Project Title: Proposal for Research to Reduce or Eliminate Mutagenic Effects from a Promising New Antischistosomal Drug.

New or Extension: New

Time Period: Two years

Estimated Total Cost: $254,333

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Narrative Summary: This project is designed to isolate and identify the metabolic products of the antischistosomal drug, CGP4540, and to eliminate mutagenic activity entirely or to reduce it to a minimum. Preliminary studies on the metabolism of CGP4540 in mice, rats, hamsters, and monkeys indicate that the drug is rapidly metabolized and gives rise to at least 5 different metabolites. Schistosomicidal metabolites are non-mutagenic but bacterial metabolism of the basic compound produces mutagenic effects. Since CGP4540 is inactive against schistosomes in vitro, isolation and identification of the active metabolites is necessary. Should this be accomplished, other compounds capable of being metabolized to the same product might conceivably be more effective. The isolation and identification of the metabolic products of the drug, both in the tissues, of the host and the parasite will be necessary for an understanding of the mode of action, the identification of active metabolites, and for improved drug formulations.

1-4-78
2. **Research Purpose and Expected Products:**

   a. **Project Objective**

   It is the aim of this project to contribute toward the development of an effective and safer drug preparation for the treatment of human subjects infected with any one of the species of schistosomes invading man, *S. hematobium, S. japonicum, or S. mansoni*. Since elimination of the intestinal bacteria of the host (produced by using antibiotics) has been found not to affect anti-schistosomal efficacy but does prevent development of mutagenic metabolites, efforts will be directed toward elucidation of the metabolism of the drug, CGP-4540. Once the metabolism of CGP-4540 has been elucidated and the contribution of intestinal bacteria to the formation of one or several mutagenic compounds has been understood, opportunities will become available to treat human schistosomiasis using a formulation of CGP-4540, or of an analog thereof, with minimal or no mutagenic risk in any of the following manners:

   a) Single oral dose of CGP-4540 preceded by the elimination of the bacterial flora (using 1 or 2 doses of a mixture of antibiotics or of succinylsulfathiazide).

   b) Use of a suitable formulation of a structural analog(s) which gives rise to a schistosomicidal, but fails to generate the formation of a mutagenic metabolite(s).

   c) Parenteral Administration of CGP-4540 to "short circuit" its exposure to the metabolic activities of the bacterial flora.

   d) Coadministration of anticarcinogenic non-toxic substances (thiols, antioxidants or Vitamin A).

   Every one of these possibilities and approaches will be fully explored in the course of this project.
b. Expected End Product

The main product of the research will be one or more formulated compounds, possibly including the basic compound, which offer sufficient promise of effective safe antischistosomal activity as to offer high expectations that the remaining preclinical toxological studies can be successfully completed and approval obtained for necessary human testing.

3. Significance and Rationale for the Research

a. Schistosomiasis is a long-term chronic, parasitic disease of man which occurs predominately among the poor in rural tropical areas. It is endemic in 70 countries or island territories which in 1972 had an estimated population of 1600 million people of whom about 500 million are exposed and of these approximately 300 million are infected. Schistosomiasis is known to occur in Mauritius, Southwest Asia, the Orient, the Americas and all countries of Africa except Lesotho.

Severity of schistosomal disease in man varies considerably from place to place and from individual to individual. Factors which appear to have the greatest importance in determining severity of clinical manifestations include the species of parasite, duration of infection, and the number of worms harbored by the individual, i.e., the intensity of infection. Since immunity to schistosomiasis in man is weak at best, repeated reinfections can and do occur. This feature contributes greatly to the ultimate development of the high intensities of infection which in time can produce
clinical disease terminating in premature death following a long disabling illness. Conditions which favor development of serious disease include high population density, high prevalence of high intensity infections, poor sanitary conditions, a high density of efficient intermediate snail hosts and frequent exposure of people to snail infested water polluted with waste from infected humans.

Important development activities contribute both to the spread and to intensification of the schistosomiasis problem. Irrigation systems and water impoundments provide ideal habitats for the intermediate hosts snails unless attention is given to the need for schistosomiasis control during the construction of such projects.

b. Understanding the need for effective, safe antischistosomal agents in control programs can best be obtained if the life cycle of the organism is understood. Mated female schistosomal worms in infected individuals lay eggs, many of which pass through tissue into the urinary bladder or rectum and are excreted in urine or feces. Tiny miracidia, hatched from those eggs that are fortunate enough to find their way into snail infested waters, seek out and penetrate susceptible snails. After further development within the snail's tissues, the intermediate snail hosts begin shedding the next stage of the schistosomal organism, namely, the forked-tailed cercariae. The life cycle of the schistosome is completed after cercaria have penetrated the skin of exposed individuals and have matured into adult worms which then begin the cycle anew by egg production.
It is quite clear from the life cycle of the schistosome that several procedures offer possibilities for control. In the past, considerable attention has been given to the destruction of snails by chemical means. More recently, the possibility of successful snail control by non-chemical means has been receiving more attention. In a few selected areas, efforts have been directed toward the interruption of human contact with infected water by providing safe sources of water for domestic and personal use. Theoretically, it also would be possible to control schistosomiasis through the safe disposal of human waste but it is difficult to succeed by this method especially among agricultural field laborers. None of the above methods, however, would reduce egg production by living worms harbored by infected individuals; nor would such methods prevent the development of schistosomal disease in individuals who already have heavy schistosome infections. Authorities on schistosomiasis increasingly are coming to believe that combination control measures are required under many circumstances. The most successful programs have been those in which both snail control and treatment of infected individuals have been employed.

The ideal antischistosomal agent remains to be developed. Because schistosomiasis is widespread, mass treatment programs are desirable but undesirable side effects of a drug used in mass treatment programs also would be widespread. A drug to be used in mass treatment campaigns should possess several attributes. It should be effective against all of the species of schistosomes that occur in any given area. Operationally, it is desirable that the drug be safe and effective when administered by
mouth, preferably in a single dose. There should be no need for close medical supervision since mass treatment campaigns require that the treatments be administered by relatively unskilled personnel. None of the currently available drugs fulfill these criteria. All are capable of producing serious medical complications and therefore must be administered only under close medical supervision. Hycanthone although highly effective in a single dose must be injected into the buttocks. Operationally this is a demerit since treatments must be given in private and personnel if both sexes employed. Niridazole although effective when administered by mouth must be given for four to seven days and is largely contra indicated for general use against S. mansoni and S. japonicum where impairment of liver function can be present. Metrifonate given in multiple oral doses, is effective only against S. hematobium and therefore would not be a fully effective drug for use in an area where both S. hematobium and S. mansoni infections are present. By contrast, oxamniquine is effective only against S. mansoni. Intramuscular administration in a single dose is preferred. The antimony compounds must be administered parenterally rather than by mouth and none has been deemed safe enough for use except under direct medical supervision.

Preliminary work with CGP 4540 indicates that it is effective against all three species of human schistosomes after the administration of a single oral dose to experimental animals. It also appears less mutagenic in one test system than are some of the currently used drugs. This is important because it appears that compounds which are highly mutagenic also have a high potential for causing cancer.
Of the available drugs, hycanthone and niridazole are carcinogens, i.e., capable of causing cancer in animals. Carcinogenicity of the antimony compounds, metrifonate and oxamniquine, according to FDA has not been determined.

