60-Day Challenge	90-Day Challenge
<u>Phenanthrene:</u>					
WR 122,455	400	HEC PO	3/5 4/5	0/5 0/5	0/5 0/5
<u>Quinazoline:</u>					
WR 158,122	640	HEC PO	5/5 5/5	5/5 4/5	4/5 4/5
WR 159,412	640	HEC PO	4/5 1/5	4/5 0/5	2/5 0/5
<u>Quinoline:</u>					
WR 102,796	400	HEC PO	2/5 3/5	3/5 3/5	3/5 1/5
WR 228,258	40	HEC PO	4/5 4/5	4/5 2/5	0/5 0/5

TABLE IX (Con't.)

Compound	Amt. Drug (mg/kg)	Vehicle**	No. Mice Cured***/Total		
			30-Day Challenge	60-Day Challenge	90-Day Challenge
<u>Sulfone:</u>					
Acedapson (DADDs)	320	HEC PO	1/5 1/5	0/5 1/5	0/5 1/5
<u>Triazine:</u>					
WR 5,473 Cycloguanil pamoate	320	HEC PO	5/5 4/5	1/5 0/5	0/5 0/5
<u>Combinations:</u>					
Cycloguanil pamoate + DADDs	320 + 320	HEC PO	4/5 4/5	1/5 2/5	1/5 0/5
Cycloguanil pamoate + WR 49,808 @ Menoctone	320 + 320	HEC PO	1/5 0/5	0/5 0/5	0/5 0/5
<u>Miscellaneous:</u>					
WR 226,337	160	HEC PO	2/5 1/5	2/5 0/5	0/5 0/5

* 5.0 x 10⁵ parasitized erythrocytes/mouse.

** HEC - aqueous 0.5% hydroxyethylcellulose - 0.1% tween-80.

PO - refined peanut oil.

*** Alive four weeks after challenge with *P. berghei*.

@ WR 49,808 administered alone at 320 mg/kg exhibited no repository activity.

A SCREENING PROCEDURE FOR THE EVALUATION OF TRYPANOSOMICIDAL ACTIVITY
OF CANDIDATE COMPOUNDS IN TRYPANOSOMA RHODESIENSE INFECTED MICE

The test system described herein was developed specifically to evaluate the trypanosomicidal activity of large numbers of candidate compounds. Based on blood-induced Trypanosoma rhodesiense infections in mice, it performs as a primary screen or as a secondary screen and confirmatory test and gives precise quantitative evaluations of chemical compounds that demonstrate potentially useful therapeutic and/or prophylactic activity in T. rhodesiense infections. Consequently, it is also a helpful guideline in the synthesis of new active agents.

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

- (1) chemicals structurally related to compounds of known value in the treatment or prevention of T. rhodesiense infections;
- (2) chemicals structurally unrelated to compounds of known value in the treatment or prevention of T. rhodesiense infections;
- (3) structural analogues of compounds that have demonstrated activity in our screening procedure and represent novel chemical groups; and
- (4) compounds known to have activity against other infectious agents.

Table 1 summarizes the number of compounds tested and the number of mice used from August 1, 1972, through September 30, 1977.

Our own colony of ICR/HA Swiss mice provided all the test animals needed in this operation. Using mice of a given age, sex and weight and a standard inoculum of the Wellcome CT-strain of T. rhodesiense, it has been possible to produce a consistently uniform disease fatal to 100 percent of untreated animals within 4-6 days.

Test compounds were administered subcutaneously in a single dose on the day of infection. Selected active compounds were administered orally.

Drug activity was assessed by comparing the maximum survival time of the treated trypanosome-infected animals to the survival time of

the untreated trypanosome-infected controls. To be classified as active, a compound must suppress the disease and produce an increase of at least 100 percent in the life span of the treated animals over that of the untreated controls. To be considered curative, treated animals must remain alive for 30 days.

METHODS*

ANIMAL HOSTS. ICR/HA Swiss mice (Mus musculus) used in this screening procedure weigh 30-32 grams, weight variations in any given experimental or control group being carefully limited to three grams. In all tests animals have been of the male sex and approximately of the same age.

Animals are housed in metal-topped plastic cages, fed a standard laboratory diet and given water ad lib. Once the mice have been given a drug they are kept in a room maintained at 84° F (+ 2° F) and a relative humidity of 66% (+ 2%).

TEST PROCEDURE. Test animals receive an intraperitoneal injection of 0.5 cc of a 1:50,000 dilution of heparinized heart blood drawn from a donor mouse infected three days earlier.

