

AGENCY FOR INTERNATIONAL DEVELOPMENT

PROJECT DATA SHEET

1. TRANSACTION CODE

A = Add  
 C = Change  
 D = Delete

Amendment Number  
2

DOCUMENT CODE  
3

2. COUNTRY/ENTITY

S&T Interregional

3. PROJECT NUMBER

931-0453.05

4. BUREAU/OFFICE

ST/H/CD

10

5. PROJECT TITLE (maximum 40 characters)

Malaria Immunology - Univ. of Hawaii

6. PROJECT ASSISTANCE COMPLETION DATE (PACD)

MM DD YY  
06 31 84

7. ESTIMATED DATE OF OBLIGATION  
(Under 'B.' below, enter 1, 2, 3, or 4)

A. Initial FY 80 B. Quarter  C. Final FY 83

8. COSTS (\$000 OR EQUIVALENT \$1 = )

A. FUNDING SOURCE	FIRST FY 80			LIFE OF PROJECT		
	B. FX	C. L/C	D. Total	E. FX	F. L/C	G. Total
AID Appropriated Total	710.5		710.5	910.5		910.5
(Grant)	( 710.5 )	( )	( 710.5 )	( 910.5 )	( )	( 910.5 )
(Loan)	( )	( )	( )	( )	( )	( )
Other U.S. 1.						
Other U.S. 2.						
Host Country						
Other Donor(s)						
<b>TOTALS</b>	<b>710.5</b>		<b>710.5</b>	<b>910.5</b>		<b>910.5</b>

9. SCHEDULE OF AID FUNDING (\$000)

A. APPROPRIATION	B. PRIMARY PURPOSE CODE	C. PRIMARY TECH CODE		D. OBLIGATIONS TO DATE		E. AMOUNT APPROVED THIS ACTION		F. LIFE OF PROJECT	
		1. Grant	2. Loan	1. Grant	2. Loan	1. Grant	2. Loan	1. Grant	2. Loan
(1) HEA	511	542		710.5		200		910.5	
(2)									
(3)									
(4)									
<b>TOTALS</b>				<b>710.5</b>		<b>200</b>		<b>910.5</b>	

10. SECONDARY TECHNICAL CODES (maximum 6 codes of 3 positions each)

11. SECONDARY PURPOSE CODE

12. SPECIAL CONCERNS CODES (maximum 7 codes of 4 positions each)

A. Code

B. Amount

13. PROJECT PURPOSE (maximum 400 characters)

The purpose of this project is to investigate the potentials for the development of a malaria vaccine by establishing the culture parameters for the in-vitro production of P. falciparum at high parasitemia, the purification of antigens produced, and the conduct of vaccination trials to elucidate the immunogenicity of merozoites produced in-vitro.

14. SCHEDULED EVALUATIONS

Interim MM YY MM YY Final MM YY  
0 2 8 3 | | | | 0 4 8 4

15. SOURCE/ORIGIN OF GOODS AND SERVICES

000  941  Local  Other (Specify)

16. AMENDMENTS/NATURE OF CHANGE PROPOSED (This is page 1 of a \_\_\_\_\_ page PP Amendment)

This amendment supplements the current project to accelerate its activities in the area of monoclonal antibodies and in-vitro culture systems which was specifically recommended by the indepth evaluation team which conducted a site visit March 7-9, 1983.

17. APPROVED BY

Signature  
George Curtin, M.D.  
Title  
Director  
Office of Health

Date Signed

MM DD YY  
06 30 84

18. DATE DOCUMENT RECEIVED IN AID/W, OR FOR AID/W DOCUMENTS, DATE OF DISTRIBUTION

MM DD YY

**AUTHORIZATION AMENDMENT**

Name of Country/Entity: Interregional Project  
No. 931-0453.05

Project: Malaria Immunity and Vaccination Research

Contractor: University of Hawaii  
DPE-0453-C-00-1026-00

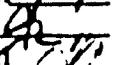
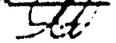
Pursuant to Section 104 of the Foreign Assistance Act of 1961, as amended, the centrally funded program, Malaria Immunity and Vaccination Research: Malaria Immunity and Vaccination, was authorized November 20, 1980. The authorization is hereby amended as follows:

1. The life-of-project cost shall be increased by \$200,000. This raises the life-of-project authorization from \$710,500 to a new total of \$910,500.
2. The authorization cited above remains in force except as hereby amended.
3. This project is included in the FY 1983 Congressional Presentation (Annex V, page 57).

