931045318490 1 PD-AAG-846 931-0453.18

AGENCY FOR INTERNATIONAL DEVELOPMENT
931045318580 (attached)
PROJECT DATA SHEET

1. TRANSACTION CODE: A (A = Add, C = Change, D = Delete) — Amendment Number 1 — DOCUMENT CODE 3

2. COUNTRY/ENTITY: DSB Interregional

3. PROJECT NUMBER: 931-0453.18

4. BUREAU/OFFICE: DS/HEA — 10

5. PROJECT TITLE (maximum 40 characters): Malaria Immunity and Vaccination (Mich.)

6. PROJECT ASSISTANCE COMPLETION DATE (PACD): MM 04 DD 30 YY 84

7. ESTIMATED DATE OF OBLIGATION (Under 'B.' below, enter 1, 2, 3, or 4)
A. Initial FY 81 B. Quarter 2 C. Final FY 83

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

A. FUNDING SOURCE	FIRST FY 81 B. FX	C. L/C	D. Total	LIFE OF PROJECT E. FX	F. L/C	G. Total
AID Appropriated Total	119		119	302		302
(Grant)	(119)	( )	(119)	(302)	( )	(302)
(Loan)	( )	( )	( )	( )	( )	( )
Other U.S. 1.						
Other U.S. 2.						
Host Country						
Other Donor(s)						
TOTALS	119		119	302		302

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) DS/HEA	511	542				302		302	
(2)									
(3)									
(4)									
TOTALS						302		302	

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)

The purpose of this project is do identify, isolate, and characterize naturally elaborated soluable exoantigens in the exhausted culture medium used to grow P. falciparum.

14. SCHEDULED EVALUATIONS
Interim MM 04 YY 82 MM 04 YY 83 Final MM 12 YY 83

15. SOURCE/ORIGIN OF GOODS AND SERVICES
[x] 000 [x] 941 [ ] Local [ ] Other (Specify)

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

BEST AVAILABLE DOCUMENT

17. APPROVED BY
Signature: John Alden — May 14, 1981
Title: Director, Office of Health
Date Signed: MM DD YY

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

Project Malaria Immunity and Vaccination, New Sub-Project with Michigan State University

Project Number: 931-0453.18

I approve the total level of A.I.D. appropriated funding planned for this project not to exceed three hundred eight thousand two hundred fifty-two dollars ($308,252), including the funding authorized above during the period FY 1981 through FY 1983, subject to the availability of funds and in accordance with A.I.D. allotment procedures.

Bernard Chapnick

Acting AA/DS, Bernard Chapnick

5/27/81

Date

Attachments:

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

Clearances:

DS/HEA, J. Alden HPD for CP Date:
DS/PO, M. Rechcigl [illegible] for Date: 5/26/81
DS/PO, A. Morales [illegible] Date: 5/26/81

MAY 26 1981

ACTION MEMORANDUM FOR THE ACTING ASSISTANT ADMINISTRATOR
FOR DEVELOPMENT SUPPORT

FROM: DS/HEA, Clifford Pease

PROBLEM:

Approval of a PAF for negotiation of a contract with Michigan State University under Project 931-0453, Malaria Immunity and Vaccination.

DISCUSSION:

This research activity was reviewed and approved at the March 3-4, 1981 meeting of the Research Advisory Committee. With reference to RAC provisions, A.I.D. technical staff has satisfactorily reviewed the budget with the proposed investigator and as it always has been, the project is entirely focused on exoantigens (see page 3 of the proposal). Copies of the RAC recommendation and the Action Memorandum for the Administrator are attached.

Funds for the activity are included in the FY 1981 Congressional Presentation and the FY 1981 OYB for the Office of Health. The proposal has been reviewed and approved by the folowing:

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 A.I.D. PR 78-4.

The contract will, therefore, be awarded to Michigan State University without consideration of other sources.

RECOMMENDATION:

That you sign the attached PAF.

Clearance:

DS/PO, A. Morales _____ date 4/24/81

A. TITLE PAGE

1. NAME AND ADDRESS OF ORGANIZATION:

   Department of Microbiology and Public Health
   Michigan State University
   East Lansing, Michigan 48824

2. TITLE OF PROPOSED RESEARCH:

   Antigenic Analysis of Cultured *Plasmodium falciparum*

3. NAME AND TITLE OR PRINCIPAL INVESTIGATOR:

   James B. Jensen, Assistant Professor

4. DATE OF SUBMISSION:

   1980

   PERIOD FOR WHICH PROPOSAL IS VALID:

   April 1, 1981 through March 31, 1983

5. DESIRED STARTING DATE:

   April 1, 1981

   DURATION OF PROPOSED PROJECT:

   3 years

6. The undersigned agrees to accept responsibilities for the scientific and technical conduct of the project and for provision of required progress reports if a grant (contract) is awarded as the result of this application.