The donor line is maintained by three-day blood passes, each animal receiving 0.1 cc of a 1:500 dilution of heparinized heart blood drawn from a three-day donor. Donors, like test animals, weigh 30-32 grams, weight variations for each pass being limited to three grams.

To check factors such as changes in the infectivity of our I. rhodesiense strain or in the susceptibility of the host, one group of infected untreated mice are included as negative controls. In order to determine the effect a drug exerts on a trypanosome infection two parameters are measured: the increase in mouse survival time and drug curative action. For comparative purposes two standard compounds, stilbamidine isethionate and 2-hydroxystilbamidine isethionate, are administered at one level each (26.5 mgs/kg) to separate groups of 10 mice. These diamidines serve as positive controls, producing definite increases in survival time and curative effects. Another function of the two positive controls involves a check on whether three procedures are performed correctly: the drug weighing, the preparation of drug solutions and suspensions, and the administration of drugs.

*Designed and developed by Dr. Leo Rane.

DRUG ADMINISTRATION. Test compounds are dissolved or suspended in peanut oil before they are administered subcutaneously. Compounds to be administered orally are mixed in an aqueous solution of 0.5% hydroxyethylcellulose - 0.1% tween-80.

Treatment consists of a single dose given subcutaneously or orally two to three hours after the injection of parasites. Deaths that occur before the fourth day, when untreated controls begin to die, are regarded as a result of action by the drug, not parasites.

Each compound is initially administered in three graded doses diluted four-fold to groups of five mice per dose level. The top dose is 424, 212 or 106 mg/kg depending on the amount of compound available for testing. Active compounds are subsequently tested at six or nine dose levels, diluted two-fold from the highest dose. Successive six-level tests are performed at respectively lower doses if necessary until the lower limit of activity is reached.

A drug that is toxic for the host at each of the three levels initially tested is retested at six dose levels diluted two-fold from the lowest toxic dose.

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 effective dose (MED) producing a significant effect. An MTD is defined as the highest dose up to 424 mg/kg causing no more than one of five animals to die from drug toxicity. The MED is defined as the minimum dose increasing the life span of treated animals by 100% over the life span of untreated controls.

Clearly inactive compounds are rejected after one test; borderline compounds after two tests. Active compounds are characterized by a dose-response curve, which establishes the spread between the MTD and the lower limit of activity by a determination of drug activity in the dose level dilution tests. Treated animals alive at the end of 30 days are considered cured.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST
TRYPANOSOMA RHODESIENSE INFECTIONS IN MICE

During the opening period of this project, June 1, 1972 - May 31, 1973, our screening procedure was developed and its reliability established. 3,030 selected compounds were screened, including a number of agents known to be effective in T. rhodesiense infections and drugs drawn from our antimalarial program. Of these, 68 demonstrated a degree of activity sufficient to produce at least 100 percent increases in the survival time of treated T. rhodesiense infected mice.

1,581 compounds were tested in the period June 1, 1973 - May 31, 1974. Of these, 185 demonstrated a degree of activity sufficient to produce at least 100 percent increases in the survival time of treated T. rhodesiense infected mice, 92 were active subcutaneously and 93 orally.

1,826 compounds were tested in the period June 1, 1974 - May 31, 1975. Of the 298 recognized as active compounds, 225 were active subcutaneously and 73 orally.

1,653 compounds were tested in the period June 1, 1975 - May 31, 1976. Of the 257 recognized as active compounds, 198 were active subcutaneously and 59 orally.

4,235 compounds were tested in the period June 1, 1976 - September 30, 1977. Of the 396 recognized as active compounds, 109 were active both orally and subcutaneously, 17 other compounds were active only orally, not subcutaneously, and 270 other compounds were active subcutaneously.

This breakdown is significant since: (1) activity evaluations provided in our screening procedure are precise and quantitative; (2) dose response curves of active compounds administered subcutaneously show a wider spread between the MTD and the MED than dose response curves of active compounds administered orally; and (3) these dose responses also reveal a wider spread of toxic effects when active compounds toxic for the host are administered subcutaneously rather than orally.

