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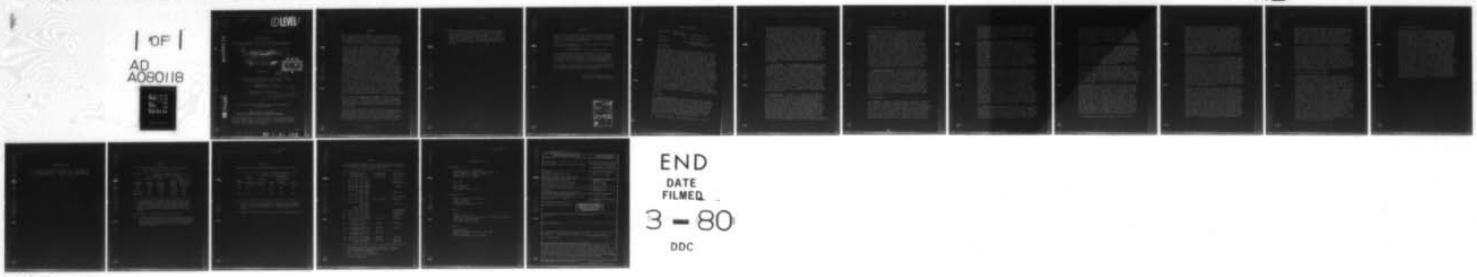

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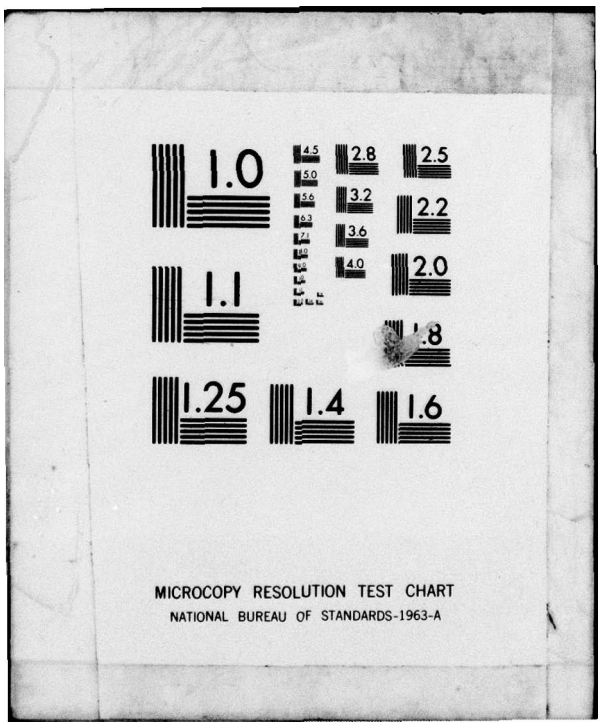
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U. S. Army Medical Research Unit (WRAIR)/Brasilia
APO Miami 34030

SUMMARY

The collaborative anti-schistosome drug testing program between the University of Brasilia and the U.S. Army Medical Research Unit (WRAIR) Brasilia continues to screen new chemical compounds for prophylactic and curative activity against schistosomiasis mansoni.

The U. S. Army's Anti-schistosome Drug Development program, Walter Reed Army Institute of Research, provides selected drugs for testing in mice. The drugs are tested for prophylactic and/or curative activity depending upon the pre-established priorities. The Primary Mortality Test (PMT) is a prophylactic test system which uses mice exposed to 3,000 or more S. mansoni cercariae. Drugs are prepared in peanut oil and administered at 640 mgs/kg to mice in a single subcutaneous inoculation two days post-exposure. Activity is determined by mouse survival beyond the survival of untreated control mice. The Primary Curative Test (PCT) is now being employed as a standard therapeutic test system and involves treating mice exposed to 160 cercariae and in which the infection is allowed to progress until mature worm development is obtained. Drugs are administered subcutaneously at 100 mgs/kg for five consecutive days (between days 33 and 37 post-exposure). Curative activity is measured by the number of live and/or dead worms, and the physical condition of living worms, found in liver squash preparations from the infected/treated mice. We have instituted standardization experiments for establishing a third test system--the Secondary Curative Test (SCT). This test is designed to complement the PCT by following up the active compounds with duration of drug action studies.