January 12, 1981
Date

James B. Jensen
Principal Investigator

7. Human Experimentation Plan for this proposal has been reviewed and approved by:

J F Bruderle
Institutional Ethics Committee

on 9-10-79.
Date

8. JAN 20 1981
Date

Stephen H. Terry
STEPHEN H. TERRY
Assistant Vice President for Finance

B. RELEVANCE OF PROPOSED WORK TO A.I.D.

1. Statement of the problem

Falciparum malaria continues to rank among the major infectious diseases with a world incidence of well over 200 million cases per annum. Africa alone accounts for nearly 1/2 of these cases with an annual mortality of 1 million, principally among children under 14 years of age (WHO Tech. Rep. Ser. No. 537, 1974, p. 9). The disease is caused by a protozoan parasite, *Plasmodium falciparum* (Apicomplexa), that infects the human erythrocyte and is transmitted by mosquitos of the genus *Anopheles*. Of all the parasitic diseases, malaria is not only the greatest killer but is also one of the most widely spread. Approximately 2,015 million people live in areas where malaria has either been eradicated or where control measures are being attempted. Some 343 million people live in endemic areas where no specific programs of protection are being developed. The majority of these people live in Africa. (Unpublished data, WHO Special Programme in Tropical Diseases, TDR.WP/76.6). Alarming as these figures are, the most disturbing aspect is the major resurgence of malaria occurring in areas of the world where control of the disease was nearly successful, namely in Sri Lanka, India, Pakistan, Central and South America. This resurgence of malaria, and the failure to consolidate the gains made against malaria in control programs is due, in part, to the emergence of strains of anophelene mosquitoes that are increasingly resistant to insecticides, chiefly the chlorinated hydrocarbons, but also the organophosphates. Another development is the spread of strains of *P. falciparum* that are resistant to antimalarial drugs (WHO Tech. Rep. Ser. No. 529, 1973, pp. 30-54). In view of this disheartening epidemiological situation, new measures for the control of malaria are obviously needed.

Among the new measures is a major effort for the development of a malaria vaccine. For decades, the hope of a malaria vaccine was frustrated by the failure to grow the etiological agent, *P. falciparum*, in culture. This obstacle was finally overcome by the work of Trager and Jensen (1976), who succeeded in growing a Southeast Asian strain of *P. falciparum* in continuous culture. Since their initial success, numerous other strains have been established *in vitro* (Jensen and Trager, 1978; Nguyen-Dinh and Trager, 1980; Chin and Collins, 1980; and per. comm. from others). Their success has stimulated considerable interest in identifying antigens from cultured parasites that may elicit a protective immune response. Initially, we believed, on good evidence, that the merozoite was the developmental stage that contained antigens most likely to be protective (Richards et al., 1977; Mitchell et al., 1977; Mitchell, 1977). However, recent experimental results by Reichmann et al. (1979) suggest that considerable analysis of all the parasite antigens must be conducted before we decide which ones are the best candidates for a vaccine. Within the A.I.D. network of research laboratories working on a malarial vaccine are several that are working to determine the best parasite stages from which to isolate protective antigens. Of these none, to my knowledge, are presently working on the soluble exoantigens - here defined as those parasite antigens that are naturally soluble (as opposed to those solubilized by using surfactants) and which are released into the supernatant fluid of *in vitro* cultures of *P. falciparum*.

2. Potential utilization of research results

Results obtained from research outlined in this proposal should extend our understanding of the antigenic composition of cultured *P. falciparum*, particularly of the soluble exoantigens. Such knowledge should facilitate the development of a malaria vaccine by identifying antigens that may elicit a protective immune response

which is the primary goal of this proposal. Increased understanding of parasite antigens may lead to refined serological techniques that should facilitate the successful monitoring of field trials of any vaccine developed.

3. Developing nation participation in research plan

In conjunction with an NIH sponsored project on the emergence of strains of *P. falciparum* resistant to chloroquine and other 4-aminoquinolines in the Sudan, Africa in which Dr. Jensen is the principal investigator, antiplasmodial serum will be collected from individuals functionally immune to malaria. This antiserum will be used as an aid in the identification of protective soluble antigens from *P. falciparum* cultures. This NIH project is being conducted in collaboration with the Sudanese Ministry of Health. Details of this project and the opportunities it presents with regard to the present proposal will be outlined in following sections.

4. Environmental effects of proposed research

None.

5. Effects on population growth, etc. if research results are implemented

There will be no direct effects on population growth, status of the poor, role of women, energy requirements or human rights as a result of the research of this proposal. The eventual development of an effective malaria vaccine may have far ranging effects on population growth and status of the poor in developing nations. However, a discussion of these would be strictly speculative at this time.

