AGENCY FOR INTERNATIONAL DEVELOPMENT

PROJECT DATA SHEET

1. TRANSACTION CODE: A (A = Add, C = Change, D = Delete)

Amendment Number

DOCUMENT CODE: 3

2. COUNTRY/ENTITY: Interregional

3. PROJECT NUMBER: 931-0453.14

4. BUREAU/OFFICE: DS/HEA 10

5. PROJECT TITLE (maximum 40 characters): Malaria Imnty & Vacntn Rsrch (USUHS) A

6. PROJECT ASSISTANCE COMPLETION DATE (PACD): MM 10 DD 31 YY 83

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

A. Initial FY 80 B. Quarter 4 C. Final FY 82

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

A. FUNDING SOURCE	FIRST FY 80 B. FX	C. L/C	D. Total	LIFE OF PROJECT E. FX	F. L/C	G. Total
AID Appropriated Total	295		295			536
(Grant)	(295)	( )	(295)	( )	( )	(536)
(Loan)	( )	( )	( )	( )	( )	( )
Other U.S. 1.						
Other U.S. 2.						
Host Country						
Other Donor(s)						
TOTAL	295		295			536

9. SCHEDULE OF AID FUNDING ($000)

A. APPROPRIATION	B. PRIMARY PURPOSE CODE	C. PRIMARY TECH. CODE 1. Grant	2. Loan	D. OBLIGATIONS TO DATE 1. Grant	2. Loan	E. AMOUNT APPROVED THIS ACTION 1. Grant	2. Loan	F. LIFE OF PROJECT 1. Grant	2. Loan
(1)	540	542				536		536	
(2)									
(3)									
(4)									
TOTALS						536		536	

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 480 characters)

Locate and characterize components of Plasmodium falciparum and its host cell which are important in immunologic protection against malaria and may be candidate antigens for vaccine development.

14. SCHEDULED EVALUATIONS

Interim MM 04 YY 82 MM YY Final MM 11 YY 83

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)

17. APPROVED BY

Signature

Title: DS/HEA, Director

Date Signed: MM 06 DD 06 YY 81

18. DATE DOCUMENT RECEIVED IN AID/W, OR FOR AID/W DOCUMENTS, DATE OF DISTRIBUTION: MM DD YY

AID 1330-4 (8-79)

PROJECT AUTHORIZATION AND REQUEST FOR ALLOTMENT OF FUNDS

PART II

Entity: DS/HEA/M

Project: Malaria Immunity and Vaccination, New Sub-Project with the Uniformed Services University of the Health Sciences

Project Number: 931-0453.14

I hereby authorize FY 1980 grant funding, not to exceed two hundred ninety-five thousand ($295,000), for a Participating Agency Service Agreement with the Uniformed Services University of the Health Sciences to conduct research on the ultrastructural and cytochemical analysis of malaria antigens.

I approve the total level of A.I.D. appropriated funding planned for this project not to exceed five hundred thirty-six thousand dollars ($536,000), including the funding authorized above, during the period FY 1980 through FY 1982. I approve further increments during that period of grant funding up to two hundred forty-one thousand dollars ($241 000), subject to the availability of funds in accordance with A.I.D. allotment procedures.

DAA/DS, Stephen Joseph, M.D.

AUG 14 1980

Date

Attachments:
A. Research Proposal
B. IEE
C. RAC Recommendation
D. Action Memorandum

Clearances:
DS/HEA: JAlden date
DS/PO: BChapnick date
DS/PO: MRechcigl date

AUG 5 1980

ACTION MEMORANDUM FOR THE DEPUTY ASSISTANT ADMINISTRATOR FOR HUMAN RESOURCES DEVELOPMENT

FROM: DS/HEA, John Alden

Problem: Approval of a PAF for negotiation of a PASA with the Uniformed Services University of the Health Sciences under Project 931-0453, Malaria Immunity and Vaccination Research.

Discussion: The subject activity was approved at the June 17-18, 1980 meeting of the Research Advisory Committee. Copies of the RAC recommendation and the Action Memorandum for the Administrator are attached.

Funds for the activity are included in the FY 1980 Congressional Presentation and the FY 1980 OYB for the Office of Health. The proposal has been reviewed and approved by the following:

Office of Health (DS/HEA)
Program Office (DS/PO)
DSB Project Review Committee
Research Advisory Committee (RAC)

The proposal has been found to be technically sound and to meet all the requirements of an unsolicited proposal contained under AID PR 78-4.

Recommendation: That you sign the attached PAF.

Attachments:
PAF, with attachments

Clearance:
DS/PO: BChapnick _____ date 8/5/80

AID PROJECT SUMMARY

A. Basic Identification and Fiscal Data

Project Title:	Malaria Immunity and Vaccination Ultrastructural and Cytochemical Analysis of Malarial Antigens
New or Extension:	New
Contractor and Address:	Uniformed Services University of the Health Sciences School of Medicine 4301 Jones Bridge Road Bethesda, Maryland 20014
Principal Investigator:	Susan G. Langreth, Ph.D. Associate Professor Department of Microbiology School of Medicine Uniformed Services University for the Health Sciences Bethesda, Maryland 20014
Duration:	Three Years
Previous Project Funding:	None
Proposed Funding:	1980 -- $295,000 1981 -- $110,000 1982 -- $132,000
Total Estimate:	-- $537,000*

B. Background

In 1965 A.I.D. began to support research on a new approach to the control of malaria: protection from the disease by immunization. The Agency has expanded its initial support of one project at the University of Illinois to a collaborative network of ten laboratories working on various aspects of malaria immunity and vaccine development. One of the A.I.D.-supported laboratories has made the most significant advance in malaria research of recent times: the continuous cultivation of the parasite *Plasmodium falciparum* in human erythrocytes *in vitro* (Trager and Jensen, 1976). A.I.D. network laboratories have also successfully immunized monkeys against human malaria (*P. falciparum*) using cultured material as a source of immunogen. In addition, A.I.D. supports vaccine development based on an alternative stage of the parasite life cycle found in the mosquito, namely the sporozoite.

*Estimated funding levels do not include institutional overhead (currently not charged by this institution), or possible import duties on capital equipment.

C. Description of the Project

The overall objective of this project is to locate and characterize those components of the parasite *Plasmodium falciparum* and its host cell which are important in the immunologic protection against malaria and which, therefore, may be candidate antigens for the development of a vaccine. The specific aims are as follows:

a. To locate both the surface and the internal malaria antigens to which immune animals produce antibodies.

b. To characterize those antigenic components structurally, cytochemically, an immunologically.

c. To determine the structure and function of the knobs (alterations on the infected erythrocyte surface produced by *Plasmodium falciparum* infection).

d. To develop assays to assess purity of blood stage antigen preparations and to screen for interstrain antigenic differences and antigenic variation *in vitro*.

D. Scope of Work

The detailed research work plans are outlined in the Research Contract Application and are to be found on pages 15 through 28. (Attached)

E. External Review

This proposal has been reviewed and enthusiastically approved by external expert consultants, including Dr. Richard Beoudoin of the U.S. Navy Medical Research Institute, Dr. Peter Contacos, Retired, U.S. Public Health Service, and Dr. Irwin W. Sherman, University of California, Riverside. These reviews are attached for information.

Site Visit

Uniformed Services University
School of Medicine
Bethesda, Maryland

At the request of your office, I made an informal site visit (Wednesday April 16, 1980) to the Department of Microbiology, Uniformed Services University School of Medicine, Bethesda, Maryland. The visit was to ascertain the interest by the University, the support facilities available, and the faculty commitment to adequately support potential research programs in the malaria vaccine area.

The overall support that this University could provide for any USAID funded contract could not, in my opinion, by surpassed my many universities. The location of the campus, with its proximity to the Naval Medical Research Institute, the National Institutes of Health, and the Walter Reed Army Institute for Research, is a tremendous asset both scientifically and intellectually.

The support facilities at USUHS almost defy description. Office and laboratory space is abundant as well as superbly designed with benches, electrical, water, and gas inputs well placed for any research to be undertaken. I was particularly impressed with the EM suites, as each suite has its own preparatory and dark-room facilities contiguously located. The lecture and general teaching laboratory facilities are better than any I have ever seen.

Overall, my discussions with faculty members and administrators convinced me of their sincere and long term interest in tropical medicine and malaria research in particular. This commitment combined with absolutely first rate back-up facilities convinces me that this institution can fully carry out a first-rate research program within AID's Malaria Immunity and Vaccination Research Collaborative Network.

Peter G. Contacos, M.D., Ph.D.
Malaria Consultant

Proposal: Ultrastructural and Cytochemical Analysis of Malarial Antigens
Principal Investigator: Susan G. Langreth
Organization: Uniformed Services University of the Health Sciences, School of Medicine, Bethesda, Maryland 20014

Evaluation of Proposal

The description of the work proposed by Dr. Langreth is well written and well documented. She has set forth in great detail a careful analysis of one important aspect of falciparum antigens which disappears in cultured parasites, i.e., the knob antigens occurring on the red cell membrane of K+ strains. There is evidence which suggests that the K+ strains are more antigenic than the K- strains and that K+ strains tend to lose the knob antigens after prolonged maintenance in cultures of the Trager Jensen type. Clarification of this point would be of central importance to the USAID program since such an antigenic change in cultured parasites could conceivably call into question the feasibility of basing an effective vaccine on parasites grown in the Trager Jensen system. On the other hand, if the knob antigens are responsible for stimulation of the immune response, and especially functional immunity, the antigens could possibly be produced by other means. Although it would be difficult to separate them from the red cell membranes directly using chemical approaches alone, they could conceivably be identified and isolated by monospecific antibody produced by monoclonal hybridomas and then one could rely on DNA hybridization technology for actual production of the protective antigens from which the vaccine could be made.

