

PD-ABD-702
ISN 75518

UNITED STATES OF AMERICA
AGENCY FOR INTERNATIONAL DEVELOPMENT
U.S.A.I.D. MISSION TO KENYA



UNITED STATES POSTAL ADDRESS
US AID MISSION TO KENYA
BOX 201
APO NEW YORK, NEW YORK 09675

INTERNATIONAL POSTAL ADDRESS
POST OFFICE BOX 30251
NAIROBI, KENYA

Dr. George K. Kinoti
Department of Zoology
University of Nairobi
P.O. Box 30197
Nairobi, Kenya

Dear Dr. Kinoti,

Subject: Grant No. 615-0259-G-SS-00-1071

Pursuant to the Authority contained in the Foreign Assistance Act of 1961, as amended, the Federal Grant and Cooperative Agreement Act of 1977, the Agency for International Development (hereinafter referred to as "A.I.D." or "Grantor" hereby grants to THE University of Nairobi (hereinafter referred to as "Grantee" the sum of \$150,000 to provide support for a research program entitled "Identification of Protective and/or Diagnostic Antigens of Schistosoma haematobium", as described in the Schedule of this grant, Attachment 1; the "Program Description", Attachment 2; and the Grantee's proposal, as amended, which is made a part of the Grant and incorporated herein by reference.

This Grant is effective and obligation is made as of the date of this letter and shall apply to commitments made by the Grantee in furtherance of program objectives during the period beginning August 27, 1991 and ending August 26, 1995.

This Grant is made to the Grantee on condition that the funds will be administered in accordance with the terms and conditions as set forth in Attachment 1 entitled, the "Schedule"; Attachment 2 entitled "Program Description"; and Attachment 3 entitled "Standard Provisions", which have been agreed to by your organization.

Please sign all copies of this letter to acknowledge your receipt and acceptance of this Grant. Keep one copy for your files and return the original and all remaining copies to this office.

Sincerely,

John R. Westley for
John R. Westley for
Grant Officer
USAID/Nairobi
Nairobi, Kenya

Attachments:

1. Schedule
2. Program Description
3. Standard Provisions and Optional Provisions
4. Original Proposal, Revisions and/or Compliance to Protocols

ACKNOWLEDGED:

Grantee Name: University of Nairobi

BY: *George K. Kinoti*

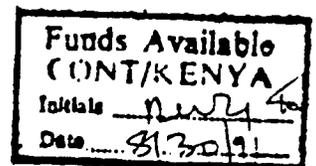
TYPED NAME: Dr. George K. Kinoti

TITLE: Professor - Zoology

Date: 16 September 1991

FISCAL DATA

| | | |
|------------------------|---|--------------------------|
| PIO/T No. | : | N/A |
| Appropriation No. | : | 72-1111021.6 |
| Budget Plan Code | : | DDSA-91-29615-KG11 |
| Allotment No. | : | 146-51-615-00-19-11 |
| Project No. | : | 936-5600 |
| Proposal No. | : | 9.398 |
| Total Estimated Amount | : | \$150,000 |
| Total Obligated Amount | : | \$150,000 |
| Funding Source | : | USAID/Nairobi |
| Project Office | : | AID/SCI |
| Voucher Paying Office | : | USAID/Nairobi Controller |



SCHEDULE

A. Purpose of Grant

No. 9.398

The purpose of this Grant is to provide support for the University of Nairobi's proposal entitled "Identification of Protective and/or Diagnostic Antigens of Schistosoma haematobium" (9.398) which is hereby incorporated by reference.

B. Period of Grant

Funds obligated hereunder are available for program expenditures for a period of four-years which includes one year for start-up and closeout from August 27, 1991 to August 26, 1995.

C. Amount of Grant and Payment

1. AID hereby obligates the amount of \$150,000 for the purposes of this Grant.
2. Payment shall be made to the Grantee in accordance with procedures set forth in Attachment 3 - Required as Applicable Standard Provision entitled "Payment - Cost Reimbursement".
3. All financial reports required by this provision shall be submitted to:

USAID/Nairobi
Office of the Controller
Nairobi, Kenya

Include PSTC Proposal No. 9.398

D. Grant Budget

The following Budget for this Grant includes local cost items, if authorized. Revisions to this Budget shall be made in accordance with Mandatory Standard Provision of this Grant entitled "Revision of Grant Budget." Within the total estimated cost of the Grant, the Grantee may adjust the line items as reasonably as necessary for the performance of the grant program.

"Reasonably as necessary" has been interpreted as up to 15 percent. Any amount greater than 15 percent would require the approval of the Project Officer.

Estimated Budget

| <u>Line Item</u> | <u>Amount</u> |
|-----------------------|---------------|
| Salaries | \$ 33,900 |
| Equipment | 50,500 |
| Materials & Supplies | 15,750 |
| Training | 22,000 |
| Travel: International | 16,600 |
| Local | 5,250 |
| Renovations | <u>5,000</u> |
| Grand Total | \$150,000 |

A Line Item Budget is attached as Attachment 2.A.

E. Reporting and Evaluation

1. Technical Reports

Reports must be sufficiently detailed to substantiate the findings and to permit a scientific evaluation of the research. Overseas collaborators shall be given fair credit for their participation in the research and a chance to review and comment on the Final Report before it is submitted. The principal investigator will share a draft of the Final Report with the A.I.D. Project Officer for comments prior to the formal submission. Publication of results in scientific journals is encouraged. Additional guidance on report preparation is given in the "Interim Guidelines on Performance Report Preparation for PSTC and CDR Projects," available from AID/SCI.

a. Performance Reports: Performance reports are required every six months. The principal investigator will submit reports stating what has been accomplished to date and detailing project management issues. A Financial Status Report will be attached to each report. Reports are due within sixty (60) days after the end of each six-month period. One copy of each report is to be submitted to the Office of the Science Advisor, Room 320 SA-18, Washington, D.C. 20523-1818; one copy to the Project Officer, Dr. Caryn Kolar Miller, S&T/HP/H/CD, Rpp, 702 SA-18, Agency for International Development, Washington, D. C. 20523-1817; one copy to the USAID Nairobi, Nairobi, Kenya; and two copies to the National Academy of Sciences, BOSTID, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

b. Final Performance Report: Within sixty (60) days after the termination of the Grant, the principal investigator will submit one copy of the Final Performance Report to the Office of the Science Advisor, A.I.D., Room 320 SA-18,

Washington, D.C., 20523-1818; one copy to the Project Officer, Dr. Caryn Kolar Miller, S&T/HP/H/CD, Rpp, 702 SA-18, Agency for International Development, Washington, D. C. 20523-1817; one copy to the USAID Nairobi, Nairobi, Kenya; one copy to Bureau for Program and Policy Coordination, Center for Development Information and Evaluation (PPC/CDIE), Room 215C, SA 18, Washington, D.C., 20523-1802; and two copies to the National Academy of Sciences, BOSTID, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

2. The Financial Status Report (SF-269) is required every six months as an attachment to the performance report and must be sent to the following Voucher Paying Office:

a. USAID Nairobi
Office of the Controller
Agency for International Development
Nairobi, Kenya

b. Voucher Identification

Grant No: 615-0259-G-SS-00-1071
Project Number: 936-5600
SCI Grant No: 9.398
Project Office: Office of the Science Advisor
Obligation No:
Budget Plan Code: DDSA-91-29615-KG11

F. Technical Backstop Officer and Investigators

1. Technical Backstop Officer

Dr. Caryn Kolar Miller
S&T/HP/H/CD, Room 702, SA-18
Agency for International Development
Washington, D.C. 20523 - 1817

2. Principal Investigator:

Dr. George K. Kinoti
Department of Zoology
University of Nairobi
P.O. Box 30197
Nairobi, Kenya

3. Co-Principal Investigator

Dr. Donald A. Harn
Assistant Professor
Department of Tropical Public Health
Harvard School of Public Health
665 Huntington Avenue,
Boston, MA., U.S.A.

G. Special Provisions

1. Compliance with Federal Guidelines and Regulatory Procedure:

- a. The recipient will implement this research activity in accordance with all relevant guidelines for U.S. Government funded research, such as:
 - (1) The National Institutes of Health (NIH) guidelines for the ethical treatment of human subjects;
 - (2) NIH and USDA guidelines for the handling of pathogenic microorganisms;
 - (3) USDA-APHIS procedures for animal and plant health inspection;
 - (4) A.I.D.'s environmental procedures; and
 - (5) Such other Federal guidelines and procedures as may apply during the course of research.
- b. All existing comparable guidelines of the host country in which the research is actually located must be followed also.
- c. Reports submitted under this activity to A.I.D. will address the cited regulatory issues. All modifications of protocols affecting these regulatory concerns must be reported. The investigators are responsible for reporting any difficulties encountered in implementing these protocols.

2. Human Subjects: Research will be conducted following the protocols described in Attachment 4 (which is the original proposal or subsequent amendments, or letter from the Principal Investigator), which insures the well-being and informed consent of human subjects. It will also be conducted in accord with the applicable procedures issued by the U.S. Government to insure ethical treatment of human subjects, and by those issued by the government of the host country in which the human subjects are to be involved.

If the protocol(s) involving human subjects is revised, it must be re-reviewed by the investigator's institutional ethical review committee, and the Project Officer and Office of the Science Advisor must be informed in writing before the revised protocol(s) is used. The revised procedures must be consonant with the guidelines of the host country and of the United States. If the patient's informed consent form is revised, a copy of new form must be submitted to both the Project Officer and the Office of the Science Advisor. A copy of the approval of the revised form by the investigator's institutional ethical review committee must also be provided to the Project Officer and the Office of the Science Advisor.