C. SCIENTIFIC ASPECTS OF PROPOSED WORK

1. Specific objectives

a. Primary aims

1) To identify, isolate and characterize "naturally elaborated" soluble exoantigens in the exhausted culture medium used to grow *P. falciparum*.

2) Identify and characterize soluble exoantigens from cultured *P. falciparum* that may play a role in protection against malaria, and thus may be a vaccine candidate by:

- immunizing rabbits, guinea pigs and *Saimiri* monkeys with cultured antigen preparations and using the resulting antiparasite serum in an *in vitro* parasite development inhibition assay;
- reacting soluble parasite antigens derived from cultured *P. falciparum* with serum obtained in Sudan from individuals functionally immune to malaria to determine which antigens may neutralize the inhibitory activity of the serum.

3) Using electron microscopic immunolabeling techniques, determine the subcellular position of those antigens that show promise as potential vaccine candidates.

2. Rationale, assumptions, theories and hypotheses, and likelyhood of success

It will be essential to analyze and characterize the antigens of P. falciparum obtained from continuous cultures of this organism before a vaccine against malaria can be developed. Soluble antigens can be roughly grouped into three catagories - (a) naturally soluble parasite antigens that may be present in the plasma of infected individuals (or culture medium) resulting from biologic activities of the parasite, loosely termed "exoantigens," such as the rhoptry contents supposedly secreted during penetration, or the merozoite surface coat sloughed as the parasite slips through the constricted ring formed during endocytosis; (b) antigenic elements released as waste products of schizogony, during segmentation, or from autolysed moribund merozoites, from the residual bodies or degenerating infected erythrocytes; or (c) particulate antigens (parasite membranes, or subcellular organelles, etc.) that are "solubilized" experimentally by surfactants. Several laboratories working on the USAID sponsored malarial vaccine program are presently working on studies dealing with the "particulate" parasite antigens such as whole merozoites, merozoite membranes, or "solubilized" parasite antigens. The studies proposed here will examine only the "exoantigens," i.e., those which appear in the supernatant fluid from cultures of P. falciparum or those aquously soluble parasite antigens readily released from parasites by freeze-thaw or other mild disruptive methods. Supposedly, these soluble antigens would approximate those to be found in the plasma of malarious patients.

Although the overall goal of the USAID sponsored malaria research is a human malaria vaccine, the present approach of immunizing Aotus monkeys, while only a model, may not identify antigens that will also protect humans. It is possible that the Aotus is not genetically equipped to recognize the falciparum antigens in a manner homologous to man and that an Aotus malaria vaccine would not work in humans. For this purpose it is proposed that for this project, human sera and lymphocytes, collected from persons functionally immune to malaria be used to aid in identifying potentially protective antigens derived from cultures. Comparison of antigens recognized by human antisera and immune lymphocytes with rabbit and monkey developed antisera will help determine the soundness of using these animals models in malaria vaccine studies. Furthermore, culture derived parasite antigens which can neutralize the inhibitory activity of human immune serum in P. falciparum cultures would naturally be a possible vaccine candidate. Finally, there simply are not enough Aotus monkeys available to conduct all the studies that will be necessary to develop a malaria vaccine unless much of the analytical work be done in vitro using anti-parasite sera derived from a source other than Aotus. In the course of my NIH sponsored investigation on the emergence of drug resistant strains of P. falciparum I will be collecting P. falciparum parasites and immune human sera throughout the Sudan, Africa's largest country. Thus with no additional expense to A.I.D. I will have access to human antiplasmodial serum.

The prospects for success of this project is good because it does not require the development of new techniques but only the systematic application of well characterized methodologies. The former hinderance of this type of project was a lack of large quantities of malarial antigens obtained from culture. This obstacle has now been overcome. Presently I have in my laboratory several cultured strains of P. falciparum from Africa (Gambia, Nigeria, and Kenya), Asia (Vietnam, The Peoples' Republic of China) and Central America (Honduras). In addition, I have recently developed several cloned lines of the Honduras derived strain and have received, through the kindness of Dr. Theodore Green, The University of Missouri, three cloned lines, C3, C5 (K+) and C6 (K-) of the FCR_3 (Gambian) strain. I am particularly encouraged by the results of preliminary experiments I have already conducted that will be described in detail in Section C.3.