TABLE 1

COMPOUNDS TESTED AND MICE UTILIZED

AUGUST 1, 1972 - SEPTEMBER 30, 1977

<u>YEAR</u>	<u>NUMBER OF COMPOUNDS</u>	<u>NUMBER OF MICE</u>
August 1, 1972 - May 31, 1973	3,030	51,405
June 1, 1973 - May 31, 1974	1,581	25,360
June 1, 1974 - May 31, 1975	1,826	33,850
June 1, 1975 - May 31, 1976	1,653	30,290
June 1, 1976 - September 30, 1977	<u>4,235</u>	<u>73,280</u>
TOTAL	12,325	214,185

SECONDARY ANTITRYPANOSOMAL PROGRAM

SPECIAL REPOSITORY TRYPANOSOME EXPERIMENT

A two year test, started in October, 1975, to determine the repository activity of three compounds (ZG 76354, AH 55296 and BG 00521), was completed. A total of 1,200 mice were divided into three groups of 400 each. The first group of mice received 708 mg/kg of ZG 76354, the second group 689 mg/kg of AH 55296, and the third 424 mg/kg of BG 00521. The mice were then challenged with trypomastigotes at various time intervals to determine the duration of repository activity. A total of 220 mice served as negative controls. The duration of suppressive activity for both ZG 76354 and AH 55296 was seven months, while BG 00521 retained activity for at least 10 months.

DRUG-RESISTANT TRYPANOSOME LINES

The resistance of Trypanosoma rhodesiense to selected anti-trypanosomal compounds can be induced by repeated drug pressure in an in vivo test system. This is achieved by infecting mice with a standard inoculum of parasites, administering the test compound in a dose just below the curative level, and passing parasites from these animals to a new set of mice when the parasitemia rises to a desirable level. Passes are made every three to four days, drug doses being increased as resistance develops at each dose level.

This type of study can establish the rate at which T. rhodesiense acquires resistance in mice to selected compounds. Cross resistance to other trypanosomicidal compounds may also be determined.

Lines of trypanosomes completely or partially resistant to the following compounds have been developed:

- 1) Suramin sodium;
- 2) Stilbamidine isethionate;
- 3) Berenil.

METHODS

ANIMALS. Male or female ICR/HA Swiss mice (Mus musculus) of approximately the same age and weight are used in all procedures. Animals are housed in groups of five, fed a standard laboratory diet and given water ad lib. Mice are kept in a room maintained at 84° F (+ 2° F) and a relative humidity of 66% (+ 2%).

DEVELOPMENT OF DRUG RESISTANT LINES. On day 0, fifteen male or female mice are divided into three groups of five animals. All animals are initially challenged intraperitoneally with drug-sensitive T. rhodesiense (Wellcome CT-strain) trypomastigotes in saline-diluted blood (1:500) drawn from a previously infected donor mouse. Group I serves as a negative control, receiving no drug. Group II receives drug either orally or subcutaneously on day 0 and day 1. Group III is given the same dose of drug by the same route on day 0 only. On day 3 or 4, fifteen new mice are infected with saline-diluted blood (1:500) from Group II. The pass is made from Group III if Group II animals demonstrate no parasites upon blood examination. These newly infected mice are similarly divided into three groups and given the same drug regimen as that just described. Passes are thus made every three or four days from the most recently infected and treated groups of animals. Drug doses are increased as resistance develops.

TEST PROCEDURE. One set of test animals is infected with drug-sensitive T. rhodesiense trypomastigotes, receiving an intraperitoneal injection of 0.5 cc of a 1:50,000 dilution of heparinized heart blood drawn from a donor mouse infected three days earlier. Other sets of mice are similarly infected with each drug-resistant line to be tested. Blood dilutions are made such that all mice infected with the resistant lines receive approximately the same number of trypomastigotes as mice infected with the sensitive line.

One group of five infected mice from the sensitive line and from each resistant line serves as a negative control, receiving no drug.

DRUG ADMINISTRATION. Test compounds are mixed in either peanut oil for subcutaneous administration or 0.5% hydroxyethylcellulose-0.1% tween-80 for oral administration. Compounds are given immediately following infection with trypomastigotes.

Each drug-resistant line is tested against the compound to which resistance has been induced so that the level of drug resistance may be determined. Drug-resistant lines are also tested for cross resistance against other selected trypanosomicidal compounds.

Compound doses are diluted two or four-fold from a level that has been projected to be fully curative. Five mice are used for each dose level.

DRUG ACTIVITY. Mortality is used as an index of drug activity. Untreated negative control mice routinely die on days 4 or 5 after inoculation of parasites. Increases in life span relative to that of negative controls at each dose level are recorded. Curative activity is used in assessing the level of resistance of selected compounds. Mice surviving for 30 days are considered cured.

The CD₅₀ (minimal dose curing at least three of five mice) is used as a basis for establishing levels of resistance and determining compound cross resistance. A cross resistance of four-fold will not be considered significant in this report as compounds were often administered at four-fold dilutions. The spread produced by such dilutions is too great using the CD₅₀ as an index of activity to attach significance to an apparent cross resistance of four-fold. A cross resistance greater than four-fold is considered significant.