In addition, however, and prior to commencement of any experimentation involving human subjects, the Grantee shall make a judgment and communicate the same to A.I.D. as to whether the regulations, procedures or facilities of the country in question are adequate to ensure the safety and free and informed consent of the human subjects. In the event such judgment is that they are not, the Grantee and A.I.D. will consult and agree on the protocol to be applied to insure the safety and free, informed consent of the subjects.

3. Laboratory Safety and Hazard Containment: Research will be conducted following the protocols described in attachment 4 (which is the original proposal or subsequent amendments, or letter from the Principal Investigator), which insure the safety of persons involved in the research. Notwithstanding, the research must be conducted following procedures issued by the U.S. Government and those issued by the government of the host country for the containment of these hazards.

If the protocols involving laboratory safety and hazard containment are revised, they must be re-reviewed by the investigator's institutional review committee(s) that approved the original protocol, and the Project Officer

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and Office of the Science Advisor must be informed in writing before the revised protocols are used. The revised procedures must be consonant with the guidelines of the country in which the laboratory is located, and of the United States. Copies of the approval of the revised protocols by the investigator's institutional review committee(s) should also be provided the Project Officer and the Office of the Science Advisor.

Similarly, the research will be conducted in the facilities described in attachment 4. If the research is moved to new facilities, or the facilities are modified in such a way to affect safety or hazard containment, a description of the new facilities must be provided to the Project Officer and to the Office of the Science Advisor before the research is affected. Any applicable institutional reviews of the facilities must be repeated, and the re-certification must be provided to the Project Officer and the Office of the Science Advisor.

4. Containment and Safe Disposal of Animal or Plant Pathogens or Pests: Research will be conducted following the protocols described in attachment 4 (which is the original proposal or subsequent amendments, or letter from the Principal Investigator), which insure the containment and safe disposal of animal or plant pathogens. Notwithstanding, the research must be conducted following procedures issued by the U.S. Government and those issued by the government of the host country for the containment of these pathogens or pests.

If any protocol is revised, it must be re-reviewed by the investigator's institutional review committee(s) that approved the original protocol, and the Project Officer and Office of the Science Advisor must be informed in writing before the revised protocols are used. The revised procedures must be consonant with the guidelines of the country in which the laboratory is located, and of the United States. Copies of the approval of the revised protocols by the investigator's institutional review committees should also be provided the Project Officer and the Office of the Science Advisor.

Similarly, the research will be conducted in the facilities described in Attachment 4. If the research is moved to new facilities, or the facilities are modified in such a way to affect safety or hazard containment, a description of the new facilities must be provided to the

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Project Officer and to the Office of the Science Advisor before the research is affected. Any applicable institutional reviews of the facilities should be repeated, and the re-certification should be provided to the Project Officer and the Office of the Science Advisor.

5. Humane Treatment of Experimental Animals:

a. Principals for the Treatment of Vertebrates: The Grantee will adhere to the following principals for the utilization, care and transportation of vertebrate animals used in testing, research and training. For guidance throughout these Principles, reference is made to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council.

- (1) Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.
- (2) The animals selected for a procedure should be an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation and in vitro biological systems should be considered.
- (3) Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain and distress in other animals.
- (4) Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

- (5) Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure, or, if appropriate, during the procedure.
 - (6) The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.
 - (7) Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.
 - (8) Where exceptions are required in relation to the provisions of these principles, the decisions should not rest with the investigators directly concerned, but should be made, with due regard to U.S. and host country regulations, by an appropriate review group such as an institutional animal research committee. Such exception should not be made solely for the purpose of teaching or demonstration.
- b. Applicable Regulations: The transportation, care and use of animals should be in accordance with the U.S. Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable U.S. Federal laws, guidelines, and policies. Notwithstanding, the research must be conducted following procedures issued by the government of the host country for the humane treatment of experimental animals.
- c. Compliance with Reviewed Protocols: Research will be conducted following the protocols described in Attachment 4 (which is the original proposal or subsequent amendments, or letter from the Principal Investigator), which insure the humane treatment of experimental animals.
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- d. Revision of Protocols: If any protocol involving the experimental animals is revised, it must be re-reviewed by the investigator's institutional review committee(s) that approved the original protocol, and the Project Officer and the Office of the Science Advisor must be informed in writing before the revised protocol(s) is used. The revised procedures must be consonant with the guidelines of the country in which the animals are affected, and of the United States. Copies of the approval of the revised protocols by the investigator's institutional review committees should also be provided the Project Officer and the Office of the Science Advisor.
- e. Facilities for Animals: The animals will be maintained in the facilities described in Attachment 4 (which is the original proposal or subsequent amendments, or letter from the Principal Investigator). Notwithstanding, the animals must be provided facilities satisfying the requirements specified by the U.S. Government and those issued by the government of the host country for the humane treatment of experimental animals. If the animals are moved to new facilities, or the facilities are modified in such a way to affect the animals, a description of the new facilities must be provided to the Project Officer and to the Office of the Science Advisor before the change is effected. Any applicable institutional reviews of the facilities must be repeated, and the re-certification should be provided to the Project Officer and the Office of the Science Advisor.

6. Intellectual Property Rights: Intellectual property rights stemming from the activities supported under this grant will be apportioned in accordance with the terms and conditions agreed upon by all partners in the Grant.

H. Standard Provisions and Optional Standard Provisions

1. While in the field, the Grantee will keep the A.I.D. field mission generally appraised of the research, but will not request administrative support except for the usual in-country introductions as may be appropriate. The Grantee will abide by Mission and host government regulations and customs as they apply to AID-supported in-country activities.

2. Project Officer approval required by paragraph (a) of optional Standard Provision 3 of this grant entitled "Air Travel and Transportation," is hereby granted for travel between the United States and all other countries described in the project description.

3. The principal investigators and essential scientific staff of grantee and co-principal investigator of the collaborating institutions may not be changed during the life of the research without prior written approval of the Office of the Science Advisor.

4. The Standard Provisions applicable to this grant are entitled "Mandatory Standard Provisions for U.S., Non-governmental Grantees" and "Optional Standard Provisions for U.S., Non-governmental Grantees" and are included herein as Attachment 3.

I. Title to Property

The title to property acquired under this Grant will vest in the Grantee in accordance with Optional Standard Provision No. 18 entitled "Title to and Use of Property (Grantee Title)". When the principal Grantee makes a subagreement under this Grant Agreement, the principal Grantee will pass to the Subgrantee the provisions of the above cited reference, as appropriate. Equipment purchased on behalf of each collaborating institution shall remain the property of that institution.

J. Publications

Acknowledgment of A.I.D.'s contribution to any publication resulting from this Grant shall be made in accordance with procedures set forth in Attachment 3 - Optional Standard Provision No. 10 entitled "Publications".

K. Closeout Procedures

This section prescribes uniform closeout procedures for this Grant.

1. The following definitions shall apply for the purpose of this section:
 - a. Closeout The closeout of a Grant is the process by which A.I.D. determines that all applicable administrative actions and all required work of the Grant have been completed by the Grantee and A.I.D.
 - b. Date of Completion The date of completion is the date on which all work under the Grant is completed, or the date on the award document, or any supplement or amendment thereto, on which A.I.D. sponsorship ends.
 - c. Disallowed Costs Disallowed costs are those charged to a Grant that A.I.D. or its representative determines to be unallowable, in accordance with the applicable Federal cost principles or other conditions contained in the Grant.
2. A.I.D. closeout procedures include the following requirements:
 - a. Upon request, A.I.D. shall make prompt payments to a Grantee of allowable reimbursable costs under the Grant being closed out.
 - b. The Grantee shall immediately refund any balance of unobligated (unencumbered) cash that A.I.D. has advanced or paid and that is not authorized to be retained by the Grantee for use in other Grants.

- c. A.I.D. shall obtain from the Grantee within 90 calendar days after the date of completion of the Grant all financial, performance, and other reports required as the condition of the Grant. A.I.D. may grant an extension when requested by the Grantee.
- d. When authorized by the Grant, A.I.D. shall make a settlement for any upward or downward adjustments, to A.I.D.'s share of costs after these reports are received.
- e. The Grantee shall account for any property acquired with A.I.D. funds, or received from the Government in accordance with the provisions of OMB Circular A-110, Attachment K.
- f. In the event a final audit has not been performed prior to the closeout of the Grant, A.I.D. shall retain the right to recover an appropriate amount after fully considering the recommendations on questioned costs resulting from the final audit.

L. Source and Origin of Goods and Services

1. The country or countries where research or other scientific/technological cooperation takes place shall be deemed to be the cooperating country for the purpose of permitting local cost financing.

2. Goods and services, except for ocean shipping, financed by A.I.D. under the project shall have their source/origin in the Cooperating Country or the United States and Code 935 countries.

3. Ocean shipping financed by A.I.D. under the project shall, except as A.I.D. may otherwise agree in writing, be financed only on flag vessels of the United States.

PROGRAM DESCRIPTION

6. TECHNICAL WORK PLAN

Objective # 1. To produce hybridomas secreting monoclonal antibodies to species and stage specific antigens of S. haematobium.

We will vaccinate mice with adult worm, cercarial, or egg antigens as described. Haematobium parasites for the production of antigenic material will be obtained from Dr. Kinoti's laboratory in Nairobi, or via an NIH contract with The Center for Tropical Disease Research at the University of Lowell, Lowell, MA. Dr. Harn is currently receiving both hamsters and snails which have been infected with the egyptian strain of S. haematobium via a previously established NIH contract. Extracts of the various

parasite stages will be prepared in aqueous or deoxycholate as previously described (9-11).

Balb/c mice will be vaccinated with the selected antigens as previously described (9-11), using from 100-200 ug of parasite protein/mouse in the primary followed by one or two identical boosts. In the primary, the antigens will be mixed with complete Freund's adjuvant, subsequent boosts will use incomplete Freund's adjuvant. Three to four days post the last boost, the spleens of vaccinated animals will be removed, single cell suspensions prepared, and then fused with Sp2 myeloma cells using polyethylene glycol as previously described (9). Hybrids secreting anti-schistosome antibodies will be detected via ELISA or by indirect immunofluorescence on living parasites or on fresh-frozen sections (9-13).