The laboratory Biomphalaria glabrata snail colony supported a daily inventory of 1,000 to 1,200 S. mansoni infected snails. The weekly cercarial collections of three to five million were more than adequate to infect prophylactic test group mice with 3,000 or more cercariae each.

During FY78, a total of 1,244 bottle number drugs were received from WRAIR and logged in at the USAMRU-Brasilia Laboratory. A total of 1,195 drugs were tested for prophylactic and/or curative activity; of these drugs 538 were tested in the PMT, 218 of these being confirmatory retests. Results of prophylactic testing were:

396 drugs negative or unconfirmed active; 136 drugs toxic; 6 drugs confirmed active. In the PCT, 657 drugs were tested, 290 being confirmatory retests. The results of curative testing were: 610 drugs negative or unconfirmed active; 32 drugs toxic; 15 drugs confirmed active. Five drugs were confirmed active in both prophylactic and curative test evaluations.

FOREWARD

The research program of the University of Brasilia and the U. S. Army Medical Research Unit (WRAIR) Brasilia is to test drugs for prophylactic and curative activity against schistosomiasis mansoni.

The research project is carried on under the following project and task number:

3M762770A802, Task 00, Work Unit 009

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", DHEW Publication Number (NIH) 73-23; as prepared by the Institute of Laboratory Animal Resources, National Research Council.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

ALUIZIO ROSA PRATA, M. D.
Professor of Tropical Medicine

BODY OF REPORT

PROJECT NO. DAOB 6525
3M762770A802: Tropical Medicine

TASK NO. 00: Tropical Medicine

WORK UNIT 009: Chemotherapeutic Studies on Schistosomiasis

DESCRIPTION:

Schistosomiasis continues to be ranked among the most important of the tropical diseases, yet we still lack suitable means for chemotherapeutic management, and have no means for chemoprophylactic management. The only drugs currently available demonstrate only partial curative efficacy and are often accompanied by adverse side effects ranging from carcinogenicity to headaches and dizziness. Considering the actual and potential global commitments of United States military and civilian personnel, the risks to infection with one of the human schistosomes remains high. Indeed the incidence of infection within foci of local indigenous populations may approach 100 percent. Consequently, a major research effort in anti-schistosome drug development is being carried out by the Walter Reed Army Institute of Research in conjunction with the University of Brasilia (USAMRU-Brasilia). The test compounds are obtained from the Division of Experimental Therapeutics (WRAIR) and are tested for prophylactic and curative activity in mice at the USAMRU-Brasilia. Our ultimate objectives are the identification of compounds with a high potential for use in the prevention and treatment of schistosomiasis mansoni.

PROGRESS:

a. Laboratory Facility. During the reporting period the laboratory operations were improved by the addition of two new items of equipment: 1) a new Mettler electronic top loading analytical balance has considerably reduced the drug preparation time and improved the accuracy of the finished product; 2) the acquisition of a new stereozoom Olympus microscope has provided the increased capability for procedures requiring these features (e.g. liver squash preparations).

b. Animal Facilities. As reported earlier, the con-

version to corncob cellulose bedding effectively eliminated the problem of mouse infection failures; this favorable situation has continued throughout the current reporting period. Early in the year, serious problems were encountered at the Bioterio of the University of Brasilia relative to the weekly supply of healthy animals in the numbers required. While our normal laboratory operations require a full complement of 400 mice per week, weighing between 18 and 23 grams, on many weeks we received no mice at all or only a few mice. These were underweight and quite often sick. Later histopathologic analyses confirmed an almost universal infection with coccidiosis concurrent with multifocal hepatitis, the combination of which with schistosome infection often led to bacterial liver abscesses. Efforts are presently underway to upgrade the mouse breeding colony and to improve the environmental conditions under which the colony is maintained.