4. Plans to Coordinate to Link Research

The Agency's Office of Health for several years has recognized schistosomiasis as a categorical disease of growing importance because of the existing close association between increased schistosomiasis transmission and the development of new water management projects, particularly irrigation systems and water impoundments. As a consequence, representatives of the Office of Health have attended a number of technical reviews of the state-of-the-art of schistosomiasis control which have been convened since 1970. One of these meeting, The Symposium on The Future of Schistosomiasis Control which was held at Tulane University in February 1972, was jointly sponsored by AID and WHO. Increased Agency support for research on schistosomiasis was stimulated in September 1974 by the address of the Secretary of State of the United States to the United Nations General Assembly which included the statement, "in coming months the United States will make specific proposals to the United Nations to initiate: *****; a concerted effort to control the disease which afflicts and debilitates over 200 million people in 70 countries -- schistosomiasis: *****." Subsequently, the Office of Health has reviewed recommendations made by authorities during the more recent technical conferences on the subject and has prepared a proposed program.
of research which accords a high priority to the testing of new drugs which show promise of being safer and more efficient than are currently available compounds. The proposed program of research on schistosomiasis has been discussed with technical personnel of the World Health Organization who have concurred with the total effort proposed and have agreed that drug testing deserves a high priority. Furthermore, WHO is in the process of establishing centers where antischistosomal drugs can be tested in infected human beings. If the pre-clinical and Phase I human testing of CGP 4540 is successfully completed, WHO would be well situated to undertake the Phase II clinical trials.

The Agency's Near East and Africa Bureaus are showing evidence of growing interest in the schistosomiasis problem. Specifically, the Near East Bureau is considering the schistosomiasis risk in a new irrigation scheme being planned for Morocco and apparently is anticipating requests for assistance in schistosomiasis control from a number of Near East countries particularly Egypt. The Africa Bureau is recruiting technical consultants to advise the Government of Swaziland on schistosomiasis control and is considering the need to incorporate plans for schistosomiasis control in new development projects such as Volta and Senegal River Basin projects.

The International Bank for Reconstruction and Development now routinely requires the incorporation of plans for schistosomiasis control in new water management schemes which it supports if they are situated in an area where schistosomiasis is a threat.

All agencies and governments now concerned with schistosomiasis control suffer from the absence of a safe, effective compound that can be used in mass treatment programs.
It is our understanding that little active research on drug testing and drug development now is in progress. Traditionally, drug development has been undertaken by the pharmaceutical industry but in the absence of a readily visible market for products, the industry generally is investing very little in the tropical disease field. In recognition of this situation, the following recommendation was made at the Tulane Conference on Schistosomiasis: "In view of the high cost of chemotherapeutic research, the U. S. Agency for International Development and/or other agencies should examine the feasibility of collaboration with industry in support of the evolution of drugs of better therapeutic index." The United States Army in the past has been a major supporter of the development of chemotherapeutic agents for prevention, treatment and control of tropical diseases but now is withdrawing its support for extramural programs. Modest support is being given by the Edna McConnell Clark Foundation for the testing of some promising compounds that have been developed by the Parke-Davis Company. We are unaware of other donors' support being given to antischistosomal drug development and understand from WHO staff that pharmaceutical industry efforts are indeed quite modest.

5. **Plan to Facilitate Utilization of Research Results**

If a nonmutagenic schistosomicidal compound is developed, the results of this research would be utilized by others, with financial support from sources other than AID, to proceed with additional pre-clinical toxicological testing which is necessary to comply with the requirements of the U.S. Food
and Drug Administration before testing in humans can be undertaken.

6. Management Considerations

A. Requirements for Non-contract Project Funding:

Because this is a highly technical field, it is desirable that experts outside this Agency assist in monitoring the project. Authorities on drug development in the Department of Health, Education, and Welfare have been requested to advise AID concerning suitable individuals but until the details are in hand, estimates of cost cannot be provided. It is our estimate that two consultants, a geneticist and a biochemical pharmacologist providing about 20 man-days per year would be adequate.

B. Evaluation Concept to be Employed

The method for project evaluation will be developed jointly by the Agency's project manager and external expert advisors.
C. Management Responsibilities:

TA/H, Joe L. Stockard, M.D.

No need is foreseen for any management responsibilities to be vested in USAIDs and no subcontracts are involved at this time.

D. Special Criteria for Selection of Research Contractor

This is a sole source procurement based upon 1) submission of an unsolicited proposal, 2) effective formulation of the basic drug has been developed by the proposer and 3) holder of the patent on the basic compound has authorized the proposer to pursue the proposed research.

E. Special Clearance and Information Distribution Requirements:

The desirability of having the prior advice and consent of the U.S.F.D.A. on the proposed work plan has been discussed with Dr. Bueding and he has taken this matter up with Dr. Edgar Martin. We have been advised by Dr. Martin that the proposed work plan would fulfill the requirements of FDA for this portion of pre-clinical testing provided the protein binding attributes of CGP 4540 are determined. Dr. Bueding concurs in this matter.

7. Technical Review

The current situation with regard to antischistosomal drugs for human use has been reviewed previously and is not repeated here. Nor does it appear desirable that the drafter of this document endeavor to deal with the state-of-the-art in biochemical pharmacology and molecular biology with which this project is concerned. Drug development is subject to Federal Government regulation and reference should be made to the attached project assessments which have been prepared by authorities in the field.
### Table I

**Effect of a single oral dose of formulated compound in mice infected with 100 cercariae 55 days prior to treatment**

<table>
<thead>
<tr>
<th>Single oral dose mg/kg</th>
<th>Strain*</th>
<th>No. of mice</th>
<th>% Reduction in no. of worms</th>
<th>% of mice with parasitological cures**</th>
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<tbody>
<tr>
<td>2.5</td>
<td>P.R.(M)</td>
<td>24</td>
<td>52</td>
<td>0</td>
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<tr>
<td></td>
<td>P.R.(M)</td>
<td>37</td>
<td>82</td>
<td>22</td>
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<tr>
<td></td>
<td>S.L.</td>
<td>14</td>
<td>85</td>
<td>26</td>
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<tr>
<td></td>
<td>Lib.(M)</td>
<td>14</td>
<td>64</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>P.R.(S.W.)</td>
<td>11</td>
<td>86</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>P.R.(M)</td>
<td>87</td>
<td>91</td>
<td>84</td>
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<tr>
<td></td>
<td>S.L.</td>
<td>28</td>
<td>92</td>
<td>85</td>
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<tr>
<td></td>
<td>Lib.(M)</td>
<td>35</td>
<td>92</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Hyc. Res.</td>
<td>17</td>
<td>92</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Lib. (C.G.)</td>
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<td>91</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Braz.</td>
<td>25</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>P.R.(M)</td>
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<td>94</td>
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<tr>
<td></td>
<td>S.L.</td>
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<td>99</td>
<td>96</td>
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<tr>
<td></td>
<td>Lib.(M)</td>
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<td>96</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>P.R.(S.W.)</td>
<td>22</td>
<td>100</td>
<td>100</td>
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<tr>
<td></td>
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<td>14</td>
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<td>Braz.</td>
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<td>100</td>
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<td>S.L.</td>
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</tr>
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<tr>
<td></td>
<td>Hyc. Res.</td>
<td>22</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*) Strain abbreviations:

- **P.R.(M):** Puerto Rican, obtained from the Museum of Zoology, U. of Michigan (Dr. Henry van der Schalie)
- **Lib.(M):** Liberian, obtained from same laboratory
- **S.L.:** St. Lucian
- **P.R.(S.W.):** Puerto Rican strain, obtained from Sterling-Winthrop Laboratories
- **Lib.(C.G.):** Liberian, maintained at Ciba-Geigy Laboratories, Basle
- **Braz.:** Brazilian (Belo Horizonte)
- **Hyc. Res.:** Hycanthone-resistant strain developed in this laboratory

**) The term "parasitological cures" is used when not a single live worm is recovered.
## TABLE II

**Effect of a single dose of formulated compound in mice infected with Schistosoma japonicum**

<table>
<thead>
<tr>
<th>Single oral dose mg/kg</th>
<th>Strain*</th>
<th>No. of mice</th>
<th>% Reduction in no. of worms</th>
<th>% of mice with parasitological cures</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Phil.</td>
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<td>100</td>
</tr>
<tr>
<td></td>
<td>Jap.</td>
<td>15</td>
<td>87</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Chin.</td>
<td>14</td>
<td>91</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>Phil.</td>
<td>20</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Jap.</td>
<td>12</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chin.</td>
<td>36</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>Phil.</td>
<td>15</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Jap.</td>
<td>22</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chin.</td>
<td>18</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*) Strains: All 3 strains were supplied by Dr. van der Schalie, Museum of Zoology, University of Michigan.

Phil.: Philippine strain

Jap.: Japanese strain

Chin.: Chinese mainland strain, originally isolated by Dr. Vogel (Tropeninstitut, Hamburg, Germany)
This section of the project statement is devoted to previous work which the proposer has conducted on CGP-4540.

The investigator received a supply of CGP-4540 from the Ciba-Geigy Corporation, Basle, Switzerland and was advised that they had found the compound to have schistosomacidal properties *in vivo* when administered as a single, high oral dose to experimental animals. He subsequently confirmed these observations in infected mice and hamsters. At a single oral dose of 300 mg/kg most, but not necessarily all, of the worms in mice infected with *S. mansoni* were eliminated. He then applied to the compound formulation principles which previously had been developed in his laboratory and proceeded to demonstrate an increase in the effectiveness of CGP-4540. This formulation consisted of a combination of reduction of particle size of the compound to an average diameter of 0.5 microns and suspension of the material in 25% glycerol, containing a surfactant based upon a long chain fatty acid. The improved results obtained with a single oral dose administered to mice infected with *S. mansoni* are presented in Table I. Similar effectiveness of the drug was demonstrated in mice infected with *S. japonicum*. This is of particular importance because at the present time effective drugs against this schistosome species are extremely limited. (Table II).

Further experiments have revealed that the formulation is equally active against schistosomal infection in another mammalian species, hamsters.

The proposer has advised us that tests conducted by Ciba-Geigy indicate that the unformulated compound is equally as active in monkeys.
and dogs as it is in mice infected with *S. mansoni* as well as in hamsters infected with *S. hematobium*.

The proposer's observation that his formulation of the compound also has high activity against immature stages (3 weeks or older) of schistosomes has considerable practical importance for operational programs. If immature forms survive treatment, they will develop into mature worms after effects of the drug have vanished thus compromising treatment of infected individuals.

Although detailed toxicological studies have not been conducted, it has been observed that mice, hamsters and uninfected monkeys tolerate well fifty times the oral therapeutic dose of the formulation. The only adverse effect noted has been a temporary loss of weight.

Formulated CGP-4540 has failed to exhibit any detectable mutagenic activity when tested in the absence or in the presence of liver microsome preparations with different bacterial tester strains. As noted previously, negative tests for mutagenicity enhanced the prospects that the drug will not produce cancer.

Preliminary studies indicate that the drug CPG-4540 is rapidly metabolized in mice, rats, hamsters and monkeys and gives rise to at least five different metabolites. Furthermore, the parent compound has been found not to be active against schistosomes in vitro. It also has been determined that any schistosomacidal metabolites produced are non-mutagenic and that mutagenic metabolite(s) of the basic compound are produced by bacterial metabolism in the intestinal tract of experimental animals rather than by the metabolic systems of the animals themselves.
A few experiments have been conducted which suggest that the compound also has potential as a prophylactic agent provided a slow release formulation can be developed.

8. Research Project Design and Methods

a. Metabolism of CGP-4540

One to three hours following the oral administration of low doses of formulated CGP-4540 (5 mg/kg) to mice infected with S. mansoni, the worms have shifted from the mesenteric veins to the liver. At this stage, they exhibit characteristic changes. Their body movements have become sluggish, their length has been reduced by 20 to 30%, and the acetabulum is completely paralyzed. By contrast, none of these changes are observed when the worms are incubated for several days in vitro in a nutrient medium containing as much as 10 μg/ml of CGP-4540. These observations indicate that CGP-4540 must be metabolized by the host to a compound which has antischistosomal properties. We shall attempt to isolate and identify this and other metabolic products of CGP-4540.

We propose to carry out studies designed to elucidate the metabolism of CGP-4540 by the host and to identify the metabolite(s) responsible for the schistosomicidal activity of the drug. Information from these studies will be used to design and synthesize compounds likely to provide increased effectiveness or to reduce undesired effects.

These studies will include a search for metabolites of CGP-4540. Such metabolites of the drug or its derivatives may be found in the liver, blood, kidney, other organs, or in the urine or feces. Since the active metabolite may be produced or remain intact in only a single tissue, many of them, as well as excreta, should be examined. To this end it will be necessary to develop methods for extraction, from the various tissues, metabolites having high, intermediate, as well as low polarity. Each range of extracts requires a different solvent extracting system. Each will be examined for metabolites which can be identified and possibly synthesized.

Techniques for the extraction, quantitation, purification, and identification of metabolic products of antischistosomal agents have been developed and employed extensively in this laboratory. These techniques include the standard solvent extraction from homogenates, column and solid phase extraction, and enzyme pretreatment where necessary. The standard methods of column and thin layer chromatography are used routinely. In addition, high pressure liquid chromatography has proven to be very useful. Although this technique
requires elaborate and expensive instrumentation, it is capable of separation and identification of complex mixtures, and of quantitation at the nanogram level.

For this study we have available a high pressure liquid chromatograph with full solvent programming, two independent pumps, various analytical and preparative columns, a dual wavelength U.V. detector and appropriate accessories, as well as a Waters 0,000 psi system with solvent programming and U.V. and refractive index detectors. During the past year these instruments have been used to determine quantitatively and to identify several metabolites of hycanthone, of a chloroindazole analog of hycanthone (IA-4) and of an antischistosomal nitrovinylfuran (SQ 18,506). The technique has the advantage that the same system for analysis can be used also for the preparation of a pure sample for further characterization by mass spectrometry, nuclear magnetic resonance and infrared spectrometry. Instrumentation for each of these techniques is available to this project either in this laboratory or in the Department of Pharmacology.