All positive hybrids will be cloned by limiting dilution, using irradiated Sp2 cells as feeder layers.

We will screen all positive antibodies to determine if any recognize species-specific antigens. This will be done by performing sequential ELISA or immunofluorescence screens against haematobium, mansoni, and japonica strains of the parasite. Hybrids which only bind to haematobium will be deemed putatively species specific.

In a similar manner, we will determine which antibodies are stage specific. Hybridoma supernatants will be screened via ELISA or immunofluorescence against egg, cercariae, schistosomula and adult stages to determine relative specificities.

We should be able to produce initial hybridomas within the first year of the proposed plan. Initial hybridomas may be produced and screened throughout the second year.

Objective # 2, Do cross-reactive or new monoclonal antibodies protect experimental animals from challenge infection?

Initially, we will screen the 3 previously described monoclonal antibodies which passively protect against challenge infection with S. mansoni (9,13,25), for the ability to passively transfer protection against S. haematobium challenge. These experiments will be performed by administering hybridoma culture supernatants, (concentrated 5-10X by Amicon filtration on YM-10 membranes) to naive hamsters either 1 or 24 hours prior to cercarial challenge (9). Controls will be isotype matched monoclonal antibodies (9,13). Infected hamsters will be perfused at 8 weeks post-challenge, and the viscera will be thoroughly examined for remaining worms. Worm burdens in experimentals will be compared to controls in order to determine significance (9).

New anti-S. haematobium monoclonal antibodies will initially be selected for binding to surface membranes of cercaria-schistosomula or adult worms by immunofluorescence as described

above. Those antibodies which recognize surface membrane epitopes of any or all of those stages will then be tested for the ability to passively transfer protection to naive hamsters. If we find that we have produced a large number of surface binding hybrids, we will initially screen them in pools of 3-4 antibodies. Additionally, we will screen these antibodies for the ability to passively transfer protection at day 0 as well as at day 5 (12).

We will be able to initiate the trials with the existing protective anti-S. mansoni antibodies within the first several months of the proposed research. The studies should be completed within 18 months. Examination of new hybridomas for protective functions will clearly be delayed by the length of time it takes for us to produce and then initially screen these hybrids. Ideally, we should be able to initiate these studies after the first year, and they will probably continue throughout the entire three years of the proposal.

Objective # 3, To identify S. haematobium antigens, using either experimental infection sera, or monoclonal antibodies, to quantitate the adult worm burdens in infected animals.

Urine samples, 25 - 50 ml, and venous blood, 5 - 10 mls., will be collected from patients at several endemic sites within Kenya. The patients will vary in age, and in known parasite burden as determined by urinary egg counts. Control urine and sera will be collected from patients living in the same areas, but who do not have active schistosome infections.

To detect parasite antigens in urine, we will initially concentrate the samples by lyophilization. We will rehydrate with distilled water such that the samples are from 100 - 1000 fold concentrated. To save small molecular weight antigens as well as large, we will remove the salt from the samples by Fic-gel P-2 minicolumn, which has an exclusion cut-off of 2000 daltons. This technique for de-salting has been used by our laboratory to remove deoxycholate from parasite extracts (9).

We will initially screen urine via direct binding dot-blot and Elisa. For these assays, urine will be applied directly to the wells or paper, then probed with polyvalent rabbit antisera to adult worms or to soluble egg antigens. We will also screen with patient sera. Control sera will be pre-bleed rabbit sera and non-infected human sera. Our second assay will be a capture assay which will be done on both urine and sera samples. In the capture assay, ELISA plates will first be coated with antisera of one species (rabbit), followed by incubation with urine, then probing with either antisera from heterologous species (man), or an enzyme conjugated rabbit antisera. This latter technique allows for the enrichment of relevant parasite antigens, greatly increasing the sensitivity.

If any urine or sera samples are positive, they will next be

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differentially screened for the presence of heterologous platyhelminth or nematode antigens. Here we will employ the capture ELISA. These tests will tell us if our unfractionated antisera detects schistosome specific antigens.

To further analyze the antigen(s) in patient urine or sera, we will perform Western blots (38). This will tell us if there is more than one molecular weight species of parasite antigen in the urine. By performing Western blots with heterologous parasite antigens we will also see which, if any, schistosome antigens are genus specific. Thus, analysis of the ELISA data, coupled with the Western blot data will tell us if there are any antigens with diagnostic potential.

If we find that there are schistosome specific antigens in the urine or serum which are recognized by patient and infected hamster or mouse sera, we will begin experiments to determine if any of these antigen(s) can be related to the adult worm burden. For these experiments, hamsters will be infected with varying numbers of cercariae, 30 - 300. Beginning at 4 weeks post-infection, mouse urine and/or sera will be collected, then tested for the presence of the candidate parasite antigen(s). Urine and sera will be collected weekly, through 25 weeks post-infection. At the end of the 25 week period hamsters will be perfused, and the adult worm burdens determined so that we can correlate numbers of worms with quantity of antigen(s). In parallel, a control group of infected hamsters will be perfused at 6, 8, 10 and 15 weeks to control for any changes in numbers of parasites during the 25 week period. These experiments will tell us: 1) how early after infection parasite antigen(s) are detectable in urine or sera; 2) how few organisms an animal can be infected with and still have detectable antigen; and 3) if the amount of antigen detected can be related to the adult worm burden.

Additionally, if the antigen in the urine correlates well with the egg excretion data of the patients, we will attempt to develop a method to capture the antigen. We will mix either urine or sera with antibody coated beads, then quantitating the antigen on the beads via an ELISA in a microtiter plate. Such a method could be easily adapted for field use.

We should be able to initiate these studies after the first year, and they would most likely continue throughout the duration of the proposal.

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| | | <u>BUDGET</u> | | | |
|-----------|---------------------------|------------------|---------------|---------------|---------------|
| <u>1.</u> | <u>Personnel</u> | <u>% of time</u> | <u>Year 1</u> | <u>Year 2</u> | <u>Year 3</u> |
| | Principal investigator | 40 | - | - | - |
| | Co-Principal investigator | | - | - | - |
| | 1 graduate assistant | 100 | 5,000 | 5,500 | 6,000 |
| | 1 graduate assistant | 100 | - | 5,000 | 5,500 |
| | 1 Junior Technician | 100 | <u>2,000</u> | <u>2,300</u> | <u>2,600</u> |
| | Total | | 7,000 | 12,800 | 14,100 |
| <u>2.</u> | Equipment | | 50,500 | - | - |
| <u>3.</u> | Materials and supplies | | 4,750 | 5,000 | 6,000 |
| <u>4.</u> | Training | | 11,000 | 11,000 | - |
| <u>5.</u> | Travel: International | | 4,150 | 4,150 | 8,300 |
| | Local | | 1,750 | 1,750 | 1,750 |
| <u>6.</u> | Renovations | | <u>6,000</u> | <u>-</u> | <u>-</u> |
| | Total \$ | | <u>85,150</u> | <u>34,700</u> | <u>30,150</u> |

BUDGET JUSTIFICATION

1. PERSONNEL

Two B.S. graduates will be identified and trained for the project. One (a biology graduate) will assist with the production of monoclonal antibodies and all experimental work with animals while the other (a biochemistry graduate) will assist with the biochemical work, i.e. characterization of monoclonals and identification of antigens. A junior technician will be appointed to provide general technical support, including production of cercariae and care of experimental animals.

2. EQUIPMENT

Equipment is required to establish a laboratory facility for hybridoma technology and essential biochemical techniques. A personal computer is requested for data analysis, report writing and keeping a financial account of the grant. A list of essential items is attached herewith.

3. MATERIALS AND SUPPLIES

These include plastics and glassware, praziquantel to treat patients after collection of urines and sera, animal feed, reagents and other laboratory supplies.

4. TRAINING

In the first year one graduate assistant will spend 5 months in Dr. Harn's laboratory at Harvard learning hybridoma techniques, followed in the second year by the second graduate. The costs include, for each person, return airfares (\$2,000) and per diem allowance at \$60.

5. TRAVEL

a. International travel: Dr. Harn will travel to Nairobi for 2-4 weeks a year to assist with the setting up of the laboratory and to help with technical problems. He has set up a similar laboratory at FIOCRUZ Goncalo, Moniz in Salvador, Bahia, Brazil. Towards the end the third year Dr. Kinoti will spend 3 weeks at Harvard reviewing progress and writing up results for publication with Dr. Harn. The costs, per one 3-week visit, are return airfare (\$ 2000), per diem allowance at \$ 100 (\$ 2100) and ground transportation (\$ 100).

b. Local travel: The main S. haematobium endemic areas are in Nyanza and Coast Provinces, 250 - 300 miles from Nairobi. As it is difficult to maintain the parasite lifecycle in the laboratory, only the snail host (Bulinus africanus or B. globosus) will be maintained and, to produce cercariae, will be infected with miracidia from urines collected from patients in the endemic areas as necessary. Sera will be collected from the same areas. All the donor patients will be treated with praziquantel. The budget will pay transportation and subsistence expenses in the field and also the expenses of transporting supplies and equipment from downtown Nairobi to the laboratory on the Chiromo Campus of the University of Nairobi.

6. RENOVATIONS: Two adjacent rooms are available for this project but they require small alterations before they can be used. One is a store ^{which} ~~and~~ requires benches, water supply, electric outlets, cupboards/drawers, basic laboratory furniture and attention to the floor to convert it into a biochemistry laboratory. The other room is a laboratory needing very minor changes and will be used for cell culture. A door is required to connect the two rooms.

parasite stages will be prepared in aqueous or deoxycholate as previously described (9-11).