Regardless, it would appear to be absolutely essential to characterize the knob antigens as thoroughly as possible. The bottom line, of course, is the demonstration that K+ antigens are responsible for protection. This can only be done in monkeys. However, monkeys are not readily available and much more information about the knob antigens is needed before an actual vaccination trial is set up. This information forms the basis of Langreth's proposal. Her approach to the problem is systematic and thorough as well as being technically sophisticated. Dr. Langreth enjoys a well-deserved reputation as an outstanding electron microscopist who goes far beyond traditional descriptive work. She uses her instrument as a powerful experimental tool which will be invaluable to the existing USAID collaborative network. At the end of the proposal she correctly emphasizes the value of analyzing all vaccine preparations using the same approach. In my opinion, this type of analysis will be necessary for any candidate vaccine destined for eventual use in man. I strongly recommend approval of this proposal and suggest that the broader scope of work be emphasized, under which Dr. Langreth can monitor the purity of candidate preparations and provide an invaluable service in the characterization of the protective antigens in such preparations. The technical expertise and experimental data obtained in analysis of K+ antigens will have wide appliation to any future vaccine preparations.

R. L. Beaudoin

R. L. Beaudoin, Ph.D.
Immunoparasitology Branch
Naval Medical Research Institute
Bethesda, Maryland 20014

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DEPARTMENT OF BIOLOGY
RIVERSIDE, CALIFORNIA 92521

March 6, 1980

Dr. J. Erickson
DS/HEA
Agency for International Development
Department of State
Washington, D.C. 20523

Dear Dr. Erickson:

Enclosed is my review of the proposal "Ultrastructural and Cytochemical Analyses of Malarial Antigens" by Dr. S. Langreth.

I hope this critique will be of some value to you.

Sincerely,

Irwin W. Sherman
Professor of Zoology

IWS:rc
Encl.

Investigator: Langreth Reviewer: Sherman

Title: Ultrastructural and Cytochemical Analyses of Malarial Antigens

Critique

This is a well-written proposal that requests 3 years support to study malarial antigens using the electron microscope and cytochemical techniques. The literature is reviewed carefully, and thoroughly.

The studies are well-conceived, and should provide valuable information for vaccine development. Indeed, without studies of the sort the PI proposes it is rather unlikely that a successful vaccine can be developed.

The PI is an accomplished microscopist and is well-versed in the *in vitro* cultivation of *P. falciparum* as well as techniques for EM localization of glycoproteins. In addition, to her own research the PI will be a valuable resource for the other AID contract (Rieckmann) at New Mexico. The association between the Rieckmann group and the PI can only strengthen both research programs.

Aim a. Immunocytochemical localization of parasite antigens on the surface of the red cell.

The PI is capable of making significant contributions. The techniques are all within her ken.

Aim b. Cytochemical characterization of antigenic structures.

Again, PI is well-qualified. Information to be obtained is relevant to vaccine purification and characterization.

Aim c. Structural analysis of the infected erythrocyte esp. knobs.

These studies are not as well-defined as those described earlier. No provision has been made for use of freeze-fracture apparatus or SEM. Of interest, but not a primary area of research.

Aim d. Determination of the function of the knobs *in vivo*.

A worthwhile study that can certainly be done well at New Mexico. This reviewer is pleased to see the PI's concern for conserving *Aotus* (p. 25). How will the PI assure that *all* parasitized RBC introduced are knobless?

Aim e and f. Ultrastructural studies of antigen preparations.

Essential for vaccine development. PI is well-qualified.

Investigator

An exceptionally talented electron microscopist who discovered the knobless condition, and who has done the finest EM study of *P. falciparum*. Well-qualified to do cytochemistry.

Budget

Adequate and modest, for the most part. Question the excessive amounts budgeted for travel.

Recommendation

Approval.

arch Proposal entitled "Ultrastructural and Cytochemical Analysis of
rial Antigens." Principal Investigator: Dr. Susan Langreth.

The proposal is highly detailed, thoughtfully prepared, and definitely has scientific merit. I certainly would endorse a project with Electron Microscopy (EM) orientation for the USAID Malaria Vaccine Research Program. I would recommend this proposal as a separate project in the USAID vaccine network for a multiplicity of reasons; foremost among them being that such EM expertise would be available for all of the network projects. Currently, only the Rockefeller University project has EM as an active part of its research project. Others in the network have to "farm out" their EM requirements — not an optimal situation. The EM requirements of the entire network should be accomplished at one source for reasons of comparability of results. If USAID is to purchase expensive EM equipment, then it should be clearly understood that the EM expertise of this proposed project will be available to the entire network for vaccine studies.

The orientation and emphasis of this proposal are focused on the overall objectives of the malaria vaccine program. The objectives complement and integrate well with the objectives of other projects of the network. EM technology is the only means of identifying and locating protective antigenic sites on and/or within the malaria parasite. In this connection, each of the projects concerned with vaccination experiments should provide Dr. Langreth with adequate serum samples from monkeys found to be totally protected by vaccination. EM technology also is the only means of unequivocally assaying the purity of vaccine preparations. Dr. Langreth's proposed studies on the *in vitro* cultured "knobby" vs. "knobless" lines of falciparum parasites are indeed important to the USAID goal of a malaria vaccine—especially as they relate to he protective antigenicity of the merozoite stage.

The proposed site of the project is ideal. I have visited the Uniformed Services University for the Health Sciences campus and found the overall academic interest and physical plant facilities to be outstanding. Please see my evaluation site visit report.

I consider the proposed budget to be indeed reasonable. Except for the large initial expense of purchasing the Electron Microscope ($160,000) the annual budget amounts to roughly $130,000 per year.

A final word about the Principal Investigator of this proposal. Dr. Langreth is a diligent, careful and very capable scientist. I have personally reviewed her contributions to the Rockefeller University project during two site visits and found them to be superior. Moreover, the fact that she has been associated with Dr. William Trager for the past 6 years speaks for itself.

PETER G. CONTACOS, M.D.
USAID CONSULTANT IN MALARIA
April 18, 1980

Research Proposal

to

The Bureau for Development Support
Agency for International Development
Department of State
Washington, D.C. 20523

Ultrastructural and Cytochemical Analysis of Malarial Antigens

from

Department of Microbiology
School of Medicine
Uniformed Services University of the Health Sciences
Bethesda, Maryland

Date of Submission: April 20, 1980

Duration: 36 months

Starting Date: August 15, 1980

Principal Investigator: Susan G. Langreth, Ph.D.

B. Relevance of Proposed Work to A.I.D.

Malaria has afflicted the human race for centuries. It remains one of the most widespread parasitic diseases in tropical and subtropical areas of the world. According to the World Health Organization, approximately 1.5 billion people are at risk from this disease (Noguer et al., 1978). Of those at risk, 352 million live in areas, primarily sub-Saharan Africa, where there are no malaria control activities whatsoever. The WHO has estimated that the current annual incidence of malaria in Africa, Asia, and Latin America is 800 million infections, resulting in 150 million cases of disease and 1.2 million deaths (Walsh & Warren, 1979). Most of these deaths will be infants and children.

The United States has supported malaria eradication/control programs throughout the world since World War II. In the 1950's and 1960's massive efforts were mounted in Asia and Latin America to eradicate malaria. These campaigns were initially successful, resulting in a reduction by 500 million of the people at risk from this disease. However, there has recently been a resurgence of malaria, partially as a result of political instability, inadequate financing coupled with inflation, complacency, and administrative difficulties. In addition, several important technical problems have arisen. Malaria parasites on all continents have developed resistance to the drugs generally in use to combat them, such as chloroquine. Mosquitoes, which transmit the disease, have developed resistance to insecticides, such as DDT, dieldren, proxpour, etc. This worldwide resurgence of malaria has been so severe that A.I.D. is currently considering an expansion of its assistance to malaria control programs.

A significant mistake made during the 1950-1960's period of malaria eradication campaigns was the neglect of funding for research (Chin, 1979). A.I.D. allocated only 0.1% of the $1.2 billion it spent on malaria programs from 1950-1972

research (Chin, 1979). The current resurgence of malaria has stimulated both A.I.D. and W.H.O. to increase their support for research programs, but total worldwide research funding for malaria remains at a low level, approximately $0.02/infected person/year (Walsh & Warren, 1979). This level is significantly lower than that for schistosomiasis, trypanosomiasis, leishmaniasis, or leprosy.

In 1965 A.I.D. began to support research on a new approach to the control of malaria: protection from the disease by immunization. The agency has expanded its initial support of one project at the University of Illinois (later University of New Mexico) to a collaborative network of ten laboratories working on various aspects of malaria immunity and vaccine development. One of the A.I.D.-supported laboratories has made the most significant advance in malaria research of recent times: the continuous cultivation of the parasite Plasmodium falciparum in human erythrocytes in vitro (Trager & Jensen, 1976). A.I.D. network laboratories have also immunized monkeys against human malaria (P. falciparum) using cultured material as a source of immunogen and obtained varying degrees of protection. The proposal herein submitted is for a malaria immunity project which would join A.I.D.'s collaborative network.

C. Scientific Aspects of Proposed Work

1. Specific Objectives

The overall objective of this project is to locate and characterize those components of the parasite *Plasmodium falciparum* and its altered host cell which are important in immunologic protection against malaria and which, therefore, may be candidate antigens for the development of a vaccine. The specific aims are as follows:

a. To locate both the surface and the internal malaria antigens to which immune animals produce antibodies.

b. To characterize those antigenic components structurally, cytochemically, and immunologically.

c. To develop assays to assess purity of blood stage antigen preparations and to screen for interstrain antigenic differences and antigenic variation *in vitro*.

d. To determine the structure and function of the knobs (alterations on the infected erythrocyte surface produced by *Plasmodium falciparum* infection).