  
James E. Sarn, M.D.  
Agency Director, ST/HP

7-6-83  
Date

Clearances:

ST/H, G. Curlin		Date: <u>6/30/83</u>
J. Royer		Date: _____
ST/PO, G. Eaton		Date: <u>7-1-83</u>

JUN 30 1983

ACTION MEMORANDUM FOR THE AGENCY DIRECTOR  
FOR HEALTH AND POPULATION

FROM: ST/H, George Curran, M.D.

Problem: Approval of a project authorization amendment to expand and accelerate a research project at the University of Hawaii (931-0453.05), under the Malaria Immunity and Vaccination Program and to raise the life-of-project funding by \$200,000.

Discussion: This contract, initiated in June 1981, has focused on the red blood cell forms of the malaria parasite to: (1) improve the in-vitro culture techniques, (2) isolate, purify and characterize P. falciparum blood stage antigens, and (3) conduct immunization trials of candidate immunogens in Aotus monkeys. An external team of scientists conducted an in-depth evaluation of the project in March 1983. This is a normal part of the malaria program internal evaluation and quality control mechanism. The evaluation team found several exciting advances that have been made by the research team, specifically in in-vitro cultivated techniques and parasite synchronization studies, and recommended that AID move forward with supplemental funds to quickly follow up the breakthroughs. This would include making the new automated culture system available to other network laboratories.

Recommendation: That you sign the attached Authorization Amendment to expand the project at the University of Hawaii to accelerate research activities in automated in-vitro culture systems and the utilization of monoclonal antibodies for antigen isolation and to increase the life-of-project funding by \$200,000.

Attachments:

1. Authorization Amendment
2. Site Visit Report - March 7-10, 1983

UNIVERSITY OF HAWAII  
SITE VISIT REPORT  
March 7-10, 1983

I. SUBSTANTIVE REVIEW

A. Project Description

The project is designed to (1) purify and characterize Plasmodium falciparum blood stage antigens and (2) perform vaccination studies in Aotus trivirgatus monkeys using purified P. falciparum antigen(s).

B. Progress to Date

1. Evaluation of past performance against specific project objectives

This project is completing the 2nd year of its 3-year projected time course. The project has made significant progress in the production of large quantities of segmenters/merozoites by (1) the development of an automated, computer controlled in vitro system consisting of a plastic production chamber capable of producing  $2-4 \times 10^{10}$  parasites/vessel/week, and (2) synchronization of cultures by the development of a colchicine treatment procedure. Partial purification of the antigens was obtained by affinity-chromatographic techniques using hyperimmune Aotus serum and a limited number of monoclonal antibodies (MAB's). The antigens were characterized using SDS-PAGE and sera from immunized monkeys. Using these techniques, 21 "different" antigens based on molecular weight have been identified.

The efforts to produce large quantities of crude antigenic material appears to have been met. The purification of the antigens through the use of immune sera and MAB's follows the original work plan but with the addition of MAB procedures. This is a logical approach to meet the project objectives. No immunization trials with purified antigens have been made due (1) to the absence of sufficient amounts of purified immunogens and (2) the AID directive to abstain from immunization trials until purified antigens are available. It is anticipated that during the remaining project period, more MAB's will be produced which are specific to many of the gel-identified molecular weight antigens and that these will be used to "purify" such antigens from culture-produced parasites. Biological characterization by immunization studies in non-primate animals will probably not be completed within the timeframe of the present project although preliminary studies may be made.

The project has resulted in (1) significant findings on the production of large numbers of synchronized parasites and (2) development of techniques for the identification of antigens using SDS-PAGE. The first will be of use to other laboratories of the USAID malaria vaccine network. The latter consists of modification of previously described procedures, some of which may be adapted by others within the network. The development of a solid phase radioimmunoassay (RIA) for the screening of monoclonals using whole fixed parasitized erythrocytes is

noteworthy and may have application to other project areas. Extensive collaboration and cooperation with other network laboratories was not apparent although exchange of parasites and procedures (through reports and publications) is evident.

2. Evaluation of results, interpretation of data, significance of findings  
Is the research able to answer or meet the expected objectives? Part of the specific objectives have been met very successfully. The *in vitro* culture system provides large amounts of parasite material which is a precondition for antigen purification studies. The characterization of those antigens is currently restricted to their molecular weight on SDS-PAGE. None of the 21 antigens recognized by immune sera has yet been correlated with protection in a GARI test.