Balb/c mice will be vaccinated with the selected antigens as previously described (9-11), using from 100-200 ug of parasite protein/mouse in the primary followed by one or two identical boosts. In the primary, the antigens will be mixed with complete Freund's adjuvant, subsequent boosts will use incomplete Freund's adjuvant. Three to four days post the last boost, the spleens of vaccinated animals will be removed, single cell suspensions prepared, and then fused with Sp2 myeloma cells using polyethylene glycol as previously described (9). Hybrids secreting anti-schistosome antibodies will be detected via ELISA or by indirect immunofluorescence on living parasites or on fresh-frozen sections (9-13).

All positive hybrids will be cloned by limiting dilution, using irradiated Sp2 cells as feeder layers.

We will screen all positive antibodies to determine if any recognize species-specific antigens. This will be done by performing sequential ELISA or immunofluorescence screens against haematobium, mansoni, and japonica strains of the parasite. Hybrids which only bind to haematobium will be deemed putatively species specific.

In a similar manner, we will determine which antibodies are stage specific. Hybridoma supernatants will be screened via ELISA or immunofluorescence against egg, cercariae, schistosomula and adult stages to determine relative specificities.

We should be able to produce initial hybridomas within the first year of the proposed plan. Initial hybridomas may be produced and screened throughout the second year.

Objective # 2, Do cross-reactive or new monoclonal antibodies protect experimental animals from challenge infection?

Initially, we will screen the 3 previously described monoclonal antibodies which passively protect against challenge infection with S. mansoni (9,13,25), for the ability to passively transfer protection against S. haematobium challenge. These experiments will be performed by administering hybridoma culture supernatants, (concentrated 5-10X by Amicon filtration on YM-10 membranes) to naive hamsters either 1 or 24 hours prior to cercarial challenge (9). Controls will be isotype matched monoclonal antibodies (9,13). Infected hamsters will be perfused at 8 weeks post-challenge, and the viscera will be thoroughly examined for remaining worms. Worm burdens in experimentals will be compared to controls in order to determine significance (9).

New anti-S. haematobium monoclonal antibodies will initially be selected for binding to surface membranes of cercaria-schistosomula or adult worms by immunofluorescence as described

above. Those antibodies which recognize surface membrane epitopes of any or all of those stages will then be tested for the ability to passively transfer protection to naive hamsters. If we find that we have produced a large number of surface binding hybrids, we will initially screen them in pools of 3-4 antibodies. Additionally, we will screen these antibodies for the ability to passively transfer protection at day 0 as well as at day 5 (12).

We will be able to initiate the trials with the existing protective anti-*S. mansoni* antibodies within the first several months of the proposed research. The studies should be completed within 18 months. Examination of new hybridomas for protective functions will clearly be delayed by the length of time it takes for us to produce and then initially screen these hybrids. Ideally, we should be able to initiate these studies after the first year, and they will probably continue throughout the entire three years of the proposal.

Objective # 3, To identify *S. haematobium* antigens, using either experimental infection sera, or monoclonal antibodies, to quantitate the adult worm burdens in infected animals.

Urine samples, 25 - 50 ml, and venous blood, 5 - 10 mls., will be collected from patients at several endemic sites within Kenya. The patients will vary in age, and in known parasite burden as determined by urinary egg counts. Control urine and sera will be collected from patients living in the same areas, but who do not have active schistosome infections.

To detect parasite antigens in urine, we will initially concentrate the samples by lyophilization. We will rehydrate with distilled water such that the samples are from 100 - 1000 fold concentrated. To save small molecular weight antigens as well as large, we will remove the salt from the samples by Bio-gel P-2 minicolumn, which has an exclusion cut-off of 2000 daltons. This technique for de-salting has been used by our laboratory to remove deoxycholate from parasite extracts (9).

We will initially screen urine via direct binding dot-blot and Elisa. For these assays, urine will be applied directly to the wells or paper, then probed with polyvalent rabbit antisera to adult worms or to soluble egg antigens. We will also screen with patient sera. Control sera will be pre-bleed rabbit sera and non-infected human sera. Our second assay will be a capture assay which will be done on both urine and sera samples. In the capture assay, ELISA plates will first be coated with antisera of one species (rabbit), followed by incubation with urine, then probing with either antisera from heterologous species (man), or an enzyme conjugated rabbit antisera. This latter technique allows for the enrichment of relevant parasite antigens, greatly increasing the sensitivity.

If any urine or sera samples are positive, they will next be

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differentially screened for the presence of heterologous platyhelminth or nematode antigens. Here we will employ the capture ELISA. These tests will tell us if our unfractionated antisera detects schistosome specific antigens.

To further analyze the antigen(s) in patient urine or sera, we will perform Western blots (38). This will tell us if there is more than one molecular weight species of parasite antigen in the urine. By performing Western blots with heterologous parasite antigens we will also see which, if any, schistosome antigens are genus specific. Thus, analysis of the ELISA data, coupled with the Western blot data will tell us if there are any antigens with diagnostic potential.

If we find that there are schistosome specific antigens in the urine or serum which are recognized by patient and infected hamster or mouse sera, we will begin experiments to determine if any of these antigen(s) can be related to the adult worm burden. For these experiments, hamsters will be infected with varying numbers of cercariae, 30 - 300. Beginning at 4 weeks post-infection, mouse urine and/or sera will be collected, then tested for the presence of the candidate parasite antigen(s). Urine and sera will be collected weekly, through 25 weeks post-infection. At the end of the 25 week period hamsters will be perfused, and the adult worm burdens determined so that we can correlate numbers of worms with quantity of antigen(s). In parallel, a control group of infected hamsters will be perfused at 6, 8, 10 and 15 weeks to control for any changes in numbers of parasites during the 25 week period. These experiments will tell us: 1) how early after infection parasite antigen(s) are detectable in urine or sera; 2) how few organisms an animal can be infected with and still have detectable antigen; and 3) if the amount of antigen detected can be related to the adult worm burden.

Additionally, if the antigen in the urine correlates well with the egg excretion data of the patients, we will attempt to develop a method to capture the antigen. We will mix either urine or sera with antibody coated beads, then quantitating the antigen on the beads via an ELISA in a microtiter plate. Such a method could be easily adapted for field use.

We should be able to initiate these studies after the first year, and they would most likely continue throughout the duration of the proposal.

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7. STAFF AND RESOURCES

A. Dr. Kinoti has wide knowledge of schistosomiasis and has done research on various aspects of it, especially epidemiology and the biology of the parasite, for over 25 years. From January to April 1988 he trained in hybridoma technology in Dr. Harn's laboratory. C.V. in Appendix.

B. Facilities for Dr. Kinoti.

Two rooms of about 350 square feet each are available in the Department of Zoology for this proposed project. However, they

will need modifications as outlined under budget justifications. Additional space of 120 square feet is available for mice and hamsters in the Zoology animal house. A separate room (80 square feet), houses the snail cycle.

C. Donald A. Harn, Ph.D.

Dr. Harn has extensive training in membrane molecular biology, and in immunochemistry. Particularly, in regards to the production of hybridomas and the characterization and purification of the antigens which they recognize, (Please see C.V. in Appendix).

D. Facilities for Dr. Harn.

The laboratory consists of a 3 room suite with approximately 1000 square feet of floor space. One of the rooms is devoted to tissue culture and contains 2 laminar flow hoods, 2 CO2 incubators, 1 refrigerator, 1 centrifuge, 1 Leitz inverted phase microscope, 1 water bath, and 1 Zimmerman electrofusion device.

The other two rooms which comprise the main laboratory contain the following: 1 floor centrifuge, 2 table top centrifuges, 3 eppendorf microfuges, 1 cytocentrifuge, 2 compound microscopes, 3 dissecting microscopes, 3 water baths, assorted electrophoresis equipment capable of (slab and tube gels, IEF, gradient gels, electro-elution, and Western blotting), 1 DNA sequencing unit, 2 -20C freezers, 1 -70C freezer, 1 liquid N2 storage tank for hybridomas, 1 ELISA reader, 1 SKATRON MASH unit, 1 LDC-Milton Roy HPLC (2 pump gradient system), 1 Gilson model 201 fraction collector.

In addition to the main laboratory, we have an environment room in which we maintain our schistosome-snail life cycles. Dr. Harn also has an office separate from the laboratory.

8. SCIENTIFIC COLLABORATION

Dr. Kinoti will be the Principle Investigator who is responsible for the project. With the help of two assistants and a junior technician, he will perform the outlined research and administer the project. From this proposal, Dr. Kinoti will be able to establish a modern laboratory working with hybridoma technology. In addition, he should also have several students and technicians who are well trained in modern immunological technology. Dr. Kinoti will benefit from Dr. Harn's expertise in hybridoma technology and biochemistry.

Dr. Harn will help set-up the facility in Nairobi, as well as assisting with the start-up of the research. Dr. Harn will also be responsible for training the research assistants. In each of the second and third years he will spend between 2 - 4 weeks in Nairobi advising and helping with technical problems. The proposed project will give Dr. Harn access to parasite material, as well as to urine and sera from patients.

9. BUDGET. (SEE ATTACHED)

10. OTHER MATTERS.

This project will not study human subjects, however, blood and urine samples will be collected will be collected from school children under medical supervision. Any children found to be infected will be treated with praziquantel under medical supervision. The Ministry of Health has approved these activities as indicated in the attached copy of a letter by Dr. Siogok in connection to an application to the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (See Appendix).

All research proposals must be approved by the Kenyan Government before the research can be carried out. This is only done when funding is assured. If this approval is accepted by AID we will seek governmental approval.