2. Rationale

The unique advantage of cytochemistry is that the chemical nature of cellular components can be determined without destroying the structural integrity and interrelationships of host and parasite, which are destroyed by preparative biochemical techniques. In fact, valid and reliable data can be obtained by cytochemical methods from heterogeneous, impure, or contaminated preparations, because all components of a given sample can be directly visualized. Using electron microscopy, cytochemistry can be extended to

the level of resolution of macromolecules. At the present stage of malaria immunity research, no one has obtained a pure fraction of any human malarial component. In addition, some components known to be antigenic, such as the knobs, can only be detected by electron microscopy. Therefore, ultrastructural analysis and cytochemistry is an essential approach to malaria immunity research.

A blood stage malaria vaccine may function by stimulating the host immune system to attack exposed foreign surfaces, such as the plasma membrane (and cell coat) of the parasite at the merozoite stage or the altered infected erythrocyte membrane (including the knobs). Immune inhibition may be the result of agglutination of merozoites, blocking invasion of merozoites, lysis of infected erythrocytes, or opsonization and phagocytosis of infected cells. Internal antigenic components may also be useful in a vaccine if antibodies to them inhibit parasite development or the function of parasite organelles, such as the merozoite's rhoptries. Using immunocytochemical techniques, by which reactive sites are located with labeled antibodies, both internal and surface antigenic components can be identified. Once found, structural and cytochemical characterization can proceed even before pure fractions are obtained. In addition, during fractionation and purification of antigens for experimental vaccine use, electron microscopy can be used to assess quality, purity, and consistency of the preparations. Thus, ultrastructural analysis and cytochemistry are powerful but, until now, underutilized approaches to malaria immunity research.

3. Literature Review

a. Infected Erythrocytes

Infection by a malaria parasite results in numerous alterations in the plasma membrane of the host erythrocyte: immunological, chemical, and

structural. In Plasmodium falciparum infections, the most dramatic alterations are the "knobs" (electron-dense inverted cuplike protrusions of the erythrocyte surface). Knobs, first observed by Trager et al. (1966), can only be visualized by electron microscopy. They are believed to be involved in the sequestration of older parasites in the deep vasculature of the heart and other organs, where P. falciparum undergoes schizogony (Miller, 1969; Gutierrez et al., 1976). The knobs have been found to be the sites of adherence of trophozoite- and schizont-infected erythrocytes to endothelial cells (Luse & Miller, 1971; Aikawa et al., 1972; Miller, 1972). The gametocytes of P. falciparum are also sequestered (in bone marrow) during most of their 10-day development. Although these sexual stages lack knobs (Kass et al., 1971), they may also produce a receptor on their host cell surfaces which is involved in their sequestration.

In a typical P. falciparum infection in its human host, one finds only young parasites (rings) in the peripheral circulation. The more mature parasites, bearing knobs on their host erythrocyte membranes, are sequestered. They are dislodged only in an immune crisis. With the advent of methods to grow P. falciparum in continuous culture in human erythrocytes (Trager & Jensen, 1976), all stages of the erythrocytic life cycle are readily obtainable. In vitro cultivated organisms are ideal for investigating the origin and fate of the knobs not only because of the availability of mature parasites but also because the cultures are isolated from the effects of the immune system. An ultrastructural study of human falciparum malaria grown in vitro has recently been completed (Langreth et al., 1978a). Knobs first appear on young trophozoite-infected erythrocytes in vitro. In vivo this is the life cycle stage at which

parasites disappear from the peripheral circulation and are sequestered, presumably by their knobs. In young trophozoites the knobs arise as inverted cuplike plaques of electron-dense material just beneath the unit (cell) membrane of the erythrocyte. The erythrocyte surface becomes distorted, forming protrusions at the sites of knob material. Older parasites (segmenters) generally have fewer but usually larger knobs than younger parasites (trophozoites). Coalescence and sloughing of knob material has been observed both in cultures and in Aotus monkey infections (Langreth et al., 1978a). Sloughed knob material may be a source of antigen released by the parasites in vivo (Wilson & Bartholomew, 1975) and in vitro (Campbell and Langreth, unpub.).

Larger knobs and coalesced knob material of older parasites often obscure the erythrocyte plasma membrane. Inability to discern this unit membrane can be the result of oblique sections, but it is also possible that the erythrocyte membrane is structurally altered at the site of a mature knob. (Immunocytochemical data also implies that the erythrocyte membrane is altered; see below). Whether or not knob materials are transmembrane integral components of the infected erythrocyte membrane can be resolved by freeze-fracture studies. The only freeze-fracture investigations on a primate malaria are those of McLaren et al. (1977, 1979) on P. knowlesi. They have found that the P face of the infected erythrocyte membrane has 20% fewer particles and that the distribution of the particles was less homogeneous (more clumped). Several metabolically labeled proteins have been found in the infected erythrocyte membranes of primate malarias (Kilejian, 1979; Kilejian & Olsen, 1979; Schmidt-Ullrich et al., 1979b). These changes may also be related to structural protein alterations

in the erythrocyte membrane, which could be detected by freeze-fracture techniques.

The Aotus trivirgatus griseimembra monkey is the animal model for human falciparum malaria (Schmidt, 1978a,b,c). Aotus monkeys that were made immune to Plasmodium falciparum infection produce antibodies to the knobs and sloughing knob material (Langreth & Reese, 1979). These antibodies were detected by an immunocytochemical sandwich labeling technique and electron microscopy. The knobs on both human and monkey infected erythrocytes bound antibody. Uninfected erythrocytes and infected ring-stage erythrocytes (knobless) failed to bind antibody. Therefore, the parasite must produce a similar antigenic change in the knobs of infected erythrocytes of both man and monkey. Three strains of P. falciparum (FCR-1/FVO, FCR-3/FMG, and FCR-4/6252), cultured in human erythrocytes, induce erythrocyte membrane alterations which have at least some common antigenicity. The knobs have also been shown to be antigenically distinct from the rest of the infected erythrocyte membrane in an heterologous system (Kilejian et al., 1977). Schmidt-Ullrich et al., (1979a) presented evidence that antibodies to two P. knowlesi-specific antigens expressed on the surface of Rhesus monkey infected erythrocytes were produced in immune animals. They concluded that presence of these antibodies was correlated with protective immunity.

It is still unknown whether anti-knob antibodies are protective in falciparum malaria. All previous blood-stage antigen preparations used to immunize monkeys, even those that were merozoite-enriched, contained knob material (Siddiqui, 1977; Mitchell et al., 1977; Reese et al., 1978; Rieckmann et al., 1978). Natural infections always involve the presence

of knob antigens. In addition, there are other immunologically important unanswered questions about the knobs. Are the knobs a site of antigenic variation, such as occurs on the infected erythrocyte surface in P. knowlesi (Brown & Brown, 1965)? Although common antigens on the knobs of different geographic strains of P. falciparum, which may be useful in a vaccine, have been demonstrated, it is essential to determine whether there are also strain-specific knob antigens and whether these play a role in immunologic protection against individual strains.

By crossed immunoelectrophoresis using immune Rhesus monkey antiserum, two groups have found antigenic components in P. knowlesi schizont-infected erythrocyte membranes (Deans et al., 1978; Schmidt-Ullrich & Wallach, 1978; Deans & Cohen, 1979; Schmidt-Ullrich et al., 1979ab). (P. knowlesi does not produce knobs). Deans & Cohen (1979), working with P. knowlesi and Kilejian & Olsen (1979), working with P. falciparum, both found new concanavalin-A-binding components in schizont-infected erythrocyte membranes, indicating the presence of new sugar groups. Schmidt-Ullrich et al. (1979b) also demonstrated by pulse-chase experiments with C^{14} glucosamine that the parasite-produced erythrocyte membrane alterations are glycoproteins. Because it is not presently possible to obtain pure fractions of P. falciparum-infected erythrocyte membranes or knobs, a direct localization of labeled (glyco)proteins in the knobs cannot be made utilizing biochemical techniques (Kilejian, 1979; Kilejian & Olsen, 1979). However, this localization can be obtained by cytochemical techniques and autoradiography.

Few attempts have been made to examine the knobs cytochemically. Miller et al. (1972) showed that the knobs do not differ from the rest of the infected erythrocyte surface with respect to binding of positively

charged ferric oxide hydrosols. In preliminary experiments, a silver methenamine method modified from that of Rambourg (1967) has been used to probe for glycoproteins in internal as well as surface structures (Langreth, unpub.). This reaction, performed on Epon-embedded sectioned material mounted on gold grids, detects aldehyde groups in polysaccharides which are exposed by periodic acid hydrolysis. Positive reactions were obtained in the parasitophorous vacuole (PV) of mature forms, particularly schizonts and segmenters, and on the knobs. This provides direct *in situ* evidence that the knobs contain glycoprotein. Even malarias which do not produce knobs do induce substantial changes in glycoproteins of the infected erythrocyte membrane (above paragraph; Sherman & Jones, 1979), but these changes have been little studied cytochemically.