What has been learned so far that is of real significance? To date, nothing of real significance in regard to antigen characterization has been learned. The characterization of the antigens will probably not go beyond the molecular weight characterization during the last remaining year of the contract. The three antigens recognized by monoclonal antibodies are not yet purified so that a more detailed analysis can not be expected. However, on a long term basis, the design of the study (monoclonal antibodies against all 21 antigens) should eventually permit the isolation of the antigens on a preparative scale, therefore allowing the biochemical characterization of any potential protective antigen(s) out of those recognized by immune sera by other laboratories in the network.

3. Use of Results

During the period covered under the current research contract, i.e. September 1981 to the time of the site visit in March 1982, the senior investigator and his staff of the Department of Tropical Medicine and Medical Microbiology of the University of Hawaii School of Medicine have published seven scientific articles and submitted two manuscripts to national and international scientific journals of high standard.

Of these, four of the published papers and one of the manuscripts described the research results of the team on their *in vitro* culture methods, giving higher numbers of the blood stages of *P. falciparum* than those achieved by other laboratories in the AID network. One paper contributed to the general knowledge of malaria immunity based on recent advances made in this multidisciplinary field. The remaining two papers and the other manuscript give case descriptions and descriptions of experiments based on a small number of *Aotus* monkeys vaccinated with a crude prototype vaccine in which two different adjuvants have been used.

Further to these publications, the senior investigator participated in a number of national and international malaria workshops, including those organized by AID and WHO.

The results of the research and the methods applied and to be developed have also been the subject of numerous seminars and lectures in the graduate and post-graduate teaching programs and curricula of the

Department. Furthermore, special research projects in malaria research related to the objectives of AID's program were chosen by graduate students as the subject of their theses leading to a MSc or the PhD degree.

In their publications, the authors acknowledged the financial support and other assistance by AID to their research projects.

Internationally, the senior author has given guest lectures in a number of foreign universities, especially those of India, in which the research on malaria and the development of a vaccine have featured prominently.

At times, the work of the Department on development of a malaria vaccine has received considerable attention by the lay press. As is often the case with popularization of scientific issues, the printed endproducts of such endeavors have led to distortions and exaggerations of the results and of the future prospects of their wide application.

In all, scientific documentation, publication and other methods of dissemination of the research carried out by the team under AID contract have been fully satisfactory.

4. Evaluation of research methodology, etc.

Have there been any improvements in methodology? There have been significant improvements in the research methodology:

- A. The most relevant improvement concerns the large scale *in vitro* culture system of *P. falciparum*. The static system has been automated in a way that the medium change via exit ports is computer controlled thus ensuring minimal disturbance of the settled RBC's. The vessel which holds 25 ml of packed RBC's is now made from polysulphone, a type of plastic withstanding  $\gamma$ -radiation and autoclaving without breaking down. No manual manipulation or opening of the system is involved in the medium change procedures thereby eliminating the risk of contamination. The system is designed to accommodate up to 12 vessels which can be controlled simultaneously.
- B. A second significant improvement concerns the synchronization of *P. falciparum*. Instead of the commonly used sorbital treatment at the start of the culture period, the cultures are exposed for 5 hours to 4 ml colchicine. With this new technique, a high degree of synchrony is achieved. In combination with the automated medium change, this method is superior in a way that the cultures can now be synchronized without taking them out of the culture vessel, therefore, again decreasing the risk of contamination.
- C. A new solid phase immunoassay provides a more reliable and faster test for the evaluation of immune sera than the previous IFA test. Whole parasitized erythrocytes (instead of sonicated extracts) are immobilized onto the microtiter plates, the antibodies to be titrated are reacted with the fixed cells and a secondary antibody coupled with  $I^{125}$ -protein A is used. However,  $10^6$  parasites per well have to be used. This high number of parasites per test

restricts the use of the RIA to those laboratories being able to produce such large amounts. Work is being done to increase the sensitivity of the test so it can be used more widely. On the other hand, this test allows the screening of a significantly higher number of sera than with the subjective IFA test.