BUDGET

| 1. | <u>Personnel</u> | <u>% of time</u> | <u>Year 1</u> | <u>Year 2</u> | <u>Year 3</u> |
|----|---------------------------|------------------|---------------|---------------|---------------|
| | Principal investigator | 40 | - | - | - |
| | Co-Principal investigator | | - | - | - |
| | 1 graduate assistant | 100 | 5,000 | 5,500 | 6,000 |
| | 1 graduate assistant | 100 | - | 5,000 | 5,500 |
| | 1 Junior Technician | 100 | <u>2,000</u> | <u>2,300</u> | <u>2,600</u> |
| | Total | | 7,000 | 12,800 | 14,100 |
| 2. | Equipment | | 50,500 | - | - |
| 3. | Materials and supplies | | 4,750 | 5,000 | 6,000 |
| 4. | Training | | 11,000 | 11,000 | - |
| 5. | Travel: International | | 4,150 | 4,150 | 8,300 |
| | Local | | 1,750 | 1,750 | 1,750 |
| 6. | Renovations | | <u>6,000</u> | <u>-</u> | <u>-</u> |
| | Total \$ | | <u>85,150</u> | <u>34,700</u> | <u>30,150</u> |

BUDGET JUSTIFICATION

1. PERSONNEL

Two B.S. graduates will be identified and trained for the project. One (a biology graduate) will assist with the production of monoclonal antibodies and all experimental work with animals while the other (a biochemistry graduate) will assist with the biochemical work, i.e. characterization of monoclonals and identification of antigens. A junior technician will be appointed to provide general technical support, including production of cercariae and care of experimental animals.

2. EQUIPMENT

Equipment is required to establish a laboratory facility for hybridoma technology and essential biochemical techniques. A personal computer is requested for data analysis, report writing and keeping a financial account of the grant. A list of essential items is attached herewith.

3. MATERIALS AND SUPPLIES

These include plastics and glassware, praziquantel to treat patients after collection of urines and sera, animal feed, reagents and other laboratory supplies.

4. TRAINING

In the first year one graduate assistant will spend 5 months in Dr. Harn's laboratory at Harvard learning hybridoma techniques, followed in the second year by the second graduate. The costs include, for each person, return airfares (\$2,000) and per diem allowance at \$60.

5. TRAVEL

a. International travel: Dr. Harn will travel to Nairobi for 2-4 weeks a year to assist with the setting up of the laboratory and to help with technical problems. He has set up a similar laboratory at FIOCRUZ Goncalo, Moniz in Salvador, Bahia, Brazil. Towards the end the third year Dr. Kinoti will spend 3 weeks at Harvard reviewing progress and writing up results for publication with Dr. Harn. The costs, per one 3-week visit, are return airfare (\$ 2000), per diem allowance at \$ 100 (\$ 2100) and ground transportation (\$ 100).

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6. RENOVATIONS: Two adjacent rooms are available for this project but they require small alterations before they can be used. One is a store ^{which} ~~and~~ requires benches, water supply, electric outlets, cupboards/drawers, basic laboratory furniture and attention to the floor to convert it into a biochemistry laboratory. The other room is a laboratory needing very minor changes and will be used for cell culture. A door is required to connect the two rooms.

MINISTRY OF HEALTH
DIVISION OF COMMUNICABLE DISEASES CONTROL



Telephone No. 2015, 2016
Telex No. 2015
Tele. 2024, 2025, 2026
Rel. No. DC. 1.1.13. (82)
and date

AFYA HOUSE
CATHEDRAL ROAD
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Nairobi
3rd May 1982

Director,
Special Programme for Research and Training,
World Health Organization
GENEVA
SWITZERLAND

RE: IDENTIFICATION OF PROTECTIVE AND/OR DIAGNOSTIC
ANTIGENS OF SCHISTOSOMA HAEMATOBIMUM

The Ministry has gone through the above proposal and studied procedure for obtaining specimens from human subject and have approved them as it has found them ethically acceptable.

T. K. Arap Siongok

for: Dr. T. K. Arap Siongok,
DIRECTOR OF MEDICAL SERVICES.

Encl.

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CURRICULUM VITAE

| | | |
|-----------------------|------------------|--|
| <u>Name</u> | George K. Kinoti | <u>Address</u> |
| <u>Nationality</u> | Kenyan | Zoology Department University of Nairobi P.O. Box 30197 Nairobi |
| <u>Marital Status</u> | Married | |

Qualifications

BSc. (London) in Zoology and Chemistry (1962)

DAP & E (London) Postgraduate Diploma in Applied Parasitology and Entomology (1964)

PhD. (London) in Parasitology (1967)

Education

Kangaru School, Embu (1953-54)

Alliance High School, Kikuyu (1955-56)

Makerere University College, Kampala, Uganda (1957-62)

London School of Hygiene & Tropical Medicine, University of London, England (1963 - 66).

Appointments

1962 Research Officer (Trainee), East African Institute for Medical Research, Mwanza (on study leave 1963 - 66)

1967 Lecturer in Zoology, Makerere University, Kampala.

1968 Lecturer in Zoology, University of Nairobi, Nairobi

1974 Senior Lecturer in Zoology, University of Nairobi

1976 Associate Professor of Zoology, University of Nairobi

1983- Professor of Zoology, University of Nairobi

1975-76 Acting Chairman, Department of Zoology, University of Nairobi

1976-86 Chairman, Department of Zoology, University of Nairobi

1980-83 Dean, Faculty of Science, University of Nairobi.

Other Current Appointments

- Local Secretary (Kenya), Royal Society of Tropical Medicine and Hygiene (1971 -)
- Member, University of Nairobi Council (1976 -)
- Member, Board of Management, Kenya Trypanosomiasis Research Institute.
- Member, Board of Management, Kenya Marine and Fisheries Research Institute.
- Chairman, Natural Sciences Committee, National Council for Science and Technology.
- Member, Research Committee, National Council for Science and Technology.
- Member, Editorial Board of the African Journal of Ecology.

Research and Teaching Interests

Parasitology, with particular interest in schistosomes

Publications

- G.K. Kinoti. A General Report of the Makerere Expedition to Lake Manyara. Makerere University Library, Kampala. (Editor and contributor of one scientific and one general article) 1961.
- G.K. Kinoti. Exploring Lake Manyara. Wildlife and Sport (Uganda) 3: 28-31 1961.
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- D.S. Brown, J.E. Jelnes, G.K. Kinoti and J.H. Ouma. Distribution in Kenya of intermediate hosts for Schistosoma. Tropical and Geographical Medicine, 33: 95-103 1981.
- J. Shah, R. Ramasamy & G.K. Kinoti. Studies of surface antigens on adult Schistosoma mansoni and Schistosoma haematobium. East African Medical Journal, 62: 776 - 783 1985.
- G.C. Coles, J.I. Bruce, G.K. Kinoti, W.T. Mutahi, E.P. Dias & N. Katz. Drug Resistance in Schistosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80: 347 1986. Correspondence.
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G.K. Kinoti & J.M. Mumo. Spurious human infection with Schistosoma bovis. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, Vol. 82 (1988). In press.

J.I. Githure, P.J. Gardener & G.K. Kinoti. Experimental infection of the naked mole-rat, Heterocephalus glaber, with Leishmania donovani. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, Vol, 82 (1988). In press.

Donald A. Harn Jr., Ph.D.
Assistant Professor of Tropical Public Health
Assistant Professor of Medicine

Affiliation

Dept. of Tropical Public Health,
Harvard School of Public Health
665 Huntington Ave.
Boston, MA. 02115

Education and Training

| | | | |
|------|-------|---|-------------------------|
| 1973 | B.A. | Biology/Chemistry | Univ. Northern Colorado |
| 1975 | M.A. | Parasitology | " |
| 1980 | Ph.D. | Membrane Molecular Biology and Immunology | U.C.L.A. |

Research and Professional Experience

1980-83 Research fellow in Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts.

1983-85 Instructor in Medicine, Harvard Medical School, Boston, MA.

1983- Associate Immunobiologist, Brigham and Women's Hospital, Department of Rheumatology and Immunology, Boston, MA.

1985- Assistant Professor of Medicine, Harvard Medical School, Boston, MA.

1986- Assistant Professor of Tropical Public Health, Harvard School of Public Health, Boston, MA.

Awards and Honors

1975 Datus M. Hammond Award for outstanding student research paper, Southern California and Rocky Mountain Conferences of Parasitology, Las Vegas, Nevada.

1978 University of California/Los Angeles (UCLA) Patent Fund Grant for Doctoral Research.

1979 UCLA Faculty Award for Distinguished Teaching Assistant.

1979 Chancellors Dissertation Fellowship.

Bibliography

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Harn, Bibliography cont.

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OMB Control No. 0412-0510
Expiration Date: 12/31/89

APPENDIX 40
MANDATORY STANDARD PROVISIONS FOR
NON-U.S., NONGOVERNMENTAL GRANTEES²

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|-----------------------------------|--|
| 1. Allowable Costs | 7. Ineligible Countries |
| 2. Accounting, Audit, and Records | 8. Debarment, Suspension, and Other Responsibility Matters |
| 3. Refunds | 9. U.S. Officials Not to Benefit |
| 4. Revision of Grant Budget | 10. Nonliability |
| 5. Termination and Suspension | 11. Amendment |
| 6. Disputes | 12. Notices |

1. ALLOWABLE COSTS (MAY 1986)

(a) The grantee shall be reimbursed for costs incurred in carrying out the purposes of this grant which are determined by the grant officer to be reasonable, allocable, and allowable in accordance with the terms of this grant and the applicable* cost principles in effect on the date of this grant, which are attached.

(1) Reasonable. Shall mean those costs that do not exceed those which would be incurred by an ordinarily prudent person in the conduct of normal business.

(2) Allocable Costs. Shall mean those costs which must be necessary to the grant.