RESULTS

DEVELOPMENT OF RESISTANCE TO SURAMIN. The development of a suramin-resistant line of T. rhodesiense by repeated drug pressure in vivo is illustrated in Table 2. Over a period of approximately ten months and 95 line passages, resistance to this naphthalene derivative was progressively induced.

The suramin-resistant line demonstrated a greater than 32-fold degree of resistance to suramin in Experiment 5 (Tables 3, 4 and 5) after a period of five months and 50 line passages. The CD₅₀ in this test was greater than 160 mg/kg. A 64-fold degree of resistance to suramin was demonstrated in Experiment 6. (Tables 3, 6 and 7) after a period of eight months and 83 line passages. The CD₅₀ in this test was greater than 320 mg/kg.

A 108-fold degree of resistance to suramin was demonstrated in Experiment 8 (Tables 3, 8 and 9) after a period of nine months and 95 line passages. The CD₅₀ in this test was 640 mg/kg. At least a partial resistance to suramin at the level is indicated by the observation that two of five mice given 640 mg/kg died on day 5, as did the negative controls.

CROSS RESISTANCE WITH SURAMIN. The cross resistance of several trypanosomocidal compounds with suramin was determined by a comparison of each compound's CD₅₀ when tested against the drug-sensitive and suramin-resistant lines, as shown in Table 3. Cross resistance levels are listed below:

<u>Compound</u>	<u>Experiment No.</u>	<u>Cross Resistance with Suramin</u>
Stilbamidine	5	4-fold
	6	16-fold
	8	4-fold
MUM 3,679 BA 62987	5	4-fold
MUM 229,005 WR 119,290	5	0
Melarsoprol	6	4-fold
	8	16-fold
Berenil	6	16-fold
	8	64-fold
BG 00521	8	128-fold

DEVELOPMENT OF RESISTANCE TO STILBAMIDINE. The development of a stilbamidine-resistant line of *T. rhodesiense* is illustrated in Table 10. Over a period of approximately 10 months and 101 line passages, resistance to this diamidine was progressively induced. It should be noted that on two occasions all mice in Groups I, II and III died before the next passage could be made. Infected blood from previous passages that had been frozen at -75° F was rapidly thawed and used to restart the resistant line.

The stilbamidine-resistant line demonstrated a 32-fold degree of resistance to stilbamidine in Experiment No. 5 (Tables 3, 4 and 5) after a period of five months and 51 line passages. The CD₅₀ in this test was 160 mg/kg. Stilbamidine was administered orally in Experiment No. 5 and subcutaneously in all subsequent experiments.

A 260-fold degree of resistance to stilbamidine was demonstrated in Experiment No. 6 (Tables 3, 6 and 7) after a period of nearly nine months and 88 line passages. The CD₅₀ in this test was 106 mg/kg.

A 32-fold degree of resistance to stilbamidine was demonstrated in Experiment No. 8 (Tables 3, 8 and 9) after a period of nearly ten months and 98 line passages. The CD₅₀ in this test was 53 mg/kg.

CROSS RESISTANCE WITH STILBAMIDINE. The cross resistance of several trypanosomicidal compounds with stilbamidine was determined by a comparison of each compound's CD₅₀ when tested against the drug-sensitive and stilbamidine-resistant lines, as shown in Table 3. Cross-resistant levels are listed below:

<u>Compound</u>	<u>Experiment No.</u>	<u>Cross Resistance with Stilbamidine</u>
Suramin	5	0
	6	0
	8	0
MUM 3,679 BA 62987	5	4-fold
MUM 229,005 WR 119,290	5	0
Melarsoprol	6	4-fold
	8	64-fold
Berenil	6	16-fold
	8	64-fold
BG 00521	8	128-fold

DEVELOPMENT OF RESISTANCE TO BERENIL. The development of a berenil-resistant line of T. rhodesiense is illustrated in Table 11. This line was not tested for its degree of resistance to berenil during this time period.

DEVELOPMENT OF RESISTANCE TO STILBAMIDINE AND BG 00521 ALONE AND IN COMBINATION. It is a well established fact in malarial infections that the development of resistance to compounds can be drastically reduced, or completely blocked, if they are administered in combination rather than alone. Based upon this rationale a similar type of experiment was designed for Trypanosoma rhodesiense parasites. Three new lines of T. rhodesiense were developed; one resistant to stilbamidine, one resistant to BG 00521, and one resistant to a combination of stilbamidine and BG 00521. The objectives for this work were two-fold; first to compare the rates of acquisition of resistance to BG 00521 vs. stilbamidine, and second to determine if the development of resistance would occur at a slower rate or, if at all, when both compounds were administered in combination.