- D. The use of metrizamide gradients ensures a high degree of purity of the labelled parasites, free of host cell membranes. The analysis of parasite antigens on SDS-PAGE using sera taken at different times during the infection (primary, secondary, postimmune sera) has been improved by establishing conditions which allow labelling of large amounts of parasites ( $8 \times 10^9$ ) with a higher specific activity. This eliminates the need to pool smaller, less radioactive preparations thus eliminating batch to batch variations.
- E. The methodology used to identify MAB's, which recognize high molecular weight proteins, has been successfully changed in that after fusion and before cloning, the MAB's are tested to determine if they bind, and therefore, remove one antigen out of the standard antigen mixture. The micromethod based upon Poisson-distribution ensures that only those lines are clones producing antibodies recognizing the antigens of interest.

Are the methods effective and efficient? The effectiveness and efficiency of the new in vitro culture methodology can be improved in several ways:

The potential capacity of the automated system has not yet been fully exploited. At present after 6-7 days, 20 ml of parasitized packed erythrocytes (equals 40 ml of whole blood containing approximately  $40 \times 5 \times 10^9$  RBC) are harvested. At a 10-20% parasitemia, this yields  $2-4 \times 10^{10}$  parasites. This yield indicates that the culture conditions are not optimal for one can expect that 48 hours after synchronization, the parasitemia increases 10-fold and at least 3-fold during the next cycle. A more frequent medium change schedule, tailored to the amount of parasites present and their developmental stage at any given time will ensure that the number of parasites (schizont) which can be harvested after only 80 hours will increase compared to the numbers harvested at present in one week. Studies are encouraged to follow up means to optimize parasitic yields using the successful automated system.

5. Evaluation of the competence of the research personnel and the institutional environment

The staff consists of the principal investigator (Dr. W. A. Siddiqui), biochemist (Dr. S. C. Kan), 3 research associates (Dr. Kevin Palmer, Mr. Kenton Kramer, and Mr. Steve Case) and a graduate assistant (Mr. George Hiu). The personnel are experienced and appear to be not only highly competent but dedicated to the project. In addition, other University of Hawaii staff members (Dr. Karen Yamaga, Dr. Leslie Tam and Ms. Joanne Olejarczyk) actively participate in the research investigations.

6. Evaluation of the physical facilities

The laboratories, located in the Leahi hospital, are spacious and appear adequate to the project needs. The Autus animal facility is sub-

standard but efforts are being made to upgrade these facilities by the addition of new animal holding cages and by animal room renovation. This need is critical. The library in the department has minimal journal representation although the university library system is obviously available. Supp. as appear to be adequate but there appears to be a shortage of certain key equipment; i.e., critical need for a good scintillation counter.

7. Contribution of the project to institutional building

There was no emphasis on the training of investigators from LDC institutions as a result of this project. However, through a NIH research support grant which will be terminated in August, 1983, such institution strengthening is being conducted in India and by training graduate students at the University of Hawaii.

C. Significance to AID Objectives

The project has as its objectives (1) purification and characterization of Plasmodium falciparum blood stage antigen(s) and (2) vaccination studies in Aotus trivigatus monkeys using a standardized falciparum antigen(s). Both objectives are key to the research strategies for AID's goal for a malarial vaccine and therefore relate to AID's interest and/or participation in the overall control aspects of the world-wide malaria problem. The priorities of the AID malaria vaccine research program in 1981 had as its two highest priorities antigenic characterization, identification of potentially protective antigens and immunization trials. In February 1983, a new set of priorities was determined and antigenic characterization continued to be the highest priority. Therefore, this project is indeed concerned with activities which are currently of high priority to AID.

D. Relation to other research

This project in several respects is similar to two other on-going AID malaria vaccine projects; namely, the projects at Scripps Clinic and Research Institute and the University of Missouri. The three projects are related in that all have antigenic characterization as the primary objective. This project differs in that the emphasis is on the asexual stage of the malaria parasite whereas the other projects have emphasis on the merozoite stage. The other primary objective of this project is immunization trials, an objective also of the project at the University of Missouri and the project in Bogota, Colombia. Currently, coordination occurs on an investigator to investigator basis and/or through the dissemination of semi-annual reports.

This project is unique among the AID research network in that activities at improving *in vitro* cultivation methodologies continue such as culture vessel design, computer automation and culture synchronization. There are a number of research groups outside the network working in these specific areas in England, France, Germany, Australia and Switzerland.