c. Snail Colony. The Biomphalaria glabrata (Paulista Strain) snail colony continues to provide Schistosoma mansoni cercariae in sufficient quantities to perform the weekly mouse exposures for drug testing and life cycle maintenance. A weekly average of 400 snails were exposed to miracidia recovered from macerated infected mouse livers. An acceptable level of prepatent mortality (22 percent) was obtained; of the survivors which were screened 42 days post-exposure, 54 percent were positive for emerging cercariae. These are maintained for future cercariae collections and a weekly average population of 1,000 to 1,200 positive snails are on hand at one time. In general approximately 45 percent of the snails exposed are later recovered with patent infections.

d. Drug Testing. A total of 1,244 bottle numbered drug samples were received from WRAIR during the reporting period. Of these, 703 compounds were designated for both prophylactic (PMT) and curative (PCT) testing; 539 compounds were designated for only curative testing; and two compounds were designated for only mortality testing. (See below for descriptions of the test systems) While these compounds were shipped and received during the year, many have not yet been tested because of our increased emphasis on the important matter of retesting older compounds which showed indications of activity or toxicity, or for which the control group data were questionable. The backlog of newer compounds is currently being diminished

as retesting is completed.

We have the capability of performing twice monthly tests in each of the two systems, prophylactic and curative. While normal PCT testing can evaluate 60 compounds at a time and PMT testing can evaluate 50 compounds at once, the mouse supply was the primary limiting factor. During the period of the above mentioned problems with the Bioterio mouse colony, we often received much reduced numbers in the weekly deliveries and on some weeks we received none. We are currently receiving approximately 75 percent of our full weekly complement of 400 mice (Swiss-Holland 40 albino, 45 ± 5 days old, weighing 18 - 23 grams). However, the test systems incorporate enough flexibility that testing can be continued using a reduced number of experimental compounds per test run. Additionally, the reduced numbers of animals provided the opportunity to perform other experimental evaluations (such as standardizations of a Secondary Curative Test) which required fewer numbers of animals. See Figure 1 which depicts the workload data for FY78.

f. Operating Personnel. The drug testing program is directed by one American Senior Investigator and supported by a staff of eight Brazilian Laboratory Assistants (one position vacant). The operating program is broken down into five work areas which are: 1) Snail Colony (two people); 2) Animal Service (two people); 3) Necropsy (one person); 4) Pharmacy (two people); and 5) Administration (one person). All individuals are cross-trained to perform the seven day work schedule of daily snail maintenance, subcutaneous and gavage drug administration, daily mouse maintenance with mortality checks, and mouse exposures to cercariae. Each individual is able to perform all duties in two other areas of work.

TEST PROCEDURES:

a. General. Whereas in past years, all drugs received were normally tested for prophylactic activity first, the current system now places a priority of prophylactic or curative testing on each compound. For those compounds received for testing in both systems, prophylactic testing is still performed first. All tests, prophylactic or curative, are performed with groups of five mice per drug per dosage schedule. all mice are individually tail-exposed 30 minutes to the numbers of cercariae required by the specific test. Drugs are routinely pre-

pared for administration in a peanut oil vehicle unless another vehicle (such as water, saline, alcohol, or cremophor) has been previously recommended. All drugs are administered subcutaneously unless orally (by gavage) has been designated. Likewise, all drugs are administered in terms of mgs per kg body weight of mouse recipient.

b. Primary Mortality Test (PMT). The PMT is a prophylactic test in that it evaluates drug activity against immature migrating larval schistosomes. Mice are exposed to 3,000 - 3,500 S. mansoni cercariae. Two days after exposure drugs are administered in a single innoculation to the five test animals per drug. The standard initial test dose is 640 mgs/kg and future testing repeats this dose with other groups at lower dosages. The 640 mgs/kg dose is a reduction of the 1280 mgs/kg dose used in earlier testing, but the experience of the past four years has indicated that nothing is gained by the higher dose in detecting active compounds, while the lower dose conserves the limited supply of compound for future testing.