The experimental design was similar to that described above for the development of other trypanosome-resistant lines. The drug levels for each drug alone and in combination for each passage are tabulated in Table 12. Based upon the results of the first check for resistance, performed with parasites from the 9th passage, it appears that a greater than 32-fold degree of resistance to BG 00521 had developed, while only a 32-fold degree of resistance to stilbamidine occurred (Tables 13, 14 and 15). Parasites in the line receiving the drugs in

combination developed a greater than 32-fold degree of resistance to BG 00521 and a 64-fold degree of resistance to stilbamidine. Thus, it appears that the development of resistance is not hindered when the drugs are administered in combination. Work is continuing with these three lines to determine the degrees of resistance with greater drug pressure.

TABLE 2

Development of a Suramin-resistant line of
Trypanosoma rhodesiense.

<u>Passage No.</u>	<u>Dose (mg/kg)@</u>
1-16	2
17-21	4
22-26	10
27-29	20
30-39	40
40-54*	50
55-71	75
72-95**	100

@ Compound administered subcutaneously.

* Resistance Test No. 5 used parasites from Passage No. 50.

**Resistance Test No. 6 used parasites from Passage No. 83.

**Resistance Test No. 8 used parasites from Passage No. 95.

TABLE 3

Activity of selected trypanosomicidal compounds against sensitive and drug-resistant lines of Trypanosoma rhodesiense.

Test No.	Compound	CD ₅₀ (mg/kg)*		
		Sensitive line	Stilbamidine-resistant line	Suramin-resistant line
5	Stilbamidine	5	160	20
	Suramin	5	1.25	>160
	MUM 3,679	6.63	>26.5	26.5
	MUM 229,005	32	8	8
6	Stilbamidine	≤ 0.41	106	6.63
	Suramin	5	5	>320
	Melarsoprol	1.66	> 6.63	> 6.63
	Berenil	0.28	> 4.44	> 4.44
8	Stilbamidine	1.66	53	6.63
	Suramin	5	5	640
	Melarsoprol	1.66	> 106	26.5
	Berenil	0.41	26.5	26.5
	BG 00521	1.66	> 212	> 212

* CD₅₀ is the minimal dose curing at least 3 of 5 mice.

Note: Stilbamidine given orally in test No. 5; otherwise, all compounds administered subcutaneously.

TABLE 4

EXPERIMENT NO. 5

<u>Drug</u>	<u>Dose (mg/kg)</u>	<u>Group No. and (No. mice cured*)</u>		
		<u>Sensitive Line</u>	<u>Stilbamidine- Resistant Line</u>	<u>Suramin- Resistant Line</u>
		1 (0)	18 (0)	35 (0)
Stilbamidine	160		19 (4)	
AX 37252	80		20 (0)	
Given P.O.	20	2 (5)	21 (0)	36 (4)
	5	3 (3)	22 (0)	37 (0)
	1.25	4 (0)		38 (0)
	0.31	5 (0)		39 (0)
Suramin	160			40 (1)
BB 62987	80			41 (1)
Given S.C.	20	6 (5)	23 (5)	42 (0)
	5	7 (3)	24 (5)	43 (0)
	1.25	8 (1)	25 (4)	
	0.31	9 (0)	26 (0)	
MUM 3,679	26.5	10 (5)	27 (2)	44 (5)
BA 62987	6.63	11 (3)	28 (0)	45 (0)
Given S.C.	1.66	12 (0)	29 (0)	46 (0)
	0.41	13 (0)	30 (0)	47 (0)
MUM 229,005	32	14 (5)	31 (5)	48 (5)
WR 119,290	8	15 (1)	32 (3)	49 (4)
Given S.C.	2	16 (0)	33 (0)	50 (0)
	0.5	17 (0)	34 (0)	51 (0)

*No. mice alive 30 days after infection.

TABLE 5

EXPERIMENT NO. 5

<u>Group No.:</u>	<u>No. mice dead/day died:</u>	<u>No. mice cured:</u>
1	4/3, 1/4	0
2		5
3	1/13, 1/17	3
4	1/3, 4/4	0
5	4/3, 1/4	0
6		5
7	1/7, 1/17	3
8	1/4, 2/7, 1/13	1
9	5/3	0
10		5
11	1/6, 1/9	3
12	1/4, 3/15, 1/17	0
13	1/3, 1/4, 3/5	0
14		5
15	1/4, 1/5, 1/8, 1/20	1
16	1/3, 4/4	0
17	5/3	0
18	3/3, 2/4	0
19	1/12	4
20	3/4, 1/5, 1/12	0
21	4/3, 1/4	0
22	1/3, 4/4	0
23		5
24		5
25	1/5, 2/7, 1/8, 1/19	0
26	4/3, 1/5	0
27	1/8, 1/15, 1/17	2
28	1/3, 4/4	0
29	1/3, 3/4, 1/5	0
30	4/3, 1/4	0
31		5
32	1/5, 1/13	3
33	2/4, 2/5, 1/12	0
34	3/3, 1/4, 1/5	0

Table 5 (cont'd.)
Experiment No. 5
Page 2.