Variants of several strains of *P. falciparum*, maintained in human erythrocytes in long-term culture, which stop producing knobs have recently been discovered (Langreth *et al.*, 1979). The surface of the infected erythrocyte of a knobless (K-) mature parasite is smooth rather than distorted as in the knob-bearing (K+) wild type. This change, detected by electron microscopy, is the first reported alteration in host or parasite as a result of *in vitro* culture. The mature forms (trophozoites, schizonts, segmenters) of K- variants cannot be concentrated by Physiogel, presumably because their smooth and flexible surfaces allow them to participate in rouleaux formation (Reese *et al.*, 1979). This altered behavior permits one to select for K+ or K- parasites by repeatedly subculturing from the Physiogel top phase (K+) or from the Physiogel bottom phase (K-). Knobless lines have been made in this manner for FCR-1, FCR-3, FCR-4, and FVO/Oak Knoll/New Mexico (Langreth *et al.*, 1979; Campbell, unpub.). The mature forms in

the K- lines are > 99% knobless, as determined by electron microscopy. Knobless parasites still have Maurer's clefts in the erythrocyte cytoplasm. However, loss of knobs is correlated with the loss of antigenicity of the infected erythrocyte surface to immune monkey serum (Langreth & Reese, 1979) and with the loss of a metabolically labeled protein (Kilejian, 1979). Loss of knobs as a result of long-term cultivation seems to occur in all strains of P. falciparum: four additional strains, which have been cultured for 9 months - 1 year are producing some knobless variants. These strains are FCR-7/Kenya, FCR-8/West Africa, FCC-1/China, and FCC-2/China (Langreth, unpub.). Loss of knobs may reflect an altered pathogenicity of the parasite in vivo. These variants may be attenuated or more virulent. In any case, the existence of the knobless variants allows one to probe the role of the knobs in the induction of antibody, in the sequestration of older parasites from the peripheral circulation and in the protection from attack by white blood cells, particularly those in the spleen.

An erythrocytic stage malaria parasite is surrounded by two membranes. The outer one, the parasitophorous vacuole membrane or PVM, originates as an invagination of the erythrocyte membrane upon invasion by a merozoite. However, the parasite rapidly alters the PVM structurally and chemically. McLaren et al. (1977, 1979) have shown that the number and distribution of intramembranous particles (indicating proteins) are altered in the PVM. Langreth (1977) has shown that the polarities of red cell membrane enzymatic activities are modified in the PVM. Portions of the PVM are deposited into the host circulation upon rupture of a mature parasite and release of merozoites. Whether or not the PVM is antigenically distinct from the erythrocyte membrane is unknown. Even if PVM is not itself a suitable vaccine

antigen, it should be characterized because it is currently a significant contaminant of isolated parasites and parasite fractions. For instance, if PVM retains erythrocyte membrane properties, its undetected presence in experimental vaccines may induce autoimmune responses.

A commonly occurring alteration of malaria parasite-infected erythrocytes are the Maurer's clefts. In P. falciparum infections, the clefts appear in the red cell cytoplasm at the late ring stage, shortly before the appearance of knobs on the erythrocyte surface (Langreth et al., 1978a). These membrane-bound cisternae are often continuous with the parasitophorous vacuole membrane (PVM) from which they probably arise. McLaren et al. (1979) have found through freeze-fracture studies that Maurer's cleft membranes are structurally similar to the PVM. Maurer's clefts can extend into the erythrocyte cytoplasm from the PV to just under the knobs; they have been found in contact with knobs in rare cases (Langreth, unpub.). The contents of the clefts are morphologically identical to the contents of the parasitophorous vacuole. Maurer's clefts may serve to transport substances (such as perhaps knob material) between the parasite and the erythrocyte surface, but at this time information about their nature and function is lacking. Schüffner's dots, a related type of membrane-bound alteration of P. vivax infected-erythrocyte cytoplasm, bind fluorescein-labeled anti-malarial antibody (Tobie & Coatney, 1961). Maurer's clefts, like PVM, are released to the bloodstream when a mature parasite ruptures its host cell. Also like PVM, Maurer's clefts must be investigated not only for their possible antigenicity but also because of their potential undetected presence as contaminants in other more suitable antigenic preparations.

b. Parasite

The only time in the erythrocytic life cycle when the malaria parasite is outside a host erythrocyte and subject to direct immunological attack is at the merozoite stage. That blood stage parasite antigens, particularly merozoite, can induce protective immunity to *P. falciparum* malaria in *Aotus* monkeys has been well demonstrated (Mitchell *et al.*, 1977; Siddiqui, 1977; Reese *et al.*, 1978; Rieckmann *et al.*, 1978). (However, the antigen preparations in all these studies were heterogeneous and probably also contained knob antigens). Protective growth- and invasion-inhibiting antibodies to *P. knowlesi* merozoites have been found in immune sera of Rhesus monkeys (Butcher & Cohen, 1972; Miller *et al.*, 1975). Miller *et al.* (1975) further showed that immune serum agglutinated merozoites by binding their cell coats and caused reduction in invasiveness. By EM immunocytochemistry it was shown that merozoites bind antibodies from *Aotus* monkeys which are immune to falciparum malaria (Langreth & Reese, 1979). The interaction with antibody resulted in changes in texture and an apparent increase in the merozoite cell coat. Adjacent merozoites were cross-linked and agglutinated by their altered cell coats. Binding of labeled antibody was heavy on the coats of both homologous and heterologous strains of parasites, indicating that the merozoite surfaces of the tested strains share at least some common antigens. However, because the strains examined were both West African (FCR-3 and FCR-4), further experiments should be performed using strains and antisera from widely different geographic regions.

Critical analysis of the various merozoite surface and internal antigens has yet to be made. For instance, what are the relative

antigenic contributions of the merozoite coat and its plasma membrane after the coat is removed? Are there strain specific antigens and where are they located? Where are the common antigens? Are merozoite antigens (coat, surface, or internal) stable or do they undergo antigenic variation? Knowledge of this kind is essential for the development of a merozoite-based vaccine effective against all strains of P. falciparum which may be encountered in the field.

The ultrastructure and invasive behavior of P. falciparum merozoites are similar to P. knowlesi merozoites (Bannister et al., 1975; Aikawa et al., 1978; Langreth et al., 1978a,b). The cell coat of the merozoite is believed to be protein or glycoprotein (Miller et al., 1975; Bannister, 1977) although its nature has not been rigorously studied. The coat is also present in the merozoite buds of P. knowlesi schizonts (Bannister, 1977). Proteins which a cell synthesizes for transport to the cell surface or for export are usually processed by the Golgi apparatus, where they are frequently glycosylated. The Golgi apparatus of P. falciparum is most prominent during schizogony, when merozoites and their coats, rhoptries, and micronemes are being formed (Langreth et al., 1978a). By localizing and tracing products processed through the Golgi, one may gain information on how the parasite controls the production and distribution of (glyco)proteins in the merozoite cell coat, the parasitophorous vacuole, Maurer's clefts, and at the erythrocyte surface. Because many of these glycoproteins appear to be antigenic, they certainly warrant characterization.

P. falciparum's Golgi apparatus also appears to be involved in the formation of rhoptries and micronemes, the organelles which are believed to secrete substances which assist a merozoite in invading an erythrocyte.

These organelles may also help merozoites escape their old host at the end of segmentation by aiding in the lysis of the parasitophorous vacuole and the erythrocyte membrane. Little is known about the nature of rhoptry or microneme contents, other than that P. berghei rhoptries were negative for acid phosphatase (Aikawa & Thompson, 1971) and that P. lophurae rhoptries contain some histidine-rich protein, which may invaginate the erythrocyte membrane (Kilejian, 1976). This histidine-rich protein, the only purified malarial protein, was used to successfully immunize ducklings against P. lophurae infections (Kilejian, 1978). Although this particular protein is present in significant amounts only in P. lophurae infections in ducks, similarly unique and antigenic proteins may be present in rhoptries and micronemes of P. falciparum. Therefore, the composition of these organelles should be investigated.

4. General Experimental Design

The research to be conducted in this project will be in the following areas:

a. Localization and characterization of both surface and internal antigenic components in blood stage Plasmodium falciparum infections. Immunocytochemical techniques will be applied to the parasite and to the infected erythrocyte.

b. Cytochemical characterization of antigenic structures, particularly the merozoite surface and the knobs, but also Maurer's clefts, the parasitophorous vacuole, and internal merozoite components, such as rhoptries. Emphasis will be on glycoprotein characterization and the radioautographic tracing of components synthesized by the parasite and processed through its Golgi apparatus.

c. Structural analysis of the infected erythrocyte membrane with particular reference to the knobs by freeze-fracture and scanning electron microscope techniques.

d. Determination of the function of the knobs *in vivo* by comparative studies of knobby (K+) and knobless (K-) lines of *P. falciparum*.

e. Ultrastructural analysis of blood stage *P. falciparum* antigen preparations for quality, integrity, and purity.

f. Periodic monitoring by immunocytochemistry for changes (antigenic variation) in long-term cultured material.

5. Methods of Procedure

Plasmodium falciparum will be maintained *in vitro* in human erythrocytes by the methods of Trager & Jensen (1976). Two strains will be used primarily: FVO/Vietnam/Oak Knoll/FCR-1 and FMG/Gambia/FCR-3. Both of these strains have knobless variant lines.

a. Immunocytochemical localization and characterization of both surface and internal antigenic components in the parasite and infected erythrocyte.

I. Correlation of inhibition assay levels with antibody-reactive sites determined by EM immunocytochemistry.

Reliable *in vitro* assays for protective antibodies based upon inhibition of parasite growth and merozoite reinvasion are now being developed (Campbell *et al.*, 1979; Reese & Motyl, 1979). By correlation of the locations of bound antibodies as determined immunocytochemically with levels of inhibitory antibody as measured by these assays, the components of the parasite and infected erythrocyte which are antigenically important in immunity to malaria can be identified. Attempts will be made to correlate high, medium and low levels of growth and reinvasion inhibition with varying intensities and locations of surface and internal immunocytochemical reactivities among the different antisera tested. The activities of different

dilutions of antisera will also be examined with both assays.

Antisera will be obtained from Aotus monkeys made immune to malaria by infection followed by drug treatment or by immunization with blood stage antigens. Antisera will also be obtained from rabbits, which have been immunized with various antigenic malarial preparations, such as culture medium supernatant (Campbell, unpub.). Rabbit-produced antimalarial antibodies can be used in growth inhibition assays since P. falciparum can be cultured in human erythrocytes in rabbit rather than human serum (Rieckmann et al., unpub.). Monoclonal antimalarial antibodies produced by hybridoma techniques will also be used as immunocytochemical probes (see below).