For every PMT group there are control groups of 1) 50 infected untreated mice, 2) 10 normal mice, and 3) five mice treated with the reference drug Niridazole (640 mgs/kg). The infected untreated control mice will begin dying on day 20 post-exposure and none will survive past day 30 in most cases. Niridazole-treated mice survive until day 49. Active drugs are those for which treated mice survive two weeks after all infected control mice are dead. At 49 days, all surviving mice (controls and drug test) are perfused (Radke, et. al., 1961) for total worm burden determination. Drugs are considered toxic at the dosage given if recipient mice die within 10 days post-treatment (12 days post-exposure). All active or toxic compounds are scheduled for later retest confirmation at the same dosage and route of administration as the initial test. If positive confirmation is obtained for activity, then further testing at different dosages by both routes (subcutaneous or oral) is scheduled.

c. Primary Curative Test (PCT). The PCT is a curative test of a compound against an established S. mansoni infection in mice exposed to 160 cercariae. Thirty-three days post-exposure drugs (100 mgs/kg) are administered daily for five consecutive days (until day 37) in the same manner as described for the PMT. Three days following the last treatment, all mice are: 1)

killed individually by cervical dislocation; 2) the livers are immediately removed; 3) the livers are made into liver squash preparations; and 4) the numbers and condition of worms in the liver are determined for each surviving animal. Control groups for each PCT run are: 1) 20 untreated infection control mice; 2) 10 Niridazole treated mice (5 at 100 mgs/kg and 5 at 160 mgs/kg); and 3) five Oxamniquine treated mice (100 mgs/kg).

Criteria for drug activity are based upon the hepatic shift of adult worms from the mesenteries to the liver. This shift is presumed to be a result of drug pressure. Not only are the total numbers of liver worms determined but the conditions of those worms are also taken into consideration. The presence of dead worms is incontrovertable evidence of drug activity, while the presence of small, abnormally developed "sick" worms possessing little movement is evidence of possible activity requiring further testing.

Untreated control animals will normally show five to 15 worms in the liver and a test animal liver worm burden of 2 - 3 times that of the mean control liver worm burden is indicative of drug activity. Oxamniquine treatment produces high dead worm burdens in the livers of infected animals, while Niridazole normally produces high living (but sick) worm burdens at 100 mgs/kg and 160 mgs/kg, with the appearance of a few dead worms at the latter dose.

While the previous year we reported the PCT as in the developmental stages, we feel that we currently have sufficient experience with the test to place considerable confidence in the results as indicators of curative drug activity. The criteria for evaluation of results varies from those used in the earlier Japan program, primarily in terms of the numbers of worms in the livers of untreated control animals. But repeated testing has indicated higher normal liver worm burdens in our infections and the differences between the two programs can probably best be explained by snail/parasite strain and/or environmental variations. Our investigations on comparative strain differences produced inconclusive results. Additionally, we have incomplete data on the Japan program strain establishment upon which we can establish valid comparisons.

d. Secondary Curative Test (SCT). In an effort to

expand our drug testing capabilities, we have initiated the standardization of an SCT to complement the PCT. The rationale of this development rests in the requirement for following up on possibly active compounds by providing additional data on the duration of action of selected compounds over a period of time. It should be emphasized that the SCT is not a primary screen, as are the PCT and PMT. Rather it is intended as a more defined evaluation of drug efficacy and emanates from the primary test systems. Consequently, it is anticipated that considerably fewer drugs, and only those that show promise will be tested. The benefits will be a more complete understanding of test drug action upon which to base future testing.

The initial secondary test runs were organized to follow the method described by Bruce et al in the unpublished W.H.O. Document 73.30 ("Schistosomiasis Drug Test Systems"). That is, mice are exposed to 160 cercariae and treated with test compound daily for five consecutive days between days 33 and 37 post-exposure. Separate perfusions of the mesenteric, portal and hepatic circulations are then performed on days 7, 14, and 21 post-treatment. Our initial tests consisted of 150 mice divided among the following five groups (30 mice per group): 1) infected, no treatment; 2) treated with Niridazole, 100 mgs/kg; 3) treated with Niridazole, 160 mgs/kg; 4) treated with Niridazole 320 mgs/kg; and 5) treated with Oxamniquine, 100 mgs/kg. Ten mice from each group were then perfused at weekly intervals as mentioned above.