<u>Group No.:</u>	<u>No. mice dead/day died:</u>	<u>No. mice cured:</u>
35	3/3, 2/4	0
36	1/5	4
37	1/4, 4/5	0
38	1/3, 4/4	0
39	1/3, 4/4	0
40	3/4, 1/5	1
41	4/4	1
42	5/4	0
43	4/3, 1/4	0
44		5
45	1/4, 2/9, 1/15, 1/24	0
46	2/4, 3/5	0
47	5/4	0
48		5
49	1/14	4
50	2/4, 2/5, 1/12	0
51	1/3, 3/4, 1/5	0

TABLE 6

EXPERIMENT NO. 6

<u>Drug</u>	<u>Dose (mg/kg)</u>	<u>Group No. and (No. mice cured*)</u>		
		<u>Sensitive Line</u>	<u>Stilbamidine- Resistant Line</u>	<u>Suramin- Resistant Line</u>
		1 (0)	19 (0)	37 (0)
Stilbamidine AX 37252	212		20 (5)	
	106		21 (3)	
	53		22 (2)	
	26.5	2 (5)	23 (0)	38 (5)
	6.63	3 (5)		39 (5)
	1.66	4 (5)		40 (1)
	0.41	5 (5)		41 (0)
Suramin	320			42 (0)
	160			43 (0)
	80			44 (0)
	20	6 (5)	24 (5)	45 (0)
	5	7 (5)	25 (5)	
	1.25	8 (0)	26 (0)	
	0.31	9 (0)	27 (0)	
Melarsoprol	6.63	10 (5)	28 (0)	46 (0)
	1.66	11 (5)	29 (0)	47 (0)
	0.41	12 (2)	30 (0)	48 (0)
	0.1025	13 (0)	31 (0)	49 (0)
Berenil	4.44	14 (5)	32 (0)	50 (0)
	1.11	15 (5)	33 (0)	51 (0)
	0.28	16 (5)	34 (0)	52 (0)
	0.07	17 (2)	35 (0)	53 (0)
	0.035	18 (0)	36 (0)	54 (0)

*No. mice alive 30 days after infection.

TABLE 7

EXPERIMENT NO. 6

<u>Group No.:</u>	<u>No. mice dead/day died:</u>	<u>No. mice cured:</u>
1	4/4, 1/5	0
2		5
3		5
4		5
5		5
6		5
7		5
8	4/6, 1/11	0
9	5/4	0
10		5
11		5
12	1/7, 1/10, 1/14	2
13	1/4, 1/5, 2/6, 1/8	0
14		5
15		5
16		5
17	1/5, 1/6, 1/7	2
18	3/5, 2/6	0
19	3/5, 1/6, 1/7	0
20		5
21	1/2, 1/19	3
22	1/7, 1/13, 1/16	2
23	2/7, 1/11, 1/12, 1/13	0
24		5
25		5
26	3/5, 1/7, 1/8	0
27	5/5	0
28	5/5	0
29	5/5	0
30	4/5, 1/6	0
31	4/5, 1/6	0
32	2/5, 3/6	0
33	5/5	0
34	5/6	0
35	4/5, 1/6	0
36	5/5	0

Table 7 (cont'd.)
Experiment No. 6
Page Two.

<u>Group No.:</u>	<u>No. mice dead/day died:</u>	<u>No. mice cured:</u>
37	4/5, 1/12	0
38		5
39		5
40	1/7, 1/9, 1/10, 1/12	1
41	5/5	0
42	4/5, 1/7	0
43	5/5	0
44	5/5	0
45	4/5, 1/6	0
46	2/6, 1/11, 1/12, 1/14	0
47	1/5, 2/6, 1/7, 1/11	0
48	4/5, 1/6	0
49	4/5, 1/11	0
50	3/6, 1/10, 1/11	0
51	3/5, 2/6	0
52	3/5, 1/14, 1/19	0
53	4/5, 1/6	0
54	3/5, 2/6	0