To locate antibody reactive sites on parasite, especially merozoite, surfaces and on infected erythrocyte surfaces, an immunocytochemical sandwich technique will be used (Langreth & Reese, 1979). All sera will be heat inactivated at 56°C for 30 min. and absorbed against normal human erythrocytes before use. Unfixed or lightly fixed (0.1% glutaraldehyde, 10 min., 4°C) parasitized erythrocytes and/or merozoites will be washed 3 x in buffered RPMI 1640, resuspended in buffer at 6% cell density and incubated with the test antiserum for 10 min. at 4°C. The samples will then be washed again 3 x with buffer and incubated for 10 min. at 4°C with a second antiserum, which is antibody to the first(test)antiserum and is conjugated to the electron-dense marker ferritin. The cells are again washed 3 x, fixed and processed for electron microscopy. Controls will include incubation with normal serum, incubation with ferritin only, and incubation with only the second antiserum.

To locate internal antigenic components, such as the contents of the parasitophorous vacuole, Maurer's clefts, rhoptries, and other parasite structures, an immunocytochemical assay must be performed on sectioned material. The sandwich technique described above, using ferritin or peroxidase as marker, will be applied to ultra-thin sections of glycolmethacrylate- or polyethylene glycol-embedded cells. For ease of handling these sections will be mounted on gold or nickel grids. The sandwich technique of Roth et al. (1978), which uses protein A-gold as the antibody marker, will also be employed. This procedure is performed on etched Epon-embedded sections mounted on nickel grids and may give superior results.

II. Determination of strain-specific and/or common antigens.

Ideally, a vaccine will be produced using antigens that are common to all geographic strains of the parasite. Therefore, once antigenic components are identified, their strain specificity must be tested. These studies will initially use FVO (Asian) and FMG (African) strains of P. falciparum. Sera from animals immunized to strain FVO will be absorbed against strain FMG (infected erythrocytes and merozoites) and vice versa. Absorbed sera will then be used in immunocytochemical assays to detect strain-specific antigens. Localization with absorbed antisera will also be correlated with inhibition of parasite growth in vitro. These kinds of absorbed sera may not be applicable to detecting the specificity of internal antigens because of technical problems, but they will be suitable for surface antigens.

A second method for determining strain specificity which is applicable to all classes of antigens is the use of antimalarial monoclonal

antibodies. Monoclonal antibodies are produced by somatic cell hybrids (hybridomas) created by the fusion of a normal antigenically stimulated spleen cell (lymphocyte) and a myeloma tumor cell. Hydridomas enable one to obtain large amounts of specific antibodies from cells which can be maintained indefinitely in culture. Some antimalarial hybridomas have already been produced (Campbell, unpub.; Miller, unpub.; Taylor, unpub.). Because each hybridoma's antibodies are highly specific, they are extremely sensitive probes for strain-specific antigens.

III. Separation and testing of erythrocyte (knob) and merozoite surface antigens.

Because all previous "merozoite" preparations used to immunize monkeys against P. falciparum contained knob material, it is unknown whether one or both antigens are required for the development of immunity. The knobless variants of the parasite will be exploited in order to separate antibodies to these two components. Knob-specific serum will be produced by absorbing isolated knobless (K-) merozoites against immune serum. Antiserum free of anti-knob antibodies will be made by absorbing knob-bearing (K+) infected erythrocytes against immune serum. After checking the completeness of these absorptions by immunocytochemistry, these two sera will be used in the GARI assay to determine the relative roles of anti-knob and anti-merozoite antibodies in inhibition in vitro. It should be stressed, however, that even if knob-specific antisera fail to inhibit in vitro, knob antibodies may still play a role in vivo. One can only investigate the role of knobs in the cellular immune response in vivo.

Knob-specific antiserum could be examined *in vivo* for its ability to prevent or cure a malaria infection. However, other experiments involving scarce and expensive *Aotus* monkeys have a higher priority (see Section d.); but, if all immune sera from both human and monkey sources screened over the next several months are found to have high titers of anti-knob antibodies, *in vivo* testing with knob-specific antisera would become more important.

b. Cytochemical characterization of antigenic structures

I. Glycoproteins

These studies will focus on the knobs and the merozoite surface and coat, but Maurer's clefts, the parasitophorous vacuole, and internal merozoite components will also be examined. Successful application of cytochemical techniques enables one to label structures with visible markers. These markers can then be used as indicators of composition and/or purity of fractionated antigenic preparations. Cytochemical tests will be applied to both K+ and K- lines of *P. falciparum* and uninfected erythrocyte controls. In addition, variations as a consequence of life cycle stage will be examined.

General cytochemical tests for glycoproteins and polysaccharides will be used first. These will include ruthenium red and Alcian blue-lanthanum nitrate staining for surface glycoproteins (Dwyer *et al.*, 1974) and the thiocarbohydrazide-silver proteinate procedure of Thiéry (1967, 1969) for internal as well as surface glycoproteins. The Thiéry reactions are done on sections and detect aldehyde groups in polysaccharides which are exposed by periodic acid hydrolysis.

Localization of lectin receptors is a neglected area of malaria research. Although Seed & Kreier (1976) found that the parasite

"surface" had few lectin binding sites relative to the erythrocyte surface in P. berghei infections, they failed to distinguish between parasite plasma membrane and the parasitophorous vacuole membrane, and they did not examine merozoites or internal components. The techniques herein described are new and have not been applied to malaria before. The technique of Horisberger & Vonlanthen (1979) will be used on the surfaces of K+ and K- infected erythrocytes and merozoites. This procedure simultaneously localizes three lectin receptors (Concanavalin A, soya bean agglutinin, and wheat germ agglutinin), using different size gold granules as markers.

A second method for detecting lectin binding sites, which can be used to detect both surface and internal carbohydrate components (Gros et al., 1977), will be through the application of glycosylated cytochemical markers (Kieda et al., 1977; Monsigny et al., 1976a,b). Samples (cells or sections) are incubated first with a lectin and subsequently with a cytochemical marker (ferritin or peroxidase) which has been glycosylated and carries a sugar specific for the multi-valent lectin.

The following lectins will be tested:

Lectin	Binding Sugars
Concanavalin A	α-D-glucopyranose (+N-acetyl) α-D-mannopyranose (+N-acetyl)
Wheat Germ Agglutinin (WGA)	D-GlcNAc, GlcNAc-(1→4)GlcNAc
Soya Bean Agglutinin (SBA)	N-acetyl-D-galactosamine, D-galactose
Peanut Agglutinin (PNA)	D-galactose, neuraminidase
Kidney Bean Agglutinin (PHA)	-NANA-D-Gal(1→3,4)GlcNAcα(1→2)Manα(1→2)Man- N-acetyl-D-galactosamine

Lectins (Cont'd)

Lectin	Binding Sugars
Bandeiraea simplicifolia Agglutinin II (BSA II)	-2-acetamido-2-deoxy-D-glucose
Gorse Seed Agglutinin (UEA)	α-L-Fucose, di-N-acetyl-chitobiose
Solanum tuberosum Agglutinin (STA)	di-N-acetyl-chitobiose tri-N-acetyl-chitotriose

II. Enzyme cytochemical reactions

The erythrocyte membrane reactions for ATPase and NADH oxidase will be applied to *P. falciparum* to determine whether the parasitophorous vacuole membrane is altered by association with the parasite as it is in *P. lophurae* infections (Langreth, 1977). Infected and uninfected erythrocyte membranes will also be compared for their enzymatic activity. Material discharged from merozoites, rhoptries and micronemes may prove to be important antigenically. Therefore, standard cytochemical tests for lytic and secretory enzyme activities will be applied to malaria parasites in order to characterize these organelles (Novikoff *et al.*, 1971).

III. Radioautography

Because the synthetic machinery of mature erythrocytes is nonfunctional, the structural and chemical changes found not only in the parasite but also in the infected erythrocyte are undoubtedly produced by the parasite. Parasite-controlled synthesis and processing of materials can be confirmed directly by EM radioautography. These experiments must be done on synchronized infections. Cultures of K+ parasites will be synchronized by a double physiogel procedure (Nguyen-Dinh & Langreth, unpub.). An asynchronous culture is subjected to physiogel treatment. The top portion (75% mature forms) is

mixed with uninfected erythrocytes. Invasion of merozoites is allowed to occur for 12 hr., at which time a second physiogel separation is made. The bottom of this preparation, containing young rings, which are 6 ± 6 hr. old, is taken as the starting material of a "synchronous" culture and plated at 8% suspension. Starting parasitemias are about 3%, which is just sufficient for electron microscopy. Once synchronized, the parasites can be pulsed and pulse-chased with radioactive precursors at various life cycle ages and for various periods of time.

The cytochemical studies will indicate which sugars are predominant in the glycoproteins of antigenic structures (knobs, merozoite cell coat, parasitophorous vacuole, etc.). The incorporation of the appropriate tritiated sugars and their subsequent transport will then be investigated. These techniques have never been applied to malaria parasites, but reliable methods have been established for other cells (Michaels & Leblond, 1976; Whur et al., 1969). Since proteins for export are usually glycosylated in the Golgi, we expect to find label in that organelle after short pulses. By these experiments, we will determine where and when in the parasite life cycle proteins are glycosylated, by what structures they are transported, and whether they appear at external sites, such as knobs and cell coat. Information on the cytochemical nature and the time and place of synthesis will assist in the isolation and purification of these potential antigens or in their elimination from fractionated material.

c. Structural analysis of the infected erythrocyte membrane with particular reference to the knobs.