E. Does the principal investigator have any other research contracts/grants from other granting agencies?

The principal investigator has a NIH grant in the amount of \$38,071 for studies on the "In vitro cultivation of human malaria parasites." This grant expires on August 31, 1983. There was a grant from WHO early in the current contract period which has expired.

II. FISCAL AND ADMINISTRATIVE REVIEW

A. Evaluation of past and current work plan budget

The dollar costs of the project are within reasonable limits based on the scope of the work. At the site visit in 1980, some equipment was requested and approved by at least 2 of 3 team members. They have not (3 years later) received such equipment. Dr. Contacos personally feels AID cannot expect optimal performance without optimal support.

There are currently supplemental requests for 1982-1983 budget (\$60,000) and the 1983-1984 (\$32,000) budget.

B. Property accountability

A complete up to date inventory of equipment was provided to the team and is on file with the AID Project Manager.

C. Examination of equipment

Equipment is put to good use. Some of the equipment is of "antique" condition and barely functional such as the scintillation counter. The animal quarters, especially caging, is below standard.

D. Staffing pattern

The staffing pattern is reasonable for the project's scope of work. The principal investigator, Dr. W. Siddiqui, contributes 15% of his time to the project at no cost. Dr. S. C. Kan, the Senior Research Associate, contributes 100% of his time. In addition, three research associates and a graduate assistant complete the AID staffing of this project. Drs. Yamaga and Tam and Ms. Olejarczyk complement the project staffing currently at no cost to AID.

E. Travel

Travel funds appear adequate.

F. Reports

Reports are now received on schedule.

### III. ANALYSIS OF FUTURE WORK PLANS

- A. The committee recommends that the research group continue to improve their in vitro culture system and the characterization of antigens derived from the culture system. In addition, it is recommended that the following specific activities should be addressed during the last year of the contract:
1. It is stressed that the characterization of antigens should be exclusively carried out using the primary strain of the AID network, the Honduras strain H-1.
  2. The full potential of the automated system should be exploited by studies aimed to increase the parasite yield in a shorter time.
  3. The three MAB's recognizing the proteins of 180, 135 and 81K should be evaluated against the spectrum of strains which are in the laboratory available now.
  4. Any monkey usage requires a prior submission and approval of a work protocol by the AID primate use committee. This is not only restricted to immunization trials, but covers all use of monkeys immunologically naive to malaria.
  5. Antigens isolated with MAB's should be put into non-primates for antibody production and then tested in a GARI test in vitro.
  6. It is recommended to de-emphasize all activities concerning the isolation of new malaria strains and their drug characterization.
  7. More in-depth characterization of isolated proteins on two-dimensional gels is desirable.
  8. To provide AID with a complete list of all Aotus monkeys, regardless of the source of their original purchase. This list should include the following details:
    - a. date of acquisition or birth (parantage)
    - b. sex
    - c. karyotype
    - d. malarial experience
    - e. pathology reports should be submitted to AID if available
  9. In case of future death of monkeys, its cause should be determined by a veterinarian to include an autopsy report.
- B. The expected input requirements are covered by the request for a supplementary budget. The request is considered reasonable. It is our understanding that the group has asked for a surplus gamma counter available through the AID malaria program. Should this instrument not be available, we recommend that the money requested for the zonal rotor should be used instead to purchase the scintillation counter. The committee is of the

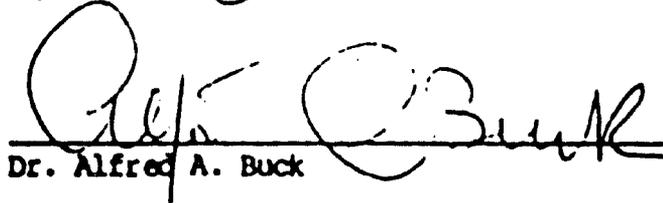
opinion that this instrument is more important for meeting the specific objectives than the zonal rotor.

Finally, the committee commends Dr. Siddiqui and his staff for the manner in which they prepared for the site visit review by way of outlines of specific objectives, activities to attain objectives, supporting data, and copies of reprints/preprints of publications. Such papers allowed for a more efficient review of work by this group.