(3) Allowable Costs. Shall mean those costs which must conform to any limitations set forth in this grant.

*NOTE: For educational institutions use OMB Circular A-21; for all other non-profit organizations use OMB Circular A-122; and for profit making firms use Federal Acquisition Regulation 31.2 and AID Acquisition Regulation 731.2.

²When these Standard Provisions are used for cooperative agreements, the following terms apply: "Grantee" means "Recipient" "Grant" means "Cooperative Agreement," and "AID Grant Officer" means "AID Agreement Officer."

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(4) Unallowable costs, direct or indirect, include but are not limited to the following examples: Advertising, bad debts, contingencies, entertainment, fines and penalties, interest, fund raising, investment management costs, losses on other awards, taxes, first class air fare unless specifically approved. Additionally, public information service costs are unallowable as indirect costs.

(b) Prior to incurring a questionable or unique cost, the grantee should obtain the grant officer's written determination as to whether the cost will be allowable.

* 2. ACCOUNTING, AUDIT, AND RECORDS (MAY 1991)

(a) The grantee shall maintain books, records, documents, and other evidence relating to the AID-sponsored project or program in accordance with generally accepted accounting principles formally prescribed by the U.S., the cooperating country, or the International Accounting Standards Committee (an affiliate of the International Federation of Accountants) to sufficiently substantive charges to this grant. Accounting records that are supported by documentation will as a minimum be adequate to show all costs incurred under the grant, receipt and use of goods and services acquired under the grant, the costs of the program supplied from other sources, and the overall progress of the program. The grantee records and subgrantee records which pertain to this grant shall be retained for a period of three years from the date of expiration of this grants and may be audited by AID and/or its representatives. The grantee shall insert this paragraph (a) in all subgrants valued in excess of \$10,000.

(b) If the grantee receives \$25,000 per year or more under this grant, the grantee agrees that it shall have an audit made of the funds provided under this grant and of the financial statements of the organization as a whole. The grantee shall select an independent auditor in accordance with the "Guidelines for Financial Audits Contracted by Foreign Recipients" issued by the AID Inspector General. The audit shall be a financial audit performed in accordance with such guidelines and in accordance with generally accepted government auditing standards issued by the Comptroller General of the United States. Audits shall be performed annually.

(c) The audit report shall be submitted to AID within 30 days after completion of the audit, but the audit shall be completed and the report submitted not later than 13 months after the close of the grantee's fiscal year. The AID Inspector General will review this report to determine whether it complies with the audit requirements of this grant. No audit costs may be charged to this grant if audits have not been made in accordance with the terms of this provision. In cases of continued inability or unwillingness to have an audit performed in accordance with the terms of this provision, AID will consider appropriate sanctions which may include suspension of all or a percentage of disbursements until the audit is satisfactorily completed.

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* (d) The grantee shall require subgrantees that receive \$25,000 or more per year under this grant to have audits performed in accordance with the requirements of this provision. The subgrantee's audit report shall be submitted to the grantee within 30 days after completion of the audit, but the audit shall be completed and the report submitted not later than 13 months after the close of the subgrantee's fiscal year. The grantee shall ensure that appropriate corrective actions are taken on the recommendations contained in subgrantees' audit reports; consider whether subgrantees' audits necessitate adjustment of its own records; and require each subgrantee to permit independent auditors to have access to records and financial statements as necessary. *

3. REFUNDS (SEPTEMBER 1990)

(a) The grantee shall remit to AID all interest earned on funds provided by AID.

(b) Funds obligated by AID but not disbursed to the grantee at the time the grant expires or is terminated shall revert to AID, except for such funds encumbered by the grantee by a legally binding transaction applicable to this grant. Any funds advanced to but not expended by the grantee at the time of expiration or termination of the grant shall be refunded to AID except for such funds encumbered by the grantee by a legally binding transaction applicable to this grant.

(c) AID reserves the right to require refund by the grantee of any amount which AID determines to have been expended for purposes not in accordance with the terms and conditions of this grant, including but not limited to costs which are not allowable in accordance with the applicable Federal cost principles or other terms and conditions of this grant. In the event that a final audit has not been performed prior to the closeout of this grant, AID retains the refund right until all claims which may result from the final audit have been resolved between AID and the grantee.

4. REVISION OF GRANT BUDGET (MAY 1985)

(a) The approved grant budget is the financial expression of the grantee's program as approved during the grant award process.

(b) The grantee shall immediately request approval from the grant officer when there is reason to believe that within the next 30 calendar days a revision of the approved grant budget will be necessary for any of the following reasons:

(1) To change the scope or the objectives of the project and/or revise the funding allocated among project objectives.

(2) Additional funding is needed.

(3) The grantee expects the amount of AID authorized funds to exceed its needs by more than \$5,000 or five percent of the AID award, whichever is greater.

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(4) The grantee plans to transfer funds budgeted for indirect costs to absorb increases in direct costs or vice versa.

(5) The grantee intends to contract or subgrant any of the work under this grant, and such contracts or subgrants were not included in the approved grant budget.

(c) Except as required by other provisions of this grant specifically stated to be an exception from this provision, the Government shall not be obligated to reimburse the grantee for costs incurred in excess of the total amount obligated under the grant. The grantee shall not be obligated to continue performance under the grant (including action under the "Termination and Suspension" provision) or otherwise to incur costs in excess of the amount obligated under the grant, unless and until the grant officer has notified the grantee in writing that such obligated amount has been increased and has specified the new grant total amount.

5. TERMINATION AND SUSPENSION (MAY 1986)

(a) For Cause. This grant may be terminated for cause at any time, in whole or in part, by the grant officer upon written notice to the grantee, whenever it is determined that the grantee has failed to comply with the conditions of the grant.

(b) For Convenience. This grant may be terminated for convenience at any time by either party, in whole or in part, if both parties agree that the continuation of the grant would not produce beneficial results commensurate with the further expenditure of funds. Both parties shall agree upon termination conditions, including the effective date and, in the case of partial terminations, the portion to be terminated. The agreement to terminate shall be set forth in a letter from the grant officer to the grantee.

(c) Suspension: Termination for Changed Circumstances. If at any time AID determines that continuation of funding for a program should be suspended or terminated because such assistance is not in the national interest of the United States or that it would be in violation of an applicable law, then AID may, following notice to the grantee, suspend this grant and prohibit the grantee from incurring additional obligations chargeable to this grant other than necessary and proper costs in accordance with the terms of this grant during the period of suspension. If the situation causing the suspension continues for 60 days or more, then AID may terminate this grant on written notice to the grantee and cancel that portion of this grant which has not been disbursed or irrevocably committed to third parties.

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(d) Termination Procedures. Upon receipt of and in accordance with a termination notice as specified in either paragraph (a) or (b) above, the grantee shall take immediate action to minimize all expenditures and obligations financed by this grant and shall cancel such unliquidated obligations whenever possible. Except as provided below, no further reimbursement shall be made after the effective date of termination. The grantee shall within 30 calendar days after the effective date of such termination repay to the Government all unexpended AID funds which are not otherwise obligated by a legally binding transaction applicable to this grant. Should the funds paid by the Government to the grantee prior to the effective date of the termination of this grant be insufficient to cover the grantee's obligations in the legally binding transaction, the grantee may submit to the Government within 90 calendar days after the effective date of such termination a written claim covering such obligations. The grant officer shall determine the amount(s) to be paid by the Government to the grantee under such claim in accordance with the applicable cost principles.

5. DISPUTES (NOVEMBER 1985)

(a) Any dispute under this grant shall be decided by the AID grant officer. The grant officer shall furnish the grantee a written copy of the decision.

(b) Decisions of the AID grant officer shall be final unless, within 30 days of receipt of the decision of the grant officer, the grantee appeals the decision to AID's Deputy Assistant to the Administrator for Management Services. Any appeal made under this provision shall be in writing and addressed to the Deputy Assistant to the Administrator for Management Services, Agency for International Development, Washington, D.C. 20523. A copy of the appeal shall be concurrently furnished to the grant officer.

(c) In order to facilitate review on the record by the Deputy Assistant to the Administrator for Management Services, the grantee shall be given an opportunity to submit written evidence in support of its appeal. No hearing will be provided.

(d) A decision under this provision by the Deputy Assistant to the Administrator for Management services shall be final.

7. INELIGIBLE COUNTRIES (MAY 1986)

Unless otherwise approved by the AID grant officer, funds will only be expended for assistance to countries eligible for assistance under the Foreign Assistance Act of 1961, as amended, or under acts appropriating funds for foreign assistance.

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8. DEBARMENT, SUSPENSION, AND OTHER RESPONSIBILITY MATTERS (MARCH 1989)

(1) The grantee certifies to the best of its knowledge and belief, that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;

(b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and

(d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

(2) The grantee agrees that, unless authorized by the Grant Officer, it will not knowingly enter into any subagreements or contracts under this grant with a person or entity that is included on the "Lists of Parties Excluded from Federal Procurement or Nonprocurement Programs". The grantee further agrees to include the following provision in any subagreements or contracts entered into under this grant:

DEBARMENT, SUSPENSION, INELIGIBILITY, AND VOLUNTARY EXCLUSION
(MARCH 1989)

The recipient/contractor certifies that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.

(3) The policies and procedures applicable to debarment, suspension and ineligibility under AID-financed transactions are set forth in 22 CFR Part 208.

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9. U.S. OFFICIALS NOT TO BENEFIT (NOVEMBER 1985)

No member of or delegate to the U.S. Congress or resident U.S. Commissioner shall be admitted to any share or part of this grant or to any benefit that may arise therefrom; but this provision shall not be construed to extend to this grant if made with a corporation for its general benefit.

10. NONLIABILITY (NOVEMBER 1985)

AID does not assume liability for any third party claims for damages arising out of this grant.