I. Freeze-fracture

Structural changes in the infected erythrocyte membrane will be examined by freeze-fracture/freeze-etch techniques. Attempts will be made to determine whether knob material is an integral part of the rbc membrane, i.e., a transmembrane protein. In addition, the particle composition and distributions in normal and K+ and K- infected erythrocyte membranes will be compared in order to determine whether other possible protein changes in the erythrocyte membrane as a result of infection by P. falciparum are expressed across the membrane. By using sorbitol-synchronized parasitized cells (Lambros & Vanderberg, 1979), correlation of the structural alterations found with life cycle stage will be attempted. Both FVO and FMG strains will be examined because of the possibility of interstrain differences.

II. Scanning electron microscopy

Scanning electron microscopy will be used to investigate interstrain differences in the size, number, distribution, and antigenicity of knobs at P. falciparum infected erythrocytes. The development of an immunocytochemical assay for scanning microscopy will be attempted. The antibody marker will be hemocyanin or gold. This assay would enable one to rapidly screen for anti-knob or other surface antibodies. Specific antibodies to surface components, such as those which may be produced by hybridomas would be particularly useful for comparing strains by SEM. The Taylor monoclonal antibody to malaria-infected erythrocyte surface will be tested in this assay.

d. Determination of the function of the knobs *in vivo*

The role of the knobs in sequestration and immunogenicity will be tested by comparing the behavior of infections of K+ and K- parasites of strain FVO of *P. falciparum* in *Aotus* monkeys. Strain FVO was chosen because, although it is chloroquine-resistant, it is well adapted to *Aotus* and its infections can be cured by appropriate drug treatment (Schmidt, 1978a,b). Monkeys will be karyotyped and grouped accordingly (Miller *et al.*, 1977). The minimum number of monkeys will be 6/experiment (3 K+ and 3 K-), but if splenectomies are to be done, more monkeys would be required per experiment.

Cloning of knobless (K-) will be necessary to obtain genotypically pure lines. This will be accomplished by dilution, micromanipulation, or single drop/mechanical cloning methods. K- variant clones will be examined by EM to be certain that each is 100% knobless and remains stable *in vitro*. K+ and K- lines will be transferred from human erythrocytes to *Aotus* erythrocytes *in vitro* as follows. Parasites in human red cells will be synchronized by sorbitol (Lambros & Vanderberg, 1979) and then allowed to mature. Mature forms (trophozoites, schizonts, and segmenters) will be concentrated by gradient procedures (Mrema *et al.*, 1979b) and subcultured with monkey erythrocytes. After cultivation for an additional 24 hr., virtually all the parasites will be in monkey erythrocytes. The knobless variant in *Aotus* erythrocytes *in vitro* will be examined by EM to make certain the K- trait is stable in the new host cell. Transferring a parasite strain from human to monkey erythrocytes *in vitro* avoids the sacrifice of a splenectomized monkey.

The behavior of the knobless variant *in vivo* is unknown: it may be attenuated or it may be more virulent or it may be identical to the

knob-bearing "wild" type in pathogenicity. The pattern, rate, and course of parasitemias of the K+ and K- lines will be monitored by Giemsa-stained blood films. Blood will also be examined by electron microscopy for the possible appearance of knobs in the K- variant. Aotus monkeys are too valuable to allow the infections to run their natural course. As soon as the outcome is obvious, animals will be cured by treatment with amopyroquin (20 mg/kg/day). If more than the minimum six monkeys per experiment are available, some animals will be splenectomized during the infections and their spleens examined histopathologically. Cured splenectomized monkeys will be added to a breeding colony. Cured intact monkeys will be rechallenged with K+ or K- parasites and will be used as sources of immune sera required for the experiments in Section a.

If K- does not revert to K+ in vivo, many questions will be answered about the role of the knobs. By sequestering mature parasitized cells, do the knobs protect from immune phagocytosis in the spleen? Are knobs necessary for sequestration of P. falciparum erythrocytic parasites? If K- parasites do sequester, is it in the same places as K+ parasites? Will monkeys survive K- infection or will it be more rapidly lethal? Are the antibodies to the knobs opsonizing in vivo so that leucocytes in the blood and spleen may select and phagocytize some knob-bearing cells? We have occasionally observed phagocytosis of opsonized infected, knobby erythrocytes in vitro (Langreth & Jensen, 1979). If anti-knob antibodies are opsonizing, the knobless variant, which binds no antibody on the infected erythrocyte surface, may be more virulent. Regardless of the outcome, information will be obtained about the function of the knobs and their relation to the host's immune response which will be essential in experimental vaccine testing.

The most critical *in vivo* experiment involving knobless "mutants" will be to determine whether immunization with K+ or K- merozoites provides equal protection against subsequent challenge with a "wild" type knob-bearing strain. If K- merozoites are found to be equally protective, knobless strains will be particularly valuable as a source of vaccine antigens because they grow more rapidly than K+ in culture (Motyl & Reese, unpub.) and because K- seems to be stable in long-term culture whereas K+ is not. If K- merozoites do not protect well, vaccine antigens may have to be prepared only from relatively short-term cultured material.

e. Ultrastructural analysis of blood stage P. falciparum antigen preparations

All previous blood stage antigen preparations, even those which were used successfully to immunize monkeys, were heterogeneous and contaminated with host cell material. No one can afford to continue to needlessly risk monkeys by exposing them to "dirty" antigens, containing such "undetected" debris. Contaminants such as red cell membranes and stroma can be particularly disastrous in an experimental vaccine preparation, because they may stimulate an autoimmune response. Other contaminants, such as broken residual bodies, may release lytic enzymes and degrade the important antigens. Because of its superior resolving power (0.4 nm), the electron microscope is a powerful, critical, and, at this time, essential tool for the assessment of antigen quality, integrity, and purity.

At a purely morphological level, the electron microscope can detect contaminants of small size or amount, such as bits of membrane, broken parasite material, hemozoin, hemoglobin (native or denatured), and red cell stroma. Whether merozoite cell coats are present or absent, whether or not rhoptry or microneme has been discharged, and whether or not membranes are intact can be determined easily at the ultrastructural level.

In addition, the ultrastructural immunocytochemical and cytochemical markers for specific components and organelles (which have been developed in sections a. and b.) can be applied to analyze the content and purity of a given antigen preparation. Once contaminants are identified, methods can be devised to eliminate them. Ultrastructural analysis of all antigen preparations fractionated from P. falciparum infections is, therefore, essential. Arrangements have already been established to perform such analysis on antigenic preparations provided by Dr. Karl Rieckmann and his collaborators and by Dr. William Trager and his collaborators.

f. Periodic monitoring by EM immunocytochemistry for changes (antigenic variation) in long-term cultured material

The appearance of knobless variants of P. falciparum after long-term cultivation can be considered an extreme case of antigenic variation (Langreth et al., 1979). Knobless was the first change observed and would not have been discovered without EM monitoring of strains. Other changes will probably occur and must be detected and analyzed. One must be certain that antigens isolated from laboratory strains of P. falciparum for use in experimental vaccines are stable with respect to corresponding antigens in strains found in the field. Every six months samples from continuously cultured strains (FVO and FMG) will be compared with cryopreserved parasites of the same strains which have cultured only briefly and with fresh isolates (maximum of 2 per year). In addition to immune Aotus serums, a series of specific antimalarial monoclonal antibodies will be used in immunocytochemical experiments to probe for antigenic changes. Antigenic components found to change their antigenicity in vitro will be inappropriate candidates for vaccine antigens, or it may be necessary to prepare vaccine antigens only from strains newly introduced into culture.

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D. Facilities and Resources

The Uniformed Services University of the Health Sciences (USUHS) provides an extremely rich environment in which to conduct malaria research because of its location adjacent to major Federal health research facilities. Close contact will be maintained with members of this malaria research community: Dr. Richard Beaudoin (Immunoparasitology, Naval Medical Research Institute), Colonel Carter Diggs (Immunology, Walter Reed Army Institute of Research), Dr. L.H. Miller (Parasitic Diseases, National Institutes of Health), Dr. Diane Taylor (Immunology, National Institutes of Health), and Dr. Michael Hollingdale (Biomedical Research Institute).

Collaborative links have been established with several members of the A.I.D.-supported malaria vaccine research network: Dr. William Trager and colleagues (Reese, Scheibel, Stanley), Dr. Karl Rieckmann and colleagues (Campbell, Mrema, Simpson), and Dr. Carlos Espinal. These arrangements will continue. Merozoite antigen preparations produced by Trager, Stanley, Rieckmann, and Mrema will be examined ultrastructurally under this proposal. Dr. Gary Campbell is making the hybridomas which will be utilized in Sections a,c, and f. If sufficient Aotus monkeys are unavailable in the U.S. to undertake the experiments on knob function in vivo, the work will be done in Colombia with Dr. Espinal.

The research program will be conducted in the Department of Microbiology, USUHS, School of Medicine. Available in the Department are an office for the principal investigator and at least 600 sq. ft. of laboratory and associated bench space, including a fume hood and air, gas, vacuum and electric lines. This laboratory is for general experimentation and to house equipment. Also available are an ultramicrotomy room, a photographic darkroom with a Durst enlarger, and space, including appropriate water and electric lines, for a transmission electron microscope. Essential equipment available at USUHS

includes critical point dryers, an automatic freeze dryer, JEOL 35 scanning electron microscope, a Balzers BAF 301 freeze-etch microtome, a vacuum evaporator with a rotating platform, and a cryostat. Animals will be housed at the centralized animal resources facility, under the direction of Dr. Richard C. Simmonds. The facility has had previous experience with Aotus monkeys.

The principal investigator, Dr. Susan G. Langreth, will spend 75% of her time on the project. Her academic appointment is Associate Professor in the Department of Microbiology. Dr. Langreth's curriculum vitae is appended to the proposal. Current research support for the principal investigator is as follows:

W.H.O. (T16/181/M2/17C), Membrane Interactions in Malaria; Principal Investigator; 30% salary + effort, 1979-81

A.I.D. Contract No. TA-C-1373, Large Scale Cultures of Human Malaria (P. falciparum) and their use for Immunization and for Antigenic Analysis of the Parasites; Dr. William Trager, Principal Investigator; 70% salary + effort, 1979-81.