Another useful tool for the study of CGP-4540 is the radioactive tracer method. Because of the relatively simple structure and the straightforward method of its synthesis, it will be possible to obtain the compound specifically labeled at various sites. This will greatly facilitate the follow-up of its fate through metabolic processes that ultimately result in its conversion to the active antischistosomal compound. Furthermore, the ability to ascertain the presence of a metabolite in a particular tissue or organ by its radioactivity before its chemical nature is known will insure that no important metabolite is overlooked. Equipment for sample preparation and high efficiency liquid scintillation counting of 3H and 14C-labeled compounds is available for these studies in our laboratories.

A bioassay system will be used to determine the presence of the active metabolite in the blood. Worms will be incubated in vitro in a nutrient medium containing blood or serum from animals that have received a large dose of CGP-4540. The presence of the active metabolite will be revealed by its effects on the worm in vitro, i.e., a paralysis of the acetabulum and of the body musculature, as well as a shortening of the worm. The quantity of this metabolite can be determined by serial dilutions, and the minimal concentration of blood or serum producing such an effect on the worms. This type of assay could guide the purification and isolation of the active antischistosomal metabolite.

Indications as to the nature of the drug metabolizing systems catalyzing the formation of active antischistosomal metabolites may be obtained by agents which are either inducing or inhibiting drug metabolizing enzymes. An example of the
former type is phenobarbital, and of the latter, SKF-525A, a selective inhibitor of cytochrome P-450. We propose to determine the effectiveness of orally administered CGP-4540 following pretreatment of mice infected with S. mansoni. Reduction or enhancement of the effectiveness of the drug by stimulation or inhibition of certain drug metabolizing enzymes would indicate the role of these enzymes and the metabolic pathways favoring the formation of the active metabolite.

Once the metabolic products have been identified in the uninfected animals, it will be necessary to determine whether the metabolism of the drug is not altered by the infection. Recent studies in this laboratory by Dr. Young-Nam Cha have shown that the activities of many, but by no means all, microsomal drug metabolizing enzymes are decreased during the infection with schistosomes and that there is a direct relationship between the severity of the infection (as determined by the number of eggs deposited in the liver) and the degree of depression of these enzyme activities. Accordingly, it will be necessary to determine whether there are qualitative and quantitative differences in the metabolism of CGP-4540 in the uninfected and infected animals, and whether the severity of the infection has an influence on the formation or elimination of the active schistosomicidal metabolite(s).

b. Development of Drug Resistance

Because of the steep dose-response curve and the uniform susceptibility of a large variety of strains of Schistosoma mansoni to CGP-4540, it is unlikely that strains resistant to this drug can develop readily. Nevertheless, this problem should receive attention since development of schistosomes resistant to another antischistosomal drug, hycanthone, has been demonstrated. After the administration of a subcurative dose of formulated CGP-4540 (3 mg/kg), which results in the elimination of 70% of the worms, the eggs produced by the surviving worms will be used to infect snails; subsequently, mice will be infected with the cercariae produced by these snails and the susceptibility of this generation of worms to CGP-4540 will be tested. In addition, following another challenge with CGP-4540, the eggs produced by the surviving worms of this generation will be used to produce a subsequent generation whose susceptibility to the drug will be tested again. Repeating this cycle for a few more generations and challenges of the respective progenies with the drug should provide information whether and to what extent resistance to the drug can be produced.
c. **Mode of Action**

There is a distinct time course in the effect of formulated CGP-4540 administered orally to mice infected with *S. mansoni*. Within one to three hours (depending on the size of the dose) after its administration to the host, there is a complete shift of schistosomes from the mesenteric veins to the liver sinuses, a phenomenon known as the "hepatic shift." This is caused by the loss of attachment of the worms to the internal wall of the mesenteric veins by means of a ventral sucker, the acetabulum. Indeed, one of the characteristics of the worms that have shifted to the liver shortly after administration of CGP-4540 is a paralysis of the acetabulum; another is a reduction in the length of the schistosomes, probably caused by a contracture of the longitudinal muscle. A third characteristic change is a reduction in the motor activity of the worms. The schistosomes remain in the liver sinuses for two to three weeks. While some of them die during this period, others remain alive and begin to shift back to the mesenteric veins. After 30 to 35 days, most of the survivors are found in the mesenteric vascular bed. However, even when a single dose as low as 7 mg/kg of the formulated drug had been administered, no live worms are detectable any more after 45 to 50 days. Hence, the recovery of some worms has only been temporary and some irreversible functional alteration must have been induced by the drug, resulting in the eventual destruction of the parasite. Thus, it would appear that at least two mechanisms are involved in the effect of the drug on the worms: (a) an immediate effect resulting in the hepatic shift within hours; (b) the long-term effects resulting in the ultimate destruction of the worms. The short- and long-term effects may not necessarily involve the same mechanisms. While all worms initially shift to the liver, some of those surviving a two- to three-week posttreatment period return to the mesenteric veins and are eliminated only after an additional period of approximately three weeks.

Preliminary studies have revealed that the short-term effects of the drug may be mediated by alterations in the neuromuscular physiology of the worm. Using cholinergic, tryptaminergic, and dopaminergic agonists and antagonists, attempts will be made to reverse the paralysis of the acetabulum and of the other muscular changes in worms removed from their host, several hours after the administration of the drug. Should any of these agents or a combination thereof produce a reversal of these changes, this would provide information about the nature of the type of neurotransmitter or receptor involved in the drug effect. The results of previous studies in this laboratory on the role of neurotransmitters in schistosomes will form the basis of investigations of this drug effect (Tomosky et al.: *J. Pharmacol, Exp. Ther.*, 190, 260, 1974).
The search for the mechanism responsible for the ultimate elimination of the worms will be facilitated by the relatively long period required for the destruction of some of the parasites. This provides an opportunity for a biochemical analysis of the worms over a period of at least four weeks. In this manner, even a slow and progressive onset of biochemical changes would be detectable over a relatively long period of time. Analysis of the worms removed at predetermined intervals will be carried out beginning several hours after the administration of the drug. This will be continued, at first daily, then every three to four days, until all the worms have been eliminated. Analyses initially will include the following:

a) Chemical analysis: glycogen, protein, DNA, RNA, and thiol groups' concentrations.

b) Histochemical examination of the female reproductive system.

c) Rate of glycolysis.

d) Determination of the following enzymatic activities: glycogen phosphorylase phosphatase, glycogen synthetase, glycogen phosphorylase, globinase.

Further studies related to the mode of action of the drug will be guided by the results of the above-proposed determinations.

d. Determination of Mutagenic Activity of CGP-4540 and of Its Metabolites

During the past few years, sensitive methods have been developed for the detection of mutagenic effects using bacterial tester strains. The usefulness of these assays resides in the predictive value of carcinogenic properties of compounds exhibiting mutagenic effects and the correlation between these two activities. There is considerable evidence that carcinogens are mutagens; at least 85% of the known carcinogens have been found to be mutagens, while virtually every noncarcinogen is also nonmutagenic. Hence, a rapid and sensitive mutagenic test system is extremely useful in pinpointing potential carcinogenic hazards to man. A very sensitive system is that developed by Ames (Mutat. Res., In Press, 1975) which uses two strains of Salmonella (TA-100 and TA-98). Both strains lack an intact cell wall and do not have an excision repair system, thereby eliminating a permeability barrier and reversible alteration of the DNA molecule. Furthermore, as shown by Ames also, some carcinogens are mutagenic only following metabolic activation by enzymes of rat liver microsomes. Therefore, a preparation containing rat liver microsomes will be included in the test system (Ames et al.: Proc. Natl. Acad. Sci. U.S.A., 70, 2281, 1973). The possibility that the host may metabolize a given
drug to a mutagenic substance will be examined by two further extensions of this test. (a) The host-mediated assay developed by Legator and Malling (Chem. Mutagens, 2, Ch. 22, 569. Pergamon Press, New York, 1971). The bacterial tester strain is introduced into the peritoneal cavity of the mammalian host following the administration of a large dose of the drug. After a predetermined period, the bacteria are removed from the host and their mutation frequencies are determined. (b) The detection of mutagenic metabolites in the urine of animals to which the drug has been administered (Commoner et al.: Nature, London, 240, 850, 1974; Durston et al., Proc. Natl. Acad. Sci. U.S.A., 71, 737, 1974). This test allows the detection of mutagenic compounds which have been produced by the metabolism of the parent drug in the host and which could have escaped detection in the in vitro test, even in the presence of microsomes.