TABLE 8

EXPERIMENT NO. 8

Drug	mg/kg	Group No. and (No. mice cured*)		
		Sensitive Line	Stilbamidine-Resistant Line	Suramin-Resistant Line
		1 (0)	19 (0)	40 (0)
Stilbamidine AX 37252	212		20 (5)	
	106		21 (4)	
	53		22 (4)	
	26.5		23 (2)	41 (5)
	6.63	2 (5)		42 (5)
	1.66	3 (4)		43 (1)
	0.41	4 (1)		44 (0)
	0.1025	5 (0)		
Suramin	640			45 (3)
	160			46 (0)
	20	6 (5)	24 (5)	47 (0)
	5	7 (5)	25 (5)	
	1.25	8 (2)	26 (1)	
Melarsoprol BG 80510	106		27 (0)	48 (4)
	26.5		28 (0)	49 (5)
	6.63	9 (5)	29 (0)	50 (0)
	1.66	10 (5)	30 (0)	51 (0)
	0.41	11 (2)		
	0.1025	12 (0)		
Berenil AH 78548	26.5		31 (4)	52 (5)
	6.63		32 (0)	53 (0)
	1.66	13 (5)	33 (0)	54 (0)
	0.41	14 (5)		
	0.1025	15 (2)		
	0.028	16 (0)		
BG 00521	212		34 (0)	55 (1)
	106		35 (0)	56 (2)
	53		36 (0)	57 (0)
	26.5		37 (0)	58 (0)
	6.63		38 (0)	59 (0)
	1.66	17 (5)	39 (0)	60 (0)
	0.41	18 (1)		

*No. mice alive 30 days after infection.

TABLE 9

EXPERIMENT NO. 8

<u>Group No.:</u>	<u>No. mice dead/day died:</u>	<u>No. mice cured:</u>
1	3/4, 2/5	0
2		5
3	1/12	4
4	1/5, 3/6	1
5	4/4, 1/5	0
6		5
7		5
8	1/5, 2/6	2
9		5
10		5
11	1/5, 2/6	2
12	5/5	0
13		5
14		5
15	2/6, 1/12	2
16	5/5	0
17		5
18	1/5, 3/6	1
19	3/4, 2/5	0
20		5
21	1/19	4
22	1/19	4
23	1/5, 2/12	2
24		5
25		5
26	2/6, 1/8, 1/20	1
27	1/4, 4/5	0
28	1/4, 3/5, 1/6	0
29	4/4, 1/5	0
30	1/4, 4/5	0
31	1/6	4
32	4/5, 1/6	0
33	2/4, 2/5, 1/6	0

Table 9 (cont'd.)
Experiment No. 8
Page Two.

<u>Group No.:</u>	<u>No. mice dead/day died:</u>	<u>No. mice cured:</u>
34	2/4, 2/6, 1/18	0
35	1/4, 3/5, 1/6	0
36	2/4, 3/5	0
37	3/4, 1/5, 1/6	0
38	5/5	0
39	2/4, 3/5	0
40	2/4, 2/5, 1/6	0
41		5
42		5
43	2/6, 2/11	1
44	5/5	0
45	2/5	3
46	3/5, 2/6	0
47	3/5, 2/6	0
48	1/18	4
49		5
50	3/5, 2/6	0
51	5/5	0
52		5
53	1/6, 2/11, 2/16	0
54	2/6, 3/12	0
55	1/4, 1/6, 1/8, 1/12	1
56	1/5, 1/6, 1/11	2
57	1/5, 4/6	0
58	3/5, 2/6	0
59	4/5, 1/6	0
60	2/5, 2/6, 1/8	0

TABLE 10

Development of a Stilbamidine-resistant line
of Trypanosoma rhodesiense.

<u>Passage No.</u>	<u>Dose (mg/kg)@</u>
1-4	1.5
5-9	3
10-14	6
15-23	20
24-29	25
30-86*	30
87-101**	37.5

@ Compound administered orally.

* Resistance Test No. 5 used parasites from Passage No. 51.

** Resistance Test No. 6 used parasites from Passage No. 88.

** Resistance Test No. 8 used parasites from Passage No. 98.

TABLE 11

Development of a Berenil-resistant line
of Trypanosoma rhodesiense.

<u>Passage No.</u>	<u>Dose (mg/kg)@</u>
1-9	0.21
10-12	0.42

@Compound administered subcutaneously.

TABLE 12

Development of lines of Trypanosoma rhodesiense resistant to BG 00521, Stilbamidine, and a combination of BG 00521 and Stilbamidine.

<u>Resistant Line</u>	<u>Passage No.</u>	<u>Dose (mg/kg)@</u>
BG 00521	1-7	0.125
	8-12*	0.25
Stilbamidine	1-7	0.125
	8-12*	0.25
Combination:		
BG 00521 (a)	1-7	0.0625 (a) + 0.0625 (b)
+		
Stilbamidine (b)	8-12*	0.125 (a) + 0.125 (b)

@ Compound administered subcutaneously.