Both grants are to the Laboratory of Parasitology at the Rockefeller University. The W.H.O. grant is not transferable and would terminate as soon as Langreth left Rockefeller.

BUDGET JUSTIFICATION

Personnel. Two technicians are requested. One technician would be responsible for electron microscope maintenance, ultramicrotomy, and photographic printing. The other technician would be involved with cultivation and general laboratory preparations. Salaries and fringe benefits are at current rates for USUHS. All of the principal investigator's salary will be provided by the University. USUHS does not currently charge overhead.

Consultant Fees. The need for expertise in malaria biochemistry is anticipated for advice on the development of cytochemical techniques from knowledge of parasite chemistry. The rate is $125/day for 4 days.

Travel- Domestic. This would be used for presenting experimental results at meetings, visiting other laboratories, and exchanging information and technic with colleagues.

Travel-Foreign. *Aotus* monkeys may not be available in the U.S. to do the proposed *in vivo* experiments. This would require that such experiments be peformed in Colombia in collaboration with Dr. Carolos Espinal (under a subcontract to Dr. Rieckmann's A.I.D. contract). One round trip coach airfare to Bogota and per diem for 10 days ($50/day) is budgeted.

Electron Microscope. The research in this proposal will require the full-time use of a modern transmission electron microscope. The existing facilities at USUHS (5 TEM's in the entire university) are insufficient to accommodate this increased demand. The initial A.I.D.-supported malaria vaccine project at the University of Illinois owned an electron microscope, but the instrument was s[illegible] to the University of Illinois when the project moved to New Mexico. An elect[illegible] microscope facility will benefit the A.I.D. network by providing a valuable technical tool which is available for collaborative studies with other research

laboratories within the malaria immunity and vaccination research program.

The requirements for an acceptable electron microscope are:

1) routine high resolving power

2) ease and speed of operation

3) contamination-free high vacuum

4) great electronic stability

5) reliability

6) ease of maintenance

7) rapid and reliable service and parts

8) proven and modern design to avoid discontinuation by company.

Several instruments fulfill these criteria: Hitachi H600, Philips 400, Zeiss 10, or JEOL 100 CX/SEG. The Philips 400 can be eliminated because of its excessive price. The Hitachi, Zeiss, and JEOL are similar in price, but the JEOL is superior in ease and reliability of operation. The JEOL 100 CX features modern, tested, and well-designed electronics and vacuum systems. The JEOL service organization has an outstanding reputation for speed and reliability. Therefore, the JEOL 100CX/SEG is the instrument of choice.

Ultramicrotome. This instrument is required for producing the ultra thin sections of Epon-embedded biological material for examination in a transmission electron microscope.

Centrifuge. The Sorvall RC3 is a refrigerated general purpose centrifuge which would be used heavily. With the omni-carrier, it has a large capacity and a top speed of 3000 rpm (2300 g). This speed will pellet merozoites as well as infected blood.

Light microscope. A compound microscope with superior optics is necessary to examine stained slides of *P. falciparum*. Therefore, Zeiss is specified.

A dissecting microscope is required for orienting specimens during embedding for electron microscopy and for trimming blocks.

Laminar flow biohazard hood and incubator. These are the major pieces of equipment required in order to culture *Plasmodium falciparum in vitro* in human erythrocytes.

Balance, pH meter, curing oven, refrigerator. This equipment is basic to any biological research laboratory.

Liquid nitrogen tank for cryopreservation. A means of storing various strains of *P. falciparum* not currently being used is essential for any laboratory doing culture work. Also, fresh isolates must be preserved for antigenic comparisons with long-term cultured material (Section f).

Darkroom developing equipment. In order to obtain consistent high quality results, photographs taken in the electron microscope must be processed under controlled conditions. The darkroom equipment needed includes a water temperature and pressure regulator, a nitrogen burst agitation system, and developing tanks and film racks.

Miscellaneous small items. This category is budgeted at $2000 for Year 1, $3000 for Year 2, and $3500 for Year 3.

Diamond knives. Diamond knives are absolutely essential for producing the consistently high quality thin sections which are required for good electron microscopy. Unfortunately, these knives are both expensive and grow dull with use. Because the number of times they can be recharged is limited, diamond knives must be considered consumable equipment. Diatome diamond knives are requested because, even though they are more expensive, their quality is far superior

to that of Dupont. Also, Dupont will guarantee only one resharpening of its product; Diatome guarantees five.

Rabbits. These animals will be used as sources of serum for the maintenance of *P. falciparum* in culture and for some of the immunocytochemical experiments in Section a.

Aotus monkeys. In order to locate and characterize the antigenic components in blood stage *P. falciparum* infections (Section a), the sera from monkeys immune to malaria is essential. The sera from immune and normal monkeys is also necessary in order to perform the immunocytochemical experiments outlined in Sections c and f. Immunization with K+ or K- merozoites and testing the behavior of K-infected erythrocytes *in vivo* (Section d) obviously cannot be done without monkeys. *Aotus* monkeys of Colombian origin will be purchased from South American Primates ($400/animal) and karyotyped by the New England Primate Center ($100/animal). They will be housed 2 to a cage (rack of 6 cages at $3600). Primate board is currently $400/animal per year. The budget in years 2 and 3 includes allowance for the board of monkeys purchased in previous years.

SUMMARY BUDGET

Category	Year 1	Year 2	Year 3	Total
Salaries	34,000	39,100	44,965	118,065
Fringe Benefits	8,500	9,775	11,240	29,515
Consultant Fees	500	575	660	1,735
Travel-Domestic	2,000	2,300	2,650	6,950
Travel-Foreign	1,500	1,725	1,985	5,210
Equipment	207,400	3,000	3,500	213,900
Supplies + Services	39,900	51,405	65,470	156,775
Publication Costs	1,200	1,380	1,590	4,170
	295,000	109,260	132,060	536,320

CURRICULUM VITAE

Susan G. Langreth

Personal History

Education

1962	B.A., Zoology	Wellesley College (Durant Scholar, General Honors, Phi Beta Kappa, Sigma Xi Associate)
1969	Ph.D., Biology	University of Chicago

Thesis Title: Studies on Cancer Crabs: I. Spermatogenesis; II. Satellite DNA's

Thesis Advisor: Hewson H. Swift

Graduate Support: National Science Foundation Predoctoral Fellowship, National Institutes of Health Traineeship in Cell Biology

Professional Experience

1966	Teaching Assistant, Invertebrate Zoology	University of Chicago, Department of Biology
1967	Teaching Assistant, Experimental Embryology	University of Chicago, Department of Biology
1968	Teaching Assistant, Cell Biology	University of Chicago, Department of Biology
1969-1970	Postdoctoral Research Associate	University of Pittsburgh School of Medicine, Department of Anatomy and Cell Biology
1970-1974	Research Associate	The Rockefeller University, Laboratory of Parasitology
1974-present	Assistant Professor	The Rockefeller University, Laboratory of Parasitology

Langreth, S.G.

Professional Societies

American Association for the Advancement of Science
American Society for Cell Biology
American Society of Tropical Medicine and Hygiene
New York Society of Electron Microscopists
New York Society for Tropical Medicine
Society of Protozoologists
Sigma Xi

Professional Service

Secretary-Treasurer, New York Society of Electron Microscopists (1976-1978)
Board of Directors, New York Society of Electron Microscopists (1978-1981)
Editorial Board, Experimental Parasitology (1979-1981)

Research Interests

Immunology of malaria
Membrane interactions of intracellular parasites
Host-parasite relationships in protozoan parasites (*Plasmodium*, *Babesia*, *Leishmania*, *Trypanosoma*)
Feeding, phagocytosis, endocytosis in protozoans
Electron microscope cytochemistry
Correlation between fine structure and function
Decapod gametogenesis

Research Support

World Health Organization Research Grant No. T16/181/M2/17(c), Principal Investigator, 1979-1981

Agency for International Development Research Contract No. Ta-C-1373, Co-investigator - Cell Biologist, 1976-present

Teaching Competence

Parasitology, protozoology, electron microscopy, cell biology, developmental biology, invertebrate zoology, histology

Langreth, S.G.

Publications

1. Langreth, S.G. 1965. Ultrastructural observations on the sperm of the crab Cancer borealis. J. Cell Biol. 27: 56A-57A. (Abstract)

2. Langreth, S.G. 1969. Spermiogenesis in Cancer crabs. J. Cell Biol. 43: 575-603. (Republished in The Meiotic Process)

3. Lynch, W.E., R.F. Brown, T. Umeda, S.G. Langreth, and I. Lieberman. 1970. Synthesis of deoxyribonucleic acid by isolated liver nuclei. J. Biol. Chem. 245: 3911-3916.

4. Trager, W., S.G. Langreth, and E.G. Platzer, 1971. Viability and fine structure of extracellular Plasmodium lophurae prepared by different methods. Program and Abstracts, Am. Soc. Parasitologists, 46th Annual Meeting. Abstract # 183.

5. Langreth, S.G., and W. Trager. 1972. Fine structure of Plasmodium lophurae developing extracellularly in vitro. Program and Abstracts, Am. Soc. Parasitologists, 47th Annual Meeting. Abstract # 17.

6. Langreth, S.G. 1972. Spermiogenesis in Cancer crabs. IN: The Meiotic Process: Pairing, Recombination and Chromosome movements. I. New York, MSS Information Corp. pp. 63-91. (Republication of J. Cell Biol. 43: 575-603)

7. Trager, W., S.G. Langreth, and E.G. Platzer. 1972. Viability and fine structure of extracellular Plasmodium lophurae prepared by different methods. Proc. Helminth. Soc. Wash., Special Issue, Basic Research in Malaria 39: 220-230.