11. AMENDMENT (NOVEMBER 1985)

The grant may be amended by formal modifications to the basic grant document or by means of an exchange of letters between the grant officer and an appropriate official of the grantee.

12. NOTICES (NOVEMBER 1985)

Any notice given by AID or the grantee shall be sufficient only if in writing and delivered in person, mailed, or cabled as follows:

To the AID grant officer, at the address specified in the grant.

To grantee, at grantee's address shown in the grant or to such other address designated within the grant.

Notices shall be effective when delivered in accordance with this provision, or on effective date of the notice, whichever is later.

(END OF MANDATORY STANDARD PROVISIONS)

1. TITLE OF PROPOSAL: Identification of Protection and/or Diagnostic Antigens of Schistosoma haematobium.

2. INSTITUTION APPLYING: University of Nairobi

3. PROJECT IDENTIFICATION NUMBER: 9.398

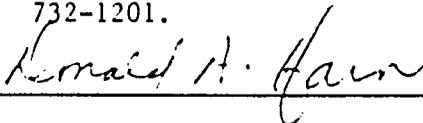
4. CO-PRINCIPAL INVESTIGATORS:

George K. Kinoti, PhD., Professor, Department of Zoology, University of Nairobi, P.O. Box 30197, Nairobi, Kenya.
Telephone: 43181

Signature: 

Donald A. Harn, PhD., Asst. Professor, Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, and Department of Rheumatology and Immunology, Harvard Medical School, Boston, MA., U.S.A.

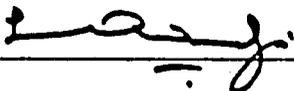
Telephone: (617) 732-1201.

Signature: 

5. INSTITUTION'S OFFICIAL RESPONSIBLE FOR GRANT ADMINISTRATION:

Professor Shem O. Wandiga, Deputy Vice-Chancellor (Administration and Finance), University of Nairobi, P.O. Box 30197, Nairobi, Kenya.

Telephone: 334244

Signature: 

6. Proposed project starting date: 1 July 1989

Duration: 3 years

Total Cost: \$ 150,000

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2. OVERALL AIM AND SPECIFIC OBJECTIVES

The overall aims of this proposal are twofold: 1) to detect antigens which may be useful in a prophylactic vaccine against schistosomiasis in Kenya; and 2) to detect antigens which are specific for adult worm products, and may therefore, eventually be used to quantitate adult worm burdens in infected patients.

We propose to study these two aims by using already existing monoclonal antibody probes, produced by Dr. Harn's laboratory, and additionally, by the production of new monoclonal antibody probes to previously undescribed schistosome antigens. The specific objectives of this proposal are: 1) To produce hybridomas secreting monoclonal antibodies to species and stage specific antigens of S. haematobium; 2) To test whether the new monoclonal antibodies or monoclonal antibodies previously produced by Dr. Harn's laboratory, are able to passively transfer immunity to S. haematobium infections in experimental animals; and 3) To identify S. haematobium antigens, using either experimental infection sera, or newly produced monoclonal antibodies, which can be used to quantitate adult worm burdens in infected animals and eventually patients.

3. RELEVANCE TO DEVELOPMENT

Effective immunization against S. haematobium and/or S. mansoni will improve the health and economic productivity of many millions of people in Africa and Latin America. Specifically, in regards to Kenya, approximately 1 million people are infected with mansoni, haematobium, or both species.

An additional benefit will be the introduction of modern immunological technologies to Kenyan scientists via 6 month training periods in Dr. Harn's laboratory in Boston. Hopefully, a core of Kenyan scientists, trained via the proposed research program, would become established, and provide a modern immunology approach to research at the University of Nairobi.

4. INNOVATIVE ASPECTS

The application of hybridoma technology within Kenya, and the training of Kenyan personnel in this technology will provide a new approach to immunology of schistosomiasis within the country.

The examination of antigens which might be cross-protective between mansoni and haematobium is new. Additionally, the proposed research will be able to look at differences between established laboratory strains of the parasites as well as strains collected from the field.

Lastly, we will be examining a new approach to determining adult worm burdens in infected animals. This will be done via the

project aimed at identifying species and/or stage specific antigens with monoclonal antibody or sera probes. If such an assay is developed, it would pave the way for determining how successful drug treatments in patients are but also, provide a means for measuring how successful future immunization protocols are in patients.

5. BACKGROUND AND RATIONALE

Schistosomiasis is a parasitic disease which poses a major public health problem for a large number of developing countries. Between 200-300 million persons are infected worldwide. To date, public health and educational attempts to help control the disease have largely failed. Currently there are two good drugs, (oxamniquine, praziquantel) which are administered after infection to kill the parasites. There are no good prophylactic measures other than avoiding contact with water harboring schistosome infected snails. In many areas of the world this is not feasible. As with other parasitic diseases, the possibility that schistosomes will develop some level of drug resistance exists. Thus, alternative, non-pharmacological approaches to control the parasite are necessary.

The development of a defined vaccine against schistosomes would augment the available drugs, and would be prophylactic by nature. That a defined vaccine can be developed was originally based on observations that animals harboring experimental, natural infections or those immunized with irradiated cercariae, develop significant levels of resistance to challenge infection (1-4). Recently, resistance to reinfection has been demonstrated in humans (5). Additionally, the development of protective monoclonal antibodies, (6-16), suggest that defined antigens, when presented properly to the host immune system, may be capable of inducing protective immunity. This last point is further demonstrated by reports describing other surface membrane antigens which have been detected by monoclonal antibodies which may be effective in vitro but do not passively transfer immunity (17-22).

Thus far, preliminary experiments with purified antigens have demonstrated that the administration of single antigens can partially protect naive mice (14,23-26). The levels of resistance obtained range from 0.0%-64%. All studies were limited in their approach, both in regards to the amounts of antigen to use, and the number of different adjuvants which were tried.

Initially, we will examine whether previously described mouse monoclonal antibodies which protect against S. mansoni, are also able to protect against S. haematobium (9-13). All three of these surface antigens are present on cercariae and/or schistosomula of both species, and these are stages which have been considered as possible targets of the host immune response due to their susceptibility to a wide variety of immune effector mechanisms (26,27). Recent studies in vivo have shown that lung and post-lung

worms are also targets of the protective immune response (28-35). The 22 kD antigen has also been found on the surface membranes of lung worms. This is the first study which we know of that will compare protective antigens that cross-react between mansoni and haematobium. A vaccine that cross-protects would be more economical than species-specific vaccines.

Although, many antigens are shared between the two strains of parasites (36,37) certain antigens have also been shown to be specific for one strain (37). To detect new antigen(s), specific to S. haematobium, we propose to produce hybridomas secreting monoclonal antibodies directed at surface membrane determinants of adults, cercariae and/or schistosomula. We will then test these antibodies for the ability to function in vitro and in vivo in various killing assays (9-13).

The third aim of this project is to develop a diagnostic assay for schistosome antigens which we hope will accomplish two things: 1), determine the adult worm burden within the host; and 2), differentially diagnose schistosome infections where eggs are not being excreted, from other helminth infections.

We hope to develop an assay which detects parasite antigen in patient urine, or blood, and is quantitative in regards to numbers of adult parasites. Such an assay would be very simple and if urine were used, non-invasive. Carlier et al. (38) were able to demonstrate that highly concentrated urine from patients contained schistosome antigen. Unfortunately no analysis was performed with relationship to intensity of infection and quantity of antigen.

The ability to quantitate the numbers of adult parasites in patients will become increasingly more important, as various laboratories begin to prepare candidate vaccines for testing in primate models. Because the lack of parasite eggs in the feces does not mean that there are no adult parasites, an alternative assay system should be developed. Thus, if a quantitative antigen assay system could be developed, it would definitely be employed should a vaccine for humans be approved for testing.

6. TECHNICAL WORK PLAN

Objective # 1. To produce hybridomas secreting monoclonal antibodies to species and stage specific antigens of S. haematobium.

We will vaccinate mice with adult worm, cercarial, or egg antigens as described. Haematobium parasites for the production of antigenic material will be obtained from Dr. Kinoti's laboratory in Nairobi, or via an NIH contract with The Center for Tropical Disease Research at the University of Lowell, Lowell, MA. Dr. Harn is currently receiving both hamsters and snails which have been infected with the egyptian strain of S. haematobium via a previously established NIH contract. Extracts of the various

Attachment

PROGRAM OF TROPICAL MEDICINE & INTERNATIONAL HEALTH

9.398



Harvard School of Public Health
Department of Tropical Public Health



Harvard Medical School
Division of Tropical Medicine

Dr. Miloslav Rechcigl, Jr.
Research Review Director
Office of the Science Advisor
Agency for International Development
Room 720, SA-18
Washington, D.C. 20523.

Sept 14, 1989

Dear Dr. Rechcigl:

Enclosed you will find our responses to the Provisos and selected comments for our proposal (9.398), submitted to AID's Program in Science and Technology Cooperation.

On behalf of Dr. Kinoti I wish to thank you for your assistance with this proposal.

Sincerely,

Donald Harn, Ph.D.
Associate Professor

Response to Provisos for USAID Proposal No: 9,398

Proposal Title: Identification of Protective and/or Diagnostic Antigens of Schistosoma haematobium.

Principal Investigators: Dr. George Kinoti and Dr. Donald Harn

Proviso 1. The methodologies for development of the diagnostic assay are inadequately reviewed and discussed, specifically the problems encountered by many other groups with respect to sensitivity and reproducibility of antigenemia assays.

Response: We agree with the reviewers that the difficult part of Objective # 3 may be in obtaining good levels of sensitivity as well as reproducible results on antigenemia levels. While there is a substantial amount of literature attesting to these problems, many of the earlier studies were testing for the presence of antibodies to specific antigens, which presents the problem of positivity long after the parasites have been eliminated from the host. In contrast, we are proposing to test for circulating antigens produced by active infection. While earlier studies in this area also had problems, the advent of monoclonal antibody technology has paved the way for extremely sensitive probes which eliminate the cross reactivities seen in previous studies using polyclonal sera.