e. Prevention of mutagenic effects

Previous studies in this laboratory have shown that different mechanisms are involved in the antischistosomal and mutagenic activities of thioxanthenes (Hulbert et al., Science, 186, 647, 1974). Therefore, conditions which reduce or eliminate the mutagenicity of a given compound or of its metabolite do not necessarily affect adversely their antischistosomal activity. During the past few years several types of agents have been reported which appear to interfere with processes initiating carcinogenesis or mutagenesis. It has been suggested that non-critical nucleophilic agents (e.g., monothiols, dithiols, methionine) might trap chemical carcinogens or mutagens and thereby prevent their reaction with DNA (Miller and Miller: J. Natl. Cancer Inst., 47, V, 1971; Crabtree: Cancer Res., 4, 668, 1944; 5, 346, 1945; 6, 553, 1946. Calcutt: Brit. J. Cancer, 15, 673, 1961, and 18, 197, 1964; Falk et al., Arch. Environm. Hlth., 9, 169, 1964. Marquardt et al.: Cancer Res., 34, 3387, 1974). Furthermore carcinogenic effects of polycyclic hydrocarbons and nitrosamines have been prevented by antioxidants and disulfiram (Wattenberg: J. Natl. Cancer Inst., 48, 1425, 1972; 50, 1541, 1973; 52, 1583, 1974) as well as by Vitamin A (Saffiotti et al.: Cancer, 20, 857, 1967. Maugh: Science, 186, 1198, 1974). After administration of a single oral large dose of formulated CGP-4540 (50 times the curative dose), the host-mediated assay with the sensitive Salmonella strain TA-100 yields slightly, but consistently, positive results, indicating that a weakly mutagenic substance has been produced by the host (negative results are obtained after elimination of the bacterial intestinal flora of the host. See below). We propose to determine whether the host-mediated assay becomes negative when the same dose of formulated CGP-4540 is coadministered with any of the above-mentioned substances which have been found to interfere, under comparable conditions, with the initiation of carcinogenesis.
f. **Dissociation of Mutagenic Activity from the Schistosomicidal Properties of Formulated CGP-4540**

Preliminary observations have indicated that the intestinal bacterial flora plays a critical role in the conversion of orally administered formulated CGP-4540 to a mutagenic substance, because, in contrast to untreated controls, after removal of these bacteria by administration of antibiotics, CGP-4540 fails to give rise to a mutagenic material in the host-mediated assay. It is of considerable interest in this connection that removal of the intestinal flora by antibiotics did not affect the antischistosomal activity of CGP-4540. Therefore, different mechanisms and metabolites are involved in the mutagenic, and the schistosomicidal effects produced by the administration of this drug. Investigation of this problem is not only of great theoretical interest, but also is critical for the development of methodologies designed to minimize or eliminate any mutagenic or carcinogenic risks associated with the administration of this antischistosomal compound. As an initial approach to this problem, we propose to isolate and to identify the metabolites of CGP-4540, administered to mice, hamsters, and monkeys whose intestinal bacterial flora have been reduced or virtually eliminated by treatment with antibiotics or with succinylsulfathiazole.

The methodologies to be used for the isolation and identification of these metabolites will be the same as outlined in Section 1 for animals whose intestinal bacterial flora has been left intact. Differences in the metabolic products of CGP-4540 in these two types of animals will reflect the contribution of the intestinal bacteria to the metabolism of the drug and to the formation of one or several mutagenic metabolites which can be eliminated by the removal of these bacteria. Further information about this problem will be sought by incubation, under anaerobic conditions, of intestinal bacteria grown in a nutrient medium with CGP-4540 and subsequent identification of the product(s). The latter may be further metabolized by the mammalian host. This will be examined also in vitro, i.e., by anaerobic and aerobic incubation of the bacterial fermentation products with preparations of intestinal mucosa or liver microsomes, or in vivo, by administration of the product(s) to the intact animal. Should these findings be confirmed and documented by the above-outlined metabolic studies, elimination of the bacterial flora (by means of antibiotics or succinylsulfathiazole) prior to the administration of a single dose of formulated CGP-4540 would eliminate a major mutagenic and carcinogenic risk inherent in the treatment of human schistosomiasis with this drug preparation.

g. **Analogs of CGP-4540 which Might Show Higher Therapeutic Indices**

Efforts will be made to produce analogs of CGP-4540 which might be non-mutagenic and show higher therapeutic indices.
h. **Antischistosomal Activity of Parenterally Administered Formulations of CGP-4540**

Preliminary observations have indicated that the antischistosomal activity of intravenously or intramuscularly administered CGP-4540 is of a high order; the minimal curative single dose has not yet been determined. The therapeutic index of this method of administration appears high because no toxic effects were observed with a dose of intravenously administered formulation exceeding the curative one by a factor of 50. There are several reasons why information about the effectiveness of parenterally administered CGP-4540 would be desirable. In the first place, there may be conditions in the field where the intramuscular route is preferable to the oral one. Secondly, unless the major route of excretion of the drug is via the bile, and if, in such an eventuality, reabsorption of a significant amount from the intestine occurs (enterohepatic circulation), parenteral administration may eliminate or at least reduce the metabolism of the drug by intestinal bacteria. This, in turn, may yield different metabolic products. If none of these were mutagenic, parenteral administration would present minimal mutagenic or carcinogenic risks even without removal of the bacterial flora.

Thirdly, introduction into mice of a subcutaneous depot of CGP-4540 suspended in sesame oil has afforded protection against percutaneous infection with cercariae of *S. mansoni* for several weeks. Thus, there is a potential for developing a prophylactic preparation of CGP-4540. However, this requires the development of conditions which preclude the formation of mutagenic substances in association with the slow release of the antischistosomal agent. The methodologies which will be used to investigate this problem are similar to those outlined above in Section 4.

i. **Summary**

It is the aim of this project to develop an effective and safe drug preparation for the treatment of human subjects infected with any one of the three species of schistosomes invading man, *S. mansoni*, *S. hematobium*, and *S. japonicum*. The formulation of CGP-4540 developed in this laboratory has been found to be highly effective and endowed with low acute host toxicity. Elimination of the intestinal bacteria of the host (produced by the use of antibiotics) does not affect antischistosomal efficacy, but fails to give rise to mutagenic metabolites. Once the metabolism of CGP-4540 in animals, with and without their bacterial intestinal flora, has been elucidated and the contribution of these microorganisms to the formation of one or several mutagenic compounds in the host has been understood by the investigations proposed above, opportunities will become available to treat human schistosomiasis using a formulation of CGP-4540, or of an analog thereof, with minimal or no mutagenic risk in any of the following manners:
a) Single oral dose of CGP-4540 preceded by the elimination of the bacterial flora (using 1 or 2 doses of a mixture of antibiotics or of succinylsulfathiazole).

b) Use of a suitable formulation of a structural analog(s) which gives rise to a schistosomicidal, but fails to generate the formation of a mutagenic, metabolite(s).

c) Parenteral administration of formulated CGP-4540 to "short circuit" its exposure to the metabolic activities of the intestinal bacterial flora.

d) Coadministration of anticarcinogenic, non-toxic substances (thiols, antioxidants or Vitamin A).