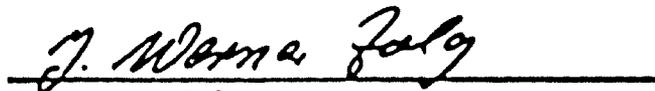
Submitted by Committee Members:

  
Dr. Peter G. Coombs

March 29, 1983  
Date

  
Dr. Alfred A. Buck

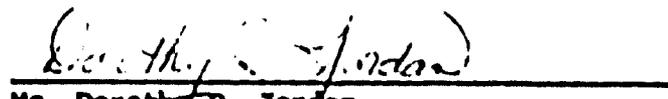
March 24 / 83  
Date

  
Dr. Werner Zalg

3/26/83  
Date

  
Dr. William Collins

28 March 1983  
Date

  
Ms. Dorothy R. Jordan

4/18/83  
Date

  
Dr. James M. Erickson  
AID Project Manager

4/18/83  
Date

SCRIPPS CLINIC AND RESEARCH FOUNDATION  
SITE VISIT REPORT  
March 11-13, 1983

I. SUBSTANTIVE REVIEW

A. Project description and background

The goal of this project is (1) to identify, isolate, and begin chemical characterization of one or more macromolecular components of the asexual erythrocytic form of Plasmodium falciparum which can be used to induce protective immunity in Aotus monkeys and (2) to attempt to establish clones of micro-organisms containing recombinant DNA which can produce the particular parasite proteins which may be useful for immunization. The project was initiated September 15, 1981 and extends through September 14, 1984.

B. Progress to date

1. Evaluation of past performance against specific project objectives

There was a delay in the initiation of active work on this project due to the lag-time required to equip and staff a new research team. The project is now staffed, well equipped, and active. The scope of the project has not deviated significantly from the stated objectives although it is difficult to separate the work of this particular project from those funded elsewhere, thus resulting in a much broader effort. The only problems appear to be time oriented and it would appear that significant progress will now be made and continue throughout the remainder of the contract period. There cannot be a logical end in sight for the project at this time since its ultimate goal is the isolation and production of the malaria vaccine. However, during the remaining period of the contract, significant progress toward the goal can be expected.

Several significant findings of the project are noteworthy. Major glycoproteins with specific molecular weights of 34, 46, 56 and 185 K daltons have been identified in ring stage parasites which have been S<sup>35</sup> methionine labeled while in the previous schizont stage of development. No biological activity has yet been demonstrated for these molecules. Two of these (46 and 185 K daltons) are of particular interest. Monoclonal antibodies which recognize the 185 K protein are now available.

Studies using a K+ clone have indicated that selection through nine preparative cycles using agglutination properties in physiolgel suggests that K- parasites can be produced from K+ cloned material in each generation. The significance of this in regard to the specific objectives is not apparent at present.

Two different approaches are being made in the genetic engineering area of the project: cloning of genomic DNA and synthesis of cDNA followed by cloning. Both areas of investigation have been started, the approaches appear sound. The project has worked closely with other projects at Scripps resulting in significant observations on the potentiation of the effect of vaccination by the activation of cell-mediated responses via complement.

This project appears to be actively pursuing collaborative studies with other workers both within and outside the AID network especially to obtain sufficient quantities of blood stage antigen(s) and additional monoclonal antibodies. The active collaboration with other workers at Scripps is particularly evident which should contribute significantly to the ultimate progress of the project. In general, little data on the specific contract objectives has been presented to the review committee making a detailed assessment of the significance of the work difficult.

2. Evaluation of results, interpretation of data, significance of findings  
The line of research should be able to answer or meet the expected objectives. However, it is premature to attempt any evaluation of the data or results at this time. The findings thus far indicate that there are 4 different glycoproteins of particular interest which may be candidates for further study. The genetic engineering aspects of the study appear sound but a judgement of the data and results would be premature at this time.
3. Use of results  
A total of six manuscripts have thus far been produced as a result of this project at Scripps. There appears to have been an active exchange of information between the staff and other members of the U.S.A.I.D. network.
4. Evaluation of research methodology
  - a. Are the techniques efficient and effective in terms of the goal of the study? The variety of equipment available, especially those which are centrally accessible, is outstanding. However, the potential of the group seems not to be fully exploited in regard to in vitro culturing of P. falciparum. To accomplish the objectives of the contract, large amounts of parasites will be required. This preparative aspect should be given immediate attention. The efficiency in fulfilling the objectives requires the acceleration of the production of MAB's needed to isolate the schizont/merozoite specific antigens.
  - b. Have there been any changes in methodology? The methods used are appropriate to answer the questions being investigated. The use of the HPLC equipment should provide data relevant for studies concerning the structure of proteins recognized by immune sera. The "schizont-labeled-ring" technique allows one to identify preferentially those proteins which are synthesized in the late schizont or merozoite stages. The availability of the

microsequencing techniques in the institute opens the possibility to characterize in detail the relevant polypeptides.