We soon learned several drawbacks to the described method. 1) The separate perfusions of liver, hepatic portal vein, and mesenteries are extremely time consuming and would necessitate the full time participation of at least three technicians for three days to complete one test run of controls and a reasonable number of test drugs. 2) Perfusion of livers is relatively meaningless, especially two and three weeks post-treatment, since dead worms within the hepatic circulation are almost impossible to dislodge and flush out. By the second week post-treatment, dead worm granulomas are already forming, as revealed in liver squash preparations performed after perfusion. Consequently, perfusion liver worm counts were lower than actually present and consisted only of living worms. 3) The exposure of mice to 160 cercariae

each (the same exposure dose as the PCT) created an undesirable mortality pattern among untreated control animals, in which 100 percent of these animals died prior to day 58 (three weeks post-treatment). In the PCT, this is of no concern since all animals are killed on day 40, prior to death from acute disease. But in the SCT it is necessary to obtain control animal survival through the third perfusion phase in order to obtain comparative worm burden determinations.

We are currently evaluating a modified SCT which consists of 1) exposure of mice to 100 cercariae (a sublethal dose for 58 days), 2) dividing each test and control group into two comparative subgroups, one for total perfusion and one for only liver squash preparations (as in the PCT), and 3) adding an additional perfusion/liver squash phase at three days post-treatment. These latter two modifications then bring the test more into line as a true secondary test by supplementing the previous primary test data under similar conditions. During the coming year we anticipate establishing the test as a standard procedure within the drug testing program.

OTHER SCHISTOSOMIASIS RESEARCH:

a. Snail Strain Studies. We last reported the differences in liver worm burdens between our PCT control data and that obtained earlier in the Japan drug testing program. A proposed reason for the abnormally high worm burdens in the Brazil program was the strain difference between the B. glabrata snail and/or schistosome maintained at the two laboratories. For comparative purposes, we obtained infected B. glabrata snails (Puerto Rican/Walter Reed Strain) from WRAIR (Kindly provided by CPT Lyford K. Greene, Department of Parasitology) and maintained them in the Brasilia laboratory. We exposed mice to cercariae collected from these snails and performed PCT and PMT tests on these different strain infections in parallel with routine test runs performed with our Paulista strain material. Both strain infections were treated identically in terms of cercariae exposure, animal maintenance, treatment with Niridazole and Oxamniquine, and data collection. No significant differences in mortality patterns (PMT), liver worm burdens (PCT) or surviving worm perfusion data (PMT and life cycle groups) were detected between the two groups. These data, resulting from 10 different experiments are currently being analyzed and evaluated.

RESULTS OF DRUG TESTING:

During FY78 a total of 1,195 selected bottle number drugs were tested for prophylactic (PMT) and/or curative (PCT) activity. Figure 2 depicts the results for the two tests. Of the compounds tested, 290 (44.1 percent) were PMT retests of previously tested drugs and 218 (40.5 percent) were PCT retests. All others were compounds which were tested for the first time. Figure 3 identifies those compounds confirmed as active in one of the two test systems. Primary reference is made only to the bottle code numbers since many (but not all) of the compounds that we receive and test are protected proprietary secrets ("commercially discreet"). We have identified the general class of compound where possible. The identified activity is based upon one or more confirmatory retests; compounds determined to be active on the first test will be confirmed by retest at the same dose by the same route of administration. Only after this confirmation is the drug considered active. Note that five compounds -- BH 08 111, BH 08 157, BH 08 166, BH 08 228 and BH 30 0333 -- have demonstrated both confirmed PMT and PCT activity. It is also worth noting that BH 30 033 has shown unconfirmed activity in a wide range of doses from 10 mgs/kg to 320 mgs/kg. This initial "block" treatment schedule must be repeated for confirmation.