Everyone of these possibilities and approaches will be fully explored in the course of the investigations proposed above.
9. **Estimated Life of Project Costs**

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<td>Paul Talalay, M.D., Distinguished Professor of Pharmacology</td>
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| Supplies: | |
| Chemicals, including radioactive compounds solvents, enzymes, etc. | $7,000 | $7,000 |
| Glassware and disposable syringes | $2,000 | $2,000 |
| Purchase of mice (60 per week) | $3,000 | $3,000 |
| Purchase of hamsters (20 per week) | $1,200 | $1,200 |
| Animal care, feed, bedding, and personnel services | $8,000** | $8,000** |
| Equipment for animals (cages, etc.) | $1,200*** | $1,200*** |
| Travel | |
| Service agreements: maintenance and servicing of equipment | $1,650 | $1,650 |
| Other: | |
| Communications, publication costs, etc. | $1,000 | $1,000 |
| TOTAL DIRECT COSTS | $83,854 | $83,854 |
| TOTAL INDIRECT COSTS | $43,604 | $43,604 |
| TOTAL DIRECT AND INDIRECT COSTS | $127,458 | $127,458 |
| TOTAL DIRECT AND INDIRECT COSTS - 1st yr. | $126,875 | $126,875 |
| - 2nd yr. | $127,458 | $127,458 |
| GRAND TOTAL PROJECT A | $254,333 | $254,333 |
10. **Work Plan and Contract Budget**

Since this is a two year project, this section is omitted in accordance with the Guidelines for Preparation of Research Project Statements.

11. **General Appraisal**

   a. **Appraisal of Project and Research Proposal**

   TA/H after reviewing the current status of chemotherapeutic agents for treatment of human schistosomiasis concludes that safer new drugs which are more satisfactory for mass treatment programs are badly needed. The compound which would be investigated during this project appears very promising and certainly warrants further pre-clinical study and the proposed efforts to improve further its safety with respect to mutagenicity. TA/H, recognizing previous recommendations that donor agencies assist pharmaceutical firms in developing antischistosomal agents for which no profitable market is apparent, considers it appropriate to support this work in an effort to control a chronic disease that is widespread and spreading further among the rural poor in many LDCs.

   TA/H, realizing the highly complex nature of this subject, has deferred to the FDA the PHS and an expert consultant parasitologist for technical appraisals of the work plan and protocols. These are attached. One matter has not been resolved unanimously. The proposal notes that four groups of pre-clinical investigations are required to comply with FDA regulations governing issuance of Investigational New Drug permits for studies in human subjects. It further indicates
that these should be pursued simultaneously during the same two year period but presents protocols for only one of the four activities. If these are pursued in consecutive order a total of seven to eight years of work will be required just to complete pre-clinical investigations preparatory to testing in normal uninfected humans. This allows considerable time for further expansion of the schistosomiasis problem. It is considered desirable, therefore, that a consensus of expert advice be obtained concerning the most satisfactory scheduling of these multiple activities. FDA and PHS indicate that the work plan is sound and suitable, respectively. It is not clear that the existing need to expedite development of a more satisfactory antischistosomal agent has been considered. FDA clearly recommends that pre-clinical testing should proceed in consecutive steps but PHS appears to understand that all activities will proceed simultaneously. Dr. Bueding has indicated that he expects to have some compounds ready for testing in primates in not more than six months after his work is initiated. If this optimistic view proves to have been warranted, then substantial reduction in the duration of pre-clinical testing may well be possible.
November 23, 1975

Joe L. Stockard, M.D.
Office of Health
Technical Assistance Bureau
Agency for International Development
Washington, D.C. 20523

Dear Dr. Stockard:

I attach a series of itemized notes concerning the Proposal for Research and Development Towards an Effective and Safe Drug for the Treatment of Human Schistosomiasis submitted to USAID by the Director of the Wellcome Laboratories for Studies on Schistosomiasis of The Johns Hopkins University.

I also include a number of comments and recommendations which I hope will fulfill the charge to me in your letter of 20 November. I have not replied directly to each of the eight questions you posed in the letter but I believe the two documents enclosed cover the points adequately.

In my view the proposal should not be supported at this time. Nevertheless, I believe that in some way the manufacturer should be pressed to explore the drug and especially the new formulation, without delay and so pave the way for systematic investigations into its usefulness.

Sincerely,

Louis J. Olivier, Ph. D.
Consultant to
Agency for International Development
Comments and Recommendations

Better drugs for treatment of schistosomiasis and for use in control campaigns are needed and it is appropriate for USAID to lend support to drug development.

The drug in question appears to be quite promising judging from the brief summary of results of oral studies in laboratory mammals given in the proposal. It isn't clear where the data were gathered, how much may have been contributed by Ciba and how much they are interested in doing in the future. One wonders at the start why Ciba hasn't gone ahead to assemble the basic data on the drug that a drug developer usually produces in justification for interest or support, especially since the drug is active against all three schistosome species. Normally, a drug company does oral and parenteral trials in several laboratory mammals and against all three species. If the results are encouraging dose regimen and formulation work is done and primates are included. The company also sets out early to get toxicology data including LD studies and acute and chronic toxicity work to determine some limits and learn a bit about what happens in the animal especially when high doses are given.

Only when such work gives cause for continued interest does one go in for more intensive study and exploration of such things as metabolism, mode of action, management of defects, etc. (See the WHO Technical Report #317 (1966) for a detailed account of the basic data normally provided by the drug developer and needed prior to advanced studies including pre-clinical testing in normal persons).

Judging from the requirements listed by the WHO Expert Group, the drug in question seems to be in a very early stage of development. Therefore, one should hesitate before investing large sums and effort in studying certain aspects of its metabolism or in attempting to avoid an apparent disadvantage (mutagenicity in one test system).

Based on the above, I conclude:

(1) that it is premature to undertake intensive study of the drug in question because much essential basic information is needed before one can decide whether the drug is active enough and safe enough to warrant further attention. (See WHO Tech. Pap. #317).

(2) that the manufacturer be asked to produce such data including data on IM use, data on toxicity and basic pharmacology. If the manufacturer is not disposed to do so then one might ask why a drug of such apparently promising features is of no interest to them.
(3) to proceed to underwrite development of the basic data would be to subsidize a manufacturer in development of a single drug, a course that doesn't seem appropriate for USAID.

(4) when the data base justifies further study of the drug, the Wellcome Laboratories for Studies on Schistosomiasis are judged specially well-qualified to undertake studies on the pharmacology, metabolism and mode of action of the drug.