That both sensitivity and specificity problems in the detection of antigens in serum and urine can be reduced or eliminated via the utilization of monoclonal antibodies is evidenced by a number of publications within the last two years, both in schistosomiasis and other helminth parasites such as filariads, (Zheng H., et al. 1987. Am. J. Trop. Med. Hyg. 36(3):554-560). In schistosomiasis it was demonstrated that via the production of species specific monoclonal antibodies, reproducible levels of serum antigenemia could be obtained, that as little as 50 pg of antigen per ml of serum and that the serum antigen levels varied far less than day to day fluctuations in fecal egg output, (N. de Jonge and A. M. Deelder 1988. Int. Conf. Parasit. p.93). Further, (N. G. van Vliet et al. 1988. Int. Conf. Parasit. p.272), demonstrated that two different antigens could be detected in the urine of patients infected with either S. mansoni or S. haematobium. In another study, (C. Ripert et al. 1988. Trop. Med. Parasit. 39:131-135), also used a monoclonal antibody against the gut associated antigens specific for schistosomes. They found that the monoclonal antibody was able to detect infection in both S. mansoni and S. haematobium infections, and that the monoclonal antibody assay appeared to be more sensitive than either fecal or urinary egg output.

Since this proposal was submitted, one of the diagnostic antigens for S. japonica has been demonstrated to be one of the heat shock proteins, Hsp 70, (Dr. Clint Carter, Vanderbilt University, personal communication). More than likely, the molecule detected in S. mansoni by N. de Jonge and Deelder 1988

(cited above), is also Hsp 70 as the reported molecular weight is 70 kDa.

Additionally, we have produced monoclonal antibodies that are specific for S. haematobium egg antigens, and do not react with S. mansoni adult, cercarial or egg antigens via ELISA or Western blot assays. Further, the Western blots demonstrated that the species specific monoclonal antibodies recognize two distinct types of antigen, one of which is a doublet with approximate Mrs. of 70 and 66 kDa. The other is a series of high molecular weight species, approximate Mrs. of 200 - 300 kDa. The epitopes recognized on the high molecular weight antigens by the monoclonal antibodies have been shown by ELISA and Western blot to be sensitive to periodate degradation and thus, are probably carbohydrate in nature. The reactivity of the antigens at Mrs. 70 and 66 kDa with monoclonal antibodies was not affected by periodate degradation.

Lastly, to further insure specificity we have obtained patient sera from Dr. Victor Tsang at the CDC in Atlanta, and Dr. Willy Piessens of the Dept, of Tropical Public Health, Harvard School of Public Health. The sera are from patients with nematode infections (ascaris, stronglyloides, trichuris), as well as enteric protozoal infections such as giardia and amoeba. We will test these sera for binding to the putatively specific antigens which our monoclonal antibodies detect.

We hope the reviewers see that substantial progress has been achieved by several groups in the areas of specificity, sensitivity and reproducibility using monoclonal antibodies as probes. Taken together with our recent progress in producing monoclonal antibodies to two distinct species specific antigens, we feel that we should be able to develop a sensitive and reproducible assay.

PROVISO # 2. The budget needs to be further justified, specifically in the category of equipment purchase.

Response: As we understand it, the concern of the reviewer's is that we are unlikely to need all of the equipment in the first year. We agree, however, the reason that we requested all equipment purchases in the first year is purely logistical. In Kenya, it typically takes 2-6 months between the time equipment is ordered and received from overseas. This is especially true of heavy equipment which comes by ship.

In terms of overall justification of the equipment we provide the following. 1) For hybridoma production: Laminar flow hood for sterile tissue culture work; inverted phase microscope to check the growth of cells; Elisa reader for quantitative analysis when testing of monoclonal antibodies; water bath for thawing of cells; floor centrifuge for spinning down cells; incubators for culturing hybridomas; -70C freezer for storage of hybridomas, fetal calf sera and certain biologicals; ph meter for determining pH of media and various buffers; balances for weighing out media and buffer components; refrigerator to store media, other biologicals and

buffers in; foot pump for vacuum aspiration of spent media from cells; pipet aid for use in the laminar flow hood in dispensing and aliquoting media and cells, works with conventional glass or plastic pipets; pipetman, purchase of 6 which would give two complete sets comprised each of 1000ul, 200ul, 20ul units; hot-stir plates (4), for preparation of media and buffers as well as for boiling samples for SDS-PAGE. 2) For SDS-PAGE and Western blot analysis of monoclonal antibodies and patient sera and urine samples: power supplies (2), for electrophoretic (SDS-PAGE) analysis; western blot units (2), each consisting of transfer cell and power pack for electrophoretic transfer of antigens to either nitrocellulose or PVDF paper; slab gel units (2), for SDS-PAGE separation of antigens, sera or urine samples; eppendorf centrifuge for preparation of samples for SDS-PAGE analysis or use in ELISA; shaking platforms (2), for incubations done during Western blot procedures, vortexes (2), for proper mixing of biologicals and conjugated antibody probes. 3) Ice machine. There is no way to provide ice in the Dept. of Zoology at the University of Nairobi. Certain biologicals, serum and urine samples must be maintained at 4C for various amounts of time. A water and ice bath is the simplest way to do this. 4) Computer. We would like to purchase an IBM PC/AT clone to maintain databases on hybridomas as well as on the epidemiological data concerning the patients.

If we must divide the equipment up into first and second year purchases, we would request that the hybridoma/tissue culture equipment definitely be purchased in the first year. If possible we would also like to purchase half of the electrophoretic equipment in the first year so that analyses of hybridomas and patient samples could at least be initiated.

PROVISO # 3. Why is the letter from Dr. Siongok concerning approval for use of human specimen samples addressed to WHO? Is the approval for the same research? Is the project also funded by WHO? The investigators should provide A.I.D. with a copy of the patient consent form to be used in the study.

Response: A copy of Dr. Siongok's letter was sent to A.I.D. to indicate the approval of the Ministry of Health. WHO decided not to fund the proposal.

In Kenya the normal procedure for the kind of study proposed here is to first obtain permission of the government authorities and then the verbal consent of the human subjects: consent forms are not used. We propose to follow the normal procedure. As we will be studying school children we will initially get permission from the government then the permission of the school headmaster, followed by an explanation of the study to the pupils, their parents and teachers. At this point we will ask for volunteers. In Dr. Kinoti's experience this is the best procedure as it leaves no room for suspicion on the part of the parents or the community who trust the teachers because they are more educated.

PROVISO #4. The investigators should provide descriptions of the

facilities and protocols to be used for the humane treatment of experimental animals. If available, documentation of the approval of the appropriate institutional review committee should be submitted.

Response: For animals used for trainees at the Harvard School of Public Health, the Harvard Medical School animal management program is accredited by the American Association for the Accreditation of Laboratory animal care, and meets National Institutes of Health standards as set forth in the "Guide for the care and Use of Laboratory Animals" (DHHS Publication No. (NIH) 85-23 Revised 1985). The Institution also accepts as mandatory the PHS "Policy on Humane care and Use of Laboratory Animals by Awardee Institutions" and NIH "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training." There is on file with the Office for Protection from Research Risks an approved Assurance of Compliance.

Unfortunately, in Kenya there are no national or institutional review committees concerned with the humane treatment of experimental animals. We will adhere to the standard's set in the U.S. National Research Council's "Guide for the care and use of Laboratory Animals.

Response to selected comments.

Comments 1, 7 and 11.

These comments can be taken together. There is no evidence that the presence of a carbohydrate epitope precludes that it is dangerous. Thus, until they are thoroughly tested we will not know. We are currently testing these antigens in Dr. Harn's laboratory, as we have recently identified and obtained one of the oligosaccharides.

We will initially work on the cross-reactive monoclonals because, as the reviewers point out, they have demonstrated protection capabilities with S. mansoni and we already have them. It should also be noted that in the response to proviso #1 we point out that we have already generated species specific monoclonal antibodies for S. haematobium. Thus, though setting up the laboratory will be the main task, several probes are already in hand.

Comment 2. Eventually we will examine whether the candidate antigens are carbohydrate or peptide, however, that may be beyond the scope of this initial project.

Comment 3. We will be screening against soluble antigens from adults and eggs as well as detergent extracts of adults and cercariae and eggs.

Comment 5. The human material is very valuable and would be difficult to duplicate in the experimental animal model. The primary reason for this is that patients are routinely infected

with a number of heterologous helminths as well as protozoan parasites. To insure specificity this type of material must be examined.

Comment 10. The laboratory will not require major renovations. The only work needed may be the installation of additional power outlets for some of the equipment.



9.398

UNIVERSITY OF NAIROBI
DEPARTMENT OF ZOOLOGY
CHIROMO

Chairman:

Cur Ref:

Your Ref: Proposal No. 9.398

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4th January, 1989

Dr. Miloslav Rechcigl, Jr.
Research Review Director
Office of the Science Advisor
Agency for International Development
WASHINGTON, DC 20523
U.S.A.

Dear Dr. Rechcigl,

Thank you very much for your letter of 6th December 1988, enclosing reviewer's comments on the proposal by Dr. Harn and me. If I may say so I am impressed with the A.I.D. review system. I find the comments pertinent and helpful.

As this is a joint proposal with Dr. Harn I need to consult him. To save time I am asking him to kindly send you our response to the concerns raised by the reviewers. I hope that this will not cause too much delay. Already several days have been lost due to closure of the University for holidays in late December/early January.

Yours sincerely,

George K. Kinoti

George K. Kinoti, PhD.
Professor of Zoology

cc: Dr. Donald A. Harn
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