5. & 6. Evaluation of the competence of the research personnel and the institutional environment

The staff appears to be well suited for the project investigation. Equipment is superb; there appears to be an ideal combination of staff, equipment, and environment conducive to the fulfillment of the contract.

7. Contribution of the project to institution building

This particular project appears to offer little in the way of LDC institutional building. However, several scientists from LDC's are actively working on this project.

C. Significance to A.I.D. Objectives

The objectives of this project both have highest priority in the recently updated strategy (February 1983) of research for the U.S.A.I.D. malaria vaccine network. This project remains an important part of the network.

D. Relation to other research

This project has objectives similar to several other AID contracts but no major duplication of effort is occurring.

E. Other research contracts/grants

The principal investigator has a grant from NIH in the amount of \$76,000 on Immunology of Malaria. This grant expires September 1983.

II. FISCAL AND ADMINISTRATIVE REVIEW

A. Evaluation of past and current work plan budget

The budget is generally adequate for the completion of contracted work. However, once the work has progressed, supplemental funding may be required.

B. Property accountability

Detailed, current inventories of capital equipment and *Lotus* monkeys are on hand to the AID Project Manager.

C. Examination of equipment

The condition of equipment is excellent. The equipment is being put to maximal use.

D. Staffing pattern

The staffing pattern is adequate for the scope of the work of this project.

E. Travel

Travel funds appear fully adequate.

F. Reports

Reports are submitted on schedule and appropriately detailed.

III. ANALYSIS OF FUTURE WORK PLANS

A. Recommendations

1. It is stressed that the characterization of antigens should be exclusively carried out using the primary strain of the AID network, the Honduras I strain.
2. The committee recommends that the research group should place emphasis on improving their ability to produce large numbers of parasites in vitro. These large numbers will be required for preparative immunoprecipitation with the goal to isolate potentially protective antigens on a preparative scale.
3. It is recommended that large-scale and/or the continual in vitro cultivation of malaria parasites be expanded now, so that parasites can be stockpiled to be available as need requires. This should ensure that during the remainder of the contract period, there will be no shortage of parasite material for use in preparative experiments.
4. Antigens isolated by MAB's or polyclonals should be put into nonprimates for antibody production and then tested for biological activity in the GARI in vitro test. The committee emphasizes the importance of the preparative aspects of the work including the testing for biological activity of immunogens in addition to the analytical aspects.
5. It is recommended that all AID research activities concerning the future cloning of K+ and K- lines, as well as the involvement of complement in the cellular immunity, should be discontinued until specifically linked to contract objectives.
6. The emphasis in the recombinant DNA work should concentrate on the synthesis and cloning of cDNA. The establishment and screening of genomic DNA libraries should be given a much lower priority compared to the cDNA aspect.

7. More in-depth characterization of isolated antigens on two dimensional gels is desirable in order to determine if the antigenic material is homogenous.
8. A statement of personel under the AID umbrella and the percentage of their time on contract research activities should be submitted to AID. In addition, the research activities to which each of these group members have been assigned should be included.
9. AID should be provided with a complete list of all Aotus monkey's regardless of the source of their original purchase. This list should include the following details:
  - a. date of aquisition or birth (parentage)
  - b. sex
  - c. karyotype
  - d. malarial experience
  - e. pathology reports (if available)
10. In case of future death of monkeys, its cause should be determined by a veterinarian including an autopsy report.
11. Any monkey usage requires a prior submission and approval of a work protocol by the AID primate use committee. This is not only restricted to immunization trials, but covers all use of monkeys immunologically naive to malaria.

B. Expected input requirements

There appears to be no additional input requirements in terms of total cost for accomplishing specific contract objectives.

It is unfortunate that this group was not adequately prepared for the site visit by way of preparing detailed outlines of specific objectives, listing the progress against these objectives, and outlining the activities being planned to attain objectives. Supporting data, as well as preprints-reprints of journal articles would have allowed for a more efficient review.

Submitted by Committee Members:

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March 29, 1983  
Date

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3/26/83  
Date

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4/18/83  
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