(5) the Laboratories do not appear well-qualified to carry out the studies on mutagenesis proposed. It would seem better to do such studies in a laboratory devoted to such work since the subject is complex, techniques are evolving rapidly and much disagreement surrounds the results and the significance of the tests to man.

(6) that demonstration of the utility of the IM route would decrease the necessity to do the proposed studies on metabolites produced in the gut.

(7) that the work proposed in Projects B, C and D should not be sponsored by USAID.

(8) that USAID should avoid commitment to aid in the basic studies of anti-schistosomal drugs normally carried out by drug houses but that USAID should consider aid in developing data which they are nor normally required to produce. Such data would include those mentioned in (4) above but also include data that can be obtained from clinical trials both in the laboratory and in the field since, for a drug developer, such studies may be beyond its means.
Itemized Notes on The Proposal

I'll start by noting my reactions to portions of the proposal item by item and then summarize my reactions and make recommendations concerning the proposal.

Page 5, line 19: Negative tests for mutagenesis are mentioned here but positive tests are described on page 12.

Page 6, line 1: This generalization is a non-sequitur. It also presents a requirement that is impossible to fulfill for any new drug. No existing test for mutagenesis is absolutely predictive for man and negative test results do not assure complete absence of mutagenic properties, only failure to detect any.

Page 6, Second Para: If the drug is metabolized to 5 or more compounds would the mutagenic potential of each be of interest?

Page 7, Development of drug resistance: This is of secondary importance and could well be deferred at least until studies show clearly the drug is usable and the active metabolite(s) is known. A resistance study must contemplate continuation through many generations and some would say it isn't economically sound - being better to wait for evidence resistance is occurring in the field. One-time testing of existing strains for resistance is another matter but isn't necessarily very significant either from the practical point of view if they are laboratory strains.

Page 11, Mutagenic activity: Bacterial test systems are useful as screening but tests with mammalian cells and especially in whole mammals are far more useful and are essential when one wants to extrapolate to man.

Page 12 & 13: This subject is of broad theoretical and practical significance. However, it seems better suited to specialists in mutagenesis and to conditions where the study would not be limited to one drug and its relatives - a mutagenesis or carcinogenesis laboratory. With respect to the drug in question, if the IM route is effective and practical the oral route could be discarded due to mutagenesis problems thus avoiding the need for the work on metabolites made in the intestines.
Page 14: Basic testing of analogs is normally a function of the manufacturer who is normally best set up to make and to do basic tests on them.

Page 15, First para: This information is routinely collected by the drug producer. It is not clear why they did not do such tests. In any case, this information is required before any large further research commitments are made. As said above, if the drug can be used parenterally, it affects the desirability and usefulness of gut studies and of the work needed to show that the use of anti-bacterial substances only with the drug is useful or desirable.

Page 15, Second para: Has Ciba done this? Normally, the manufacturer would handle such studies.

Page 15, Last para: This paragraph makes several assumptions that may be open to question:

(1) "Low toxicity" - This seems to be based on a very small amount of data judging from the test.

(2) "Elimination" of intestinal bacteria is assumed to be possible and fully effective. If so, is it safe, practical and economic?

(3) "The proposed studies would produce a formulation or drug combination with "minimal" risk. What is, or may be accepted as "minimal"?

Page 16, a: This would not be needed if the IM route is chosen. This could open up a new series of problems. At the least the preparation would be complex and it might be hard to clear through drug control agencies. Are there precedents?

Page 17: Parts of Project A and most, if not all, of Projects B, C and D are usually performed by the discovering company and patent owner.

Page 18, line 9: Among a number of authorities, it is not considered essential, or even preferable, that an anti-schistosomal drug be given orally. The oral route has advantages but it is not quite so "sure,"it requires absorption from the intestine, and it may cause intestinal irritation. The IM route offers its own problems but it is simple, rapid and sure in the sense that one knows exactly what the patient received.
- **lines 17-21:** This seems to imply that a candidate drug must pass all mutagenesis tests put forward. Such a barrier would have to be recognized as a formidable challenge to drug development. Moreover, to "eliminate" mutagenic and carcinogenic hazards is obviously a scientific and technical impossibility since one cannot prove a negative but only reduce the probability by testing. It seems the assumption is made that the candidate drug would be free from the two hazards if the proposed studies were made. Not so.

**Pages 19 and 20:** It appears that the major work on the project would be done by persons qualified in metabolism and enzyme studies. One does not see capability to undertake mutagenesis studies, especially of the scope and significance of those proposed.
MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service

TO: Director
Technical Assistance Bureau/H
Agency for International Development
Attention: Dr. Joe Stockard

FROM: Director
Office of International Health

DATE: DEC 11 1975

SUBJECT: "Proposal for Research and Development Towards an Effective and Safe Drug for the Treatment of Human Schistosomiasis" - Requested Appraisal

In response to Dr. Stockard's letter of November 20, to Dr. de Caires, the Proposal has been reviewed by CDC, FDA, NIAID/NIH and OIH. These individual appraisals have been consolidated and form the basis of a PHS response to the eight points raised:

1. There is a great need for a safe and effective anti-schistosomal drug, or drugs. Ideally, for mass use, single dose therapy is eminently desirable. Administration orally would be cheaper than parenterally.

2. The work plan for pre-clinical testing is sound. The duration may well exceed two years, plus write-up and reporting time.

3. Protocols are satisfactory for the development, if the data so warrant, of an IND submission to the FDA, but note comment under (5).

4. Imposed mutagenic and carcinogenic test systems are adequate.

5. Based on a single dose treatment the work plan appears complete, but it must be kept in mind that, even with mass administration, reinfections will occur in people exposed to infested water. Such persons may therefore require one or more "repeat" doses. Chronic toxicity tests will therefore be needed with this contingency in mind, of two-years duration and initiated after termination of other shorter term tests.

6. Pre-treatment with antibiotics or other chemicals will seriously complicate mass treatment, operationally and in costs. Multiple intestinal pathogens may well be the rule, rather than the exception, in some areas/countries. Reduction of mutagenic "risks" will have to be carefully weighed against demonstrable benefits, especially in S. japonicum disease.

7. Cost estimates in the two-year time-frame, appear reasonable, but
that duration may be exceeded, especially if chronic toxicity studies (two years) are to be undertaken.

8. The research and protocol are of high quality. However, we must be cautious in estimating the speed with which even a meritorious scientific plan can be translated into practical treatment modes for populations at risk. After two or more years of effort, if the drug does indeed pass these rigorous hurdles, how many more years will be required for tests of clinical investigation under a wide variety of environmental and other conditions? Will such an effort and expenditure absorb a major portion of funds that AID would put into other aspects of schistosomiasis research and control? Should not the manufacturer fund such research of a very promising and exciting compound with a vast potential market? Has the interest of the Clark Foundation been explored, for example? These are difficult questions, but do have a bearing on your Agency's role.

The Food and Drug Administration should be approached for expertise to assist AID in monitoring the study. Funding should include provision for site-visits and consultations. Some additional, routine safety tests have been suggested by FDA pharmacologists; these are considered minor and readily acceptable to the investigator:

S. Paul Ehrlich, Jr., M.D.
Joe L. Stockard, M.D.
Office of Health
Technical Assistance Bureau
Department of State
Agency for International Development
Washington, D. C. 20523

Dear Dr. Stockard:

This refers to your letter of November 20, 1975. Enclosed is my memorandum of and comments to Dr. Bueding's submission.