* Resistant Test No. 7 used parasites from Passage No. 9.

(a) BG 00521 (WR 163,577)

(b) Stilbamidine

TABLE 13

Activity of BG 00521 and stilbamidine against sensitive
and drug-resistant lines of
Trypanosoma rhodesiense.

<u>Compound</u>	<u>CD₅₀ (mg/kg)*</u>			
	<u>Sensitive Line</u>	<u>BG 00521- Resistant Line</u>	<u>Stilbamidine- Resistant Line</u>	<u>Combination@- Resistant Line</u>
BG 00521	1	32	32	32
Stilbamidine	0.5	16	16	32

*CD₅₀ is the minimal dose curing at least 3 of 5 mice.

@BG 00521 and stilbamidine (1:1).

Note: All compounds administered subcutaneously.

TABLE 14

EXPERIMENT NO. 7

Drug	Dose (mg/kg)	Group No. and (No. mice cured*)			
		Sensitive Line	Resistant Lines		Combo@
			BG 00521	AH 55296	
		1 (0)	12 (0)	29 (0)	46 (0)
WR 163,577 BG 00521	32		13 (0)	30 (0)	47 (0)
	16		14 (0)	31 (0)	48 (0)
	8		15 (0)	32 (0)	49 (0)
	4	2 (5)	16 (0)	33 (0)	50 (0)
	1	3 (4)	17 (0)	34 (0)	51 (0)
	0.5	4 (1)	18 (0)	35 (0)	52 (0)
	0.25	5 (1)	19 (0)	36 (0)	53 (0)
	0.125	6 (0)	20 (0)	37 (0)	54 (0)
Stilbamidine AH 55296	32		21 (5)	38 (5)	55 (5)
	16		22 (4)	39 (3)	56 (1)
	8		23 (0)	40 (0)	57 (1)
	4	7 (5)	24 (0)	41 (0)	58 (1)
	1	8 (5)	25 (0)	42 (0)	59 (0)
	0.5	9 (4)	26 (0)	43 (0)	60 (0)
	0.25	10 (0)	27 (0)	44 (0)	61 (0)
	0.125	11 (0)	28 (0)	45 (0)	62 (0)

*No. mice alive 30 days after infection.

@BG 00521 and stilbamidine (1:1)

TABLE 15

EXPERIMENT NO. 7

<u>Group No.:</u>	<u>No. mice dead/day died:</u>	<u>No. mice cured:</u>
1	5/4	0
2		5
3	1/24	4
4	2/11, 2/12	1
5	1/4, 2/5, 1/6	1
6	5/4	0
7		5
8		5
9	1/24	4
10	1/4, 3/5, 1/6	0
11	3/4, 2/5	0
12	5/4	0
13	2/4, 3/5	0
14	3/4, 2/5	0
15	2/4, 3/5	0
16	5/4	0
17	5/4	0
18	5/4	0
19	4/4, 1/5	0
20	4/4, 1/6	0
21		5
22	1/5	4
23	4/5, 1/16	0
24	3/4, 1/4, 1/6	0
25	5/4	0
26	5/4	0
27	5/4	0
28	5/4	0
29	5/4	0
30	5/4	0
31	4/4, 1/5	0
32	5/4	0
33	5/4	0
34	5/4	0

Table 15 (cont'd.)
Experiment No. 7
Page Two.

<u>Group No.:</u>	<u>No. mice dead/day died:</u>	<u>No. mice cured:</u>
35	5/4	0
36	3/4, 2/5	0
37	4/4, 1/5	0
38		5
39	1/8, 1/11	3
40	1/4, 3/5, 1/16	0
41	5/4	0
42	5/4	0
43	5/4	0
44	5/4	0
45	5/4	0
46	5/4	0
47	3/4, 2/5	0
48	3/4, 2/5	0
49	3/4, 1/5, 1/6	0
50	4/4, 1/11	0
51	5/4	0
52	3/4, 2/5	0
53	3/4, 2/5	0
54	4/4, 1/5	0
55		5
56	2/5, 1/13, 1/16	1
57	2/5, 1/13, 1/20	1
58	3/4, 1/13	1
59	5/4	0
60	3/4, 2/5	0
61	5/4	0
62	2/4, 3/5	0

A C K N O W L E D G M E N T

The personnel at the Rane Laboratory participating in this Chemotherapy of Malaria project deserve a tremendous degree of credit for an excellent performance.

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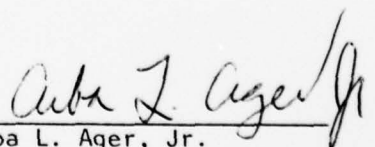
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