8. Langreth, S.G. 1973. Plasmodium lophurae: host-parasite membrane cytochemistry. Progress in Protozoology, 4th International Congress on Protozoology. P. de Puytorac and J. Grain, eds. Clermont-Ferrand, France, U.E.R. Sciences Clermont, p. 242. (Abstract)

9. Langreth, S.G., and W. Trager. 1973. Fine structure of the malaria parasite Plasmodium lophurae developing extracellularly in vitro. J. Protozool. 20: 606-613.

10. Dwyer, D.M., S.G. Langreth, and N.K. Dwyer. 1974. Evidence for a polysaccharide surface coat in the developmental stages of Leishmania donovani: a fine structure-cytochemical study. Z. Parasitenk. 43: 227-249.

11. Langreth, S.G., and A.E. Balber. 1974. Ferritin uptake and digestion in Trypanosoma brucei: a fine structure study. J. Cell Biol. 63: 185a. (Abstract)

12. Langreth, S.G., and A.E. Balber. 1975. Protein uptake and digestion in bloodstream and culture forms of Trypanosoma brucei. J. Protozool. 22: 40-53.

13. Langreth, S.G., and W. Trager. 1975. Feeding mechanisms in extracellular Babesia microti and Plasmodium lophurae. J. Protozool. 22: 23A. (Abstract)

14. Langreth, S.G. 1975. Trophozoite growth in an intraerythrocytic malaria parasite, Plasmodium lophurae. J. Cell Biol. 67: 234a. (Abstract)

15. Langreth, S.G. 1976. Feeding mechanisms in extracellular Babesia microti and Plasmodium lophurae. J. Protozool. 23: 215-223.

16. Langreth, S.G., J.B. Jensen, and W. Trager. 1977. Fine structure of human malaria parasites from continuous culture. Fifth International Congress of Protozoology, S. H. Hutner, ed. New York, V Internat. Congress Protozool. Abstract #146.

17. Langreth, S.G., R.T. Reese, J.B. Jensen, and W. Trager. 1977. Development of human malaria parasites in continuous culture. J. Cell Biol. 75: 94a. (Abstract)

18. Langreth, S.G. 1977. Electron microscope cytochemistry of host-parasite membrane interactions in malaria. Bull. W.H.O. 55: 171-178.

19. Langreth, S.G., J. B. Jensen, R.T. Reese, and W. Trager. 1978. Fine structure of human malaria in vitro. J. Protozool. 25: 243-252.

20. Langreth, S.G., and R.T. Reese. 1978. Antigenicity of the infected erythrocyte surface in falciparum malaria. J. Cell Biol. 79: 89a. (Abstract)

21. Langreth, S.G., P. Nguyen-Dinh, and W. Trager. 1978. Plasmodium falciparum: merozoite invasion in vitro in the presence of chloroquine. Exp. Parasit. 46: 235-238.

22. Reese, R.T., S.G. Langreth, and W. Trager. 1979. Isolation of stages of the human parasite Plasmodium falciparum from culture and from animal blood. Bull. W.H.O. (In Press)

23. Langreth, S.G., R.T. Reese, M.R. Motyl, and W. Trager. 1979. Plasmodium falciparum: loss of knobs on the infected erythrocyte surface after long-term cultivation. Exp. Parasit. 48: 213-219.

24. Langreth, S.G., R.T. Reese. 1979. Antigenicity of the infected-erythrocyte and merozoite surfaces in falciparum malaria. J. Exp. Med. 150: 1241-1254.

Attachment B
L.P.A.

BEST AVAILABLE DOCUMENT

ENVIRONMENTAL THRESHOLD DETERMINATION

TO: AA/DS, Mr. Sander Levin

THRU: DS/PPU, Mr. John Cumming

FROM: DS/H, Lee Howard, M.D.

SUBJECT: Environmental Threshold Determination

Project Title: Malaria Immunity & Vaccination
Project #: 931-0453
Specific Activity (if applicable) _____
REFERENCE: Initial Environmental/Examination (IEE) contained in Smith to Howard memo dated January 24, 1978

On the basis of the Initial Environmental/Examination (IEE) referenced above and attached to this memorandum I recommend that you make the following determination:

X 1. The proposed agency action is not a major Federal action which will have a significant effect on the human environment.

_____ 2. The proposed agency action is a major Federal action which will have a significant effect on the human environment, and:

_____ a. An Environmental Assessment is required; or

_____ b. An Environmental Impact Statement is required.

The cost of and schedule for this requirement is fully described in the referenced document.

_____ 3. Our environmental examination is not complete. We will submit the analysis no later than _____ with our recommendation for an environmental threshold decision.

Approved: _____
Sander M. Levin

Disapproved: _____

Date: _____

Health and Population

Ultrastructural and Cytochemical Analysis of Malaria Antigens (New) - Uniformed Services University of the Health Sciences. Duration of project, 3 years; total estimated cost, $537,000.

Project Summary and Background: This is a new research project which has been proposed by one of the more talented members of the team which made one of the most significant breakthroughs to date in malaria research. The researcher, who is moving her base of operation to the Uniformed Services University (The Armed Forces Medical College) in the suburbs of Washington, is a highly qualified electron microscopist, the only one in the A.I.D. malaria network. She would provide services in her specialty to other members of the network as well as conduct her own research.

The objective of the research is to locate those components of the parasite Plasmodium falciparum and its altered host cell which are important in immunologic protection against malaria. These components, which may be candidate antigens for a vaccine, would then be characterized structurally, cytochemically, and immunologically. Assays to determine the purity of antigen preparation will also be developed.

The project was reviewed and approved without modification by members of the interbureau technical program committee on health.

RAC Discussion: The RAC noted the unique qualifications of the researcher and her importance to the malaria network. The project was considered to be essential to the development of a vaccine. Discussion of the proposal was entirely favorable.

RAC Recommendation: That the proposal be approved as requested.

Related Research Projects Funded by AID: This project is a part of an AID malaria research program consisting of ten projects carried out by a network of eight institutions. The network was established by AID on the advice of RAC to avoid duplication of work and to coordinate the various research components toward the accomplishment of a single well defined goal, i.e. the development of a vaccine against human malaria. The projects in the malaria research network and the institutions responsible for them are listed below:

In-vitro Production Methods of Human Malaria Parasites (New York University)

In-vitro Production of Gametocytes (New York University)

Evaluation of Blood Stage Antigens (University of New Mexico)

Cultivation of Human Malaria Parasites (Rockefeller University)

In-vitro Cultivation of Malaria Parasites (University of Hawaii)

Merozoite Antigen Vaccine Research and Development (Parke-Davis Co.)

In-vitro Cultivation of Malaria Parasites (Gorgas Memorial Institute)

In-vitro Cultivation of Malaria Parasites in Cell Culture (Parke-Davis Co.)

Primate Studies in Central and South America Contributing to Development of a Malaria Vaccine (Instituto Nacional de Salud - Bogota, Colombia)

Antigenic Analysis of Cultured *Plasmodium falciparum* (Michigan State University)

Isolation and Evaluation of Ma' .a Immunogens (University of Missouri)

USGHS

ACTION MEMORANDUM FOR THE ADMINISTRATOR

THRU: ES

FROM: AA/DS, Sander Levin

Problem: Your approval is requested for the implementation of eleven research projects along the lines of recommendations of the AID Research Advisory Committee (RAC).

Discussion: We are submitting for your approval eleven research projects following the recommendations of the RAC which reviewed the project proposals at its meeting on June 17-18. Five proposals are for new projects and six proposals are for extension of ongoing activities. The recommendation relating to a twelfth project ("The Effect of Insect Pathogens on the Ability of Mosquitos to Transmit Malaria") is a follow-on of the March RAC meeting. It is included for your information only since you approved the project earlier.

All eleven projects are included within the program activities described in the FY 80 Congressional Program Presentation and all have been approved by the regional bureaus. A list of the projects and funds proposed for their implementation is attached (TAB A).

Appended at TAB B are for each project: a summary of the project with a brief statement of the substance of the RAC discussion, the RAC recommendation, (potential) utilization of project results and information on related research funded by AID.

In brief, the RAC endorsed two projects as proposed and recommended approval of nine projects subject to specified modifications and conditions. We basically agree with the RAC recommendations and shall incorporate them into the Project Authorizations (PAFs) for my signature if you give your approval. In the case of the tissue culture project (Colorado State University) we are proposing to modify the RAC recommendation for a three-year approval at $500,000 (vice the $708,000 originally proposed) to make a three-year authorization with a two year funding at $500,000, with an evaluation after a year by an expert panel including RAC representatives to determine the future course of the project.

The Initial Environmental Examinations (IEEs) for the eleven recommended projects have been completed and threshold determinations have been made that none of these projects will have a significant effect on human environment.

Your approval of the eleven projects listed at TAB A constitutes the authorization to initiate negotiations of contracts pursuant to the RAC recommendations in accordance with applicable provisions of AID Procurement Regulations and Procedures and as required by PD-47 (Supp. A 2HB1, September 1972).

Recommendation: That you approve the implementation of eleven RAC endorsed projects listed at TAB A.

APPROVED: _____

DISAPPROVED: _____

DATE: _____

Attachments:

TAB A - List of projects and funds proposed for implementation

TAB B - Project summaries with RAC recommendations

TAB C - RAC statement regarding the Auditor General's Report on the Washington University research project

Clearances:

GC:NHolmes		Date _____
GC/THFA:ARichstein		Date _____
AA/PPC:AShakow		Date _____
DS/DAA/PN:EBeard	DRAFT	Date 7/15/80
DS/DAA/HRD:SJoseph	DRAFT	Date 7/9/80
DS/PO:IChaynick	DRAFT	Date 7/3/80

DS/PO/RES:MRechelgl:cev:7/02/80:X59011