Hereafter are my answers to your specific questions:

1. There is a need for new antischistosomal drugs.

2. The work plan is suitable.

3. The preclinical safety studies which are routinely required for FDA acceptability need elaboration. Otherwise the protocols are acceptable.

4. The proposed mutagenic and carcinogenic test systems are acceptable.

5. See item #3.

6. I would object to any method which would associate antibiotic administration with an antischistosomal in mass treatment. (note that the pertaining animal experiment described in the submission does not suggest the use of such combination treatment in medical practice.

7. The budget is acceptable, but minor budget items may need expansion. The planned duration of the project is reasonable, but carcinogenicity testing may require additional time.

8. Please refer to the enclosed memorandum.

Please let me know if any clarification is needed and if I can be of any further assistance.

Sincerely yours,

Edgar J. Martin, M.D.
Division of Anti-Infective Drug Products
Bureau of Drugs
Introduction

The available antischistosomal drugs are all inadequate for one or several of the following reasons:

- Low or nil efficacy for certain species or strains of schistosomes (in man and animals).
- Severe (sometimes fatal) acute toxicity, in man, at therapeutic doses.
- Teratogenicity (animals).
- Mutagenicity (bacteria, mammalian cell cultures).
- Carcinogenicity (animals).
- Inadequacy of information.

Therefore, the development of a product which minimizes or is free of these drawbacks is needed.

Review

(Pages 1 thru 5) The product whose study is proposed in the submission, CGP-4540 (or its suitable derivatives) is hereafter called the DRUG. In various formulations a single dose of the DRUG is reported to be effective for several strains of the 3 principal schistosome species as tested in mice, hamsters, dogs and monkeys. With one of the formulations 100% efficacy was obtained by a dose of 20 mg/kg in mice and hamsters. This dose compares very favorably with other products of this class. Mutagenicity was not detected in bacterial strains using the technique of Ames et al. Detailed toxicologic studies have not yet been done. Mice, hamsters and monkeys "tolerated well 50 times the oral therapeutic dose," an observation which neither I nor the sponsor consider as conclusive.

Comment to pages 1 thru 5: Available information shows that the DRUG is effective (see tables on pages 2 and 4), probably not mutagenic, and not acutely fatal at 50 times the therapeutic dose in animals. This information encourages in depth study of the DRUG.

(Pages 6 thru 16) The DRUG has been found active in vivo but not in vitro. Therefore, the submission proposes the search for and isolation of active metabolites. Other observations suggested that the intestinal flora may transform the DRUG into a mutagen. Therefore, analogs will be synthesized and tests will be conducted to discover whether they or their metabolites
have advantages over the mother substance. Another antischistosomal compound has been found to cause development of drug resistance of the parasite. Tests will be made to see if this phenomenon occurs with the DRUG too. The mode of action of the DRUG on schistosomes will be studied. It will be tested whether and how the DRUG (or its best analog or active metabolite) can be administered parenterally.

The principal investigator and his team and facilities have prominent stature in the field of antischistosomal drug development.

(Page 17) After efficacy in lower animals and lack of mutagenicity have been established, the efficacy of the drug in primates will be tested. (Note that certain antischistosomals which had been found effective in mice have failed in primates and man: K.J. Schnitzer & F. Hawking, Experimental Chemotherapy, Academic Press, 1963, vol. 1, pp 769-790). Thereafter, the routine tests for safety of the DRUG should be performed. They should include LD50 in 2 lower animal species; organ studies including histology; cardio-vascular measurements; blood and bone marrow chemistry and morphology in dogs and primates; kidney and liver function tests in primates and at least one lower species; neurologic tests; teratology, including testicular studies: the search of the highest no-effect-dose in various parameters; protein binding of the drug. The animal species used for each of these studies should be judiciously selected. I and consultant pharmacologists will be available to discuss the details of each study protocol with the investigator. Some of the safety studies can be done simultaneously with the efficacy study. A study on carcinogenicity should be initiated as soon as all other tests demonstrate the DRUG as acceptable. Should there be alternatives for the selection of an effective metabolite, special attention should be given to those in whom the original nitro group has not been modified to a potentially cancerogenic structure.

(Page 17) The periods of the tentative time table are reasonable and within the range of routine work in pre-clinical testing. However, additional time (and funding) should be allowed for the animal test on carcinogenicity. This test would require about 2 years but can be initiated only after successful termination of the other tests.

(Pages 19 thru 22) The budget requests are reasonable and within the range of similar projects. However, additional funds should be allowed for on-site visits of the monitors. This item should provide for trips for both the principal investigator and the monitor, for selecting and, later, monitoring the subcontractors.

General Summary

The subject of the proposal aims at fulfilling a real need in chemotherapy. The information from the preliminary studies show that the substance, or substances, which the project will investigate are promising. The proposal has been expertly written. I suggest some additional experiments which
are of a minor nature. Details for the routine safety testing will have to be stipulated. In view of the status and experience of the investigator as a pharmacologist I expect no difficulties in these matters.

The investigator and his collaborators and facilities qualify for most of the aspects of the project. The investigator qualifies for selecting subcontractors for the experiments for which he is not equipped.

The budget request is commensurate with the size of the work proposed and needed. However, some minor extensions of the funding should be anticipated.

I evaluate Dr. Bueding's project as important and recommend that it be funded.

Edgar J. Martin, M.D.
Division of Anti-Infective Drug Products (HFD-140)

NOTE:
I propose presently as a consultant pharmacologist:

Dr. Marion deV. Cotten
Editor-in-Chief
J. Pharmacol. Exp. Therapy
Rt. 3, Box 229
Sylvania, Georgia 30467

(912) 863-4343
This PAF amendment incorporates all elements of project 0642 into a single authorization and adds $43,000 to the life of project funding. $254,000 was authorized and obligated in FY 1976, prior to the use of the PAF form. The original PAF (signed August 11, 1977) authorized $130,000 in FY 1977 and $313,000 in FY 1978 for a specific sub-project, the Lowell University Primate tests. The additional $43,000 in this amendment will provide for continuation of related work at Johns Hopkins University during the full period of the work at Lowell.
Project Authorization and Request for Allotment of Funds:
Part II

Entity: Development Support Bureau
Project: Antischistosomal Drug Testing
Project Number: 931-0642

This amendment authorizes the additional $43,000 needed to fund the
activity at Johns Hopkins University which is needed, along with the
activity at Lowell University to complete the test of safety and
efficacy of a new antischistosomal drug, as authorized by the RAC in
April, 1976 (JHU portion). The RAC at the April meeting recommended:
That the proposal be negotiated at a level sufficient to elucidate
the efficacy, pharmacology, and toxicology of the antischistosomal
drug CGP-4340 in primates, when administered alone or concurrently
with a single dose of erythromycin base.

The $43,000 requested is an essential part of that level, providing for
the necessary pharmacological and pathological work at JHU to accompany
non-human primate testing at Lowell.

Clearances:
DS/RES, M. Rechcigl Date 12/1/77
DS/PPU, J. Gunning Date 12/1/77

Reference: Howard to Belcher Action Memorandum dated 12/21/77
Attachments: Howard to Farrar Action Memorandum dated 8/9/77
w/attachments
Summary of project funding.