LITERATURE CITED

1. Radke, M. G., Berrios-Duran, L. A., and Moran, K. : A Perfusion Procedure (Perf-O-Suction) for Recovery of Schistosome Worms. J. Parasit. 47: 366-368, 1961.

FIGURE 1

FY 78 Workoad Data for USAMRU-Brasilia Expressed in Terms of Numbers of Mice Infected

	Numbers of Mice Infected			
	Control	Test	Total	
Procedure	Groups*	Groups*	Number	Percent**
PMT	760	2,735	3,495	27.6
PCT	560	3,350	3,910	30.9
Life Cycle	N/A	N/A	2,956	23.3
Other***	N/A	N/A	2,300	18.2

* = "Control Groups" include those animals designated as uninfected/untreated, infected/untreated, and infected/treated with reference drug(s) control animals. "Test Groups" include only infected animals treated only with Bottle Numbered Test compounds.

** = Percent of total mouse exposure effort.

*** = Includes standardization of the Secondary Curative Test (SCT), comparisons of Walter Reed and Brazilian strains of infection and other schistosomiasis research efforts performed in support of UnB research programs.

FIGURE 2

Drug Test Results for the USAMRU-Brasilia During FY 78

Test System	Total Tests Performed*	Number of Drugs Tested**			
		Confirmed Active	Toxic	Negative/ Unconfirmed Active	Total
PMT	547	6	136	396	538
PCT	670	15	32	610	657

* = 1 test = five mice treated with one drug at one dosage by one route of administration. Total number of tests does not include untreated or reference drug control groups.

** = Number of WRAIR/USAMRU-Brasilia bottle number compounds.

FIGURE 3

A List of Bottle Number Drugs Found to be Active Against Schistosomiasis mansoni by the Mouse Mortality test and Curative Test Systems During FY78

No.	WRAIR/BRASILIA Bn Number Drug	Prophylactic Test (PMT)*	Curative Test (PCT)*
1.	AD 22 662 (BR 1258)		320 gav
2.	AH 91 461 (BR 1296)		200/320 gav
3.	BC 42 725 (BR 1533)D BH 73 029 (BR 5104)D		40 gav
4.	BD 58 040 (BR 1254) (BR 1025) BE 19 593 (BR 4498)		160 gav
5.	BE 21 922 (BR 1311)		320 SQ & gav
6.	BE 21 931 (BR 1312)	640 SQ FY75+	320 gav
7.	BG 41 577 (BR 1626) (BR 1522) (BR 4502) Nitrovinyl-Furan		40 SQ
8.	BG 52 598 (BR 2079)		80/160 gav
9.	BG 75 064 (BR 2456)		100/200 SQ
10.	BG 81 679 (BR 4405)		100 SQ
11.	BH 08 111 (BR 2889) Organic Tin	640 SQ	100 SQ
12.	BH 08 157 (BR 2893) Organic Tin	640 SQ	100 SQ
13.	BH 08 166 (BR 2894) Organic Tin	640 SQ	100 SQ
14.	BH 08 200 (BR 2897) Organic Tin	640 SQ	
15.	BH 08 228 (BR 2899)	640 SQ	100 SQ
16.	BH 30 033 (BR 3809) Isothiocyanate	1280 SQ	100 SQ (10-320)

* = Confirmed by repeat testing; dosages in mgs/kg; SQ = Subcutaneous administration; gav = oral administration by gavage.

+ = Year activity first reported.

D = Discreet Compound

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18. SUPPLEMENTARY NOTES

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

(U) Brazil; (U) Drug Development; (U) Schistosoma mansoni; (U) Chemotherapy

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

The University of Brasilia and USAMRU (WRAIR) Brasilia tested 1,195 bottle number compounds for either the prophylactic or curative activity against schistosomiasis mansoni in mice. In the prophylactic test, six active drugs were identified our of 538 bottle number drugs tested. In the curative test, 15 active drugs were identified out of 657 bottle number drugs tested. Five drugs were active in both the prophylactic and curative tests.

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