3. Literature review

For many decades the hope of a malaria vaccine was frustrated principally by the failure to grow the etiological agent of malignant malaria, P. falciparum in culture. This major obstacle was overcome by the work of Trager and Jensen (1976), who succeeded in growing a Southeast Asian strain of P. falciparum in continuous culture. Since their initial success, several other strains have been established in vitro (Jensen and Trager, 1977; Siddiqui, 1979; Nguyen-Dinh and Trager, 1979; Chin and Collins, 1980 and per. comm. from others). The techniques for cultivation have been refined, simplified, and disseminated to many laboratories in the USA, United Kingdom, The Peoples' Republic of China, Nigeria, Sudan, Brazil, Columbia, France, Switzerland, Israel, Denmark, Canada, Thailand, India, and Malaysia. These laboratories, and others, have used the cultures to further studies in immunology, biochemistry, ultrastructural development, pharmacology, biology, and physiology of these parasites. To list all of the references on these studies would be exhaustive, but with regard to the present proposal, parasites from cultures have been used to make a crude vaccine that in Aotus trivirgatus monkeys was sufficiently immunogenic to offer encouragement that a malaria vaccine might be possible (Reese et al., 1978). Although not derived from continuous cultures, P. falciparum, merozoites from human (Mitchell et al., 1977) and Aotus (Siddiqui, 1977) infections were even more successful in protecting Aotus monkeys from usually fatal infections. In addition, Siddiqui's studies have demonstrated that adjuvants more acceptable than Freund's complete adjuvant for use in humans may be used.

Despite these encouraging results, much work needs to be done before even an experimental vaccine can be developed. The etiologies of many pathogenic sequelae of malaria are not known. Among these, several are potentially relevant to the production of a vaccine to malaria from cultured parasites. For example, it has long been recognized that an episode of malaria produces an anemia far in excess of that which can be accounted for by parasitic destruction of erythrocytes (McGregor, 1971). Many mechanisms for this phenomenon have been postulated (Holz et al., 1977) but one persistant hypothesis is that an autoimmune reaction is responsible (Zuckerman, 1964, 1977; Musoke et al., 1977). Preliminary experiments conducted by R. Reese and me at the Rockefeller University have shown that antigen preparations, taken from cultures of P. falciparum, contaminated with erythrocyte membranes, produced a fatal hemolytic anemia in Aotus monkeys after they were challenged with viable parasites (Reese, Jensen and Trager, unpublished). Electron microscopic examination of these preparrations showed them to contain a large number of erythrocyte membrane fragments, most of which displayed the "knob-like" surface alterations characteristic of P. falciparum infected cells (see Langreth et al., 1978 for detailed discussion). These altered red cell membranes were shown to be highly immunogenic (Kilejian et al., 1977; Langreth and Reese, 1979) and thus, we purposely did not attempt to remove them from our antigen preparation with the rationale that perhaps immunologic responses in the vaccinated monkeys might specifically destroy the infected erythrocytes because of the antigens present on their surfaces. However, soon after the immunized animals had been challenged with viable parasites, their hematocrits began to drop precipitously, producing rapidly fatal hemolytic anemia. The hematocrits of the immunized monkeys were measured at 4% and 6% respectively, on the day they died.

That the "knob-like" alterations on the contaminating erythrocyte membrane fragments were causually related to the fatal anemia is conjectural, but suggestive, especially in view of the fact that subsequent antigen preparations, in which most of these membranes were removed by centrifugation in a density gradient, produced less profound, but still serious anemias in the immunized monkeys (Reese et al., 1978).

The non-immunized control monkeys did not receive human erythrocyte membranes and thus the anemia seen in the immunized monkeys may have been due to the contaminating erythrocyte membranes in the antigen preparation. On the other hand, malaria infections always cause a significant drop in hematocrit which is greater than can be accounted for by direct parasite destruction of the red cells. Thus, unrefined antigen preparations from cultures of P. falciparum probably contain elements that elicit, or initiate a sequential series of events leading to hemolytic anemia in Aotus monkeys. Support for this hypothesis comes from the studies of Facer, (Facer et al., 1979; Facer, 1980a; Facer, 1980b) who has detected Coomb's antibody associated with the erythrocytes of anemic children with past or present infections of P. falciparum. Facer stated that the nature of the antigens on the surface of the erythrocytes that bound the immunoglobulins was not known but may have been schizont derived. An alternative hypothesis is that the antigenic knob material, which is sloughed from the schizont containing erythrocytes (Langreth et al., 1978), and which is unusually "sticky," forming agglutinated masses in infected-erythrocyte membrane preparations (Kilejian, per. comm.), may adhere to uninfected red cells, sensitizing them and making them susceptible to immune lysis. Further support for this hypothesis comes from the observation that inoculations containing knob contaminated membranes do not appear to produce anemia, but once such an immunized animal becomes infected, at the time of challenge, the growing parasites produce knob material which, when sloughed from the infected cells, becomes associated with uninfected erythrocytes that are subsequently attacked by "anti-knob" antibody which may then fix complement or act as opsonins leading to destruction of uninfected erythrocytes.

Secondly, malarial infections produce a marked hypergammaglobulinanemia of which only a small portion is protective antibody. Much evidence has been gathered to show that part of the immunologic activity is actually counter-productive, producing immunosuppression instead of immunity (McGregor, 1972; Salaman et al., 1969; Williamson and Greenwood, 1978). It is therefore reasonable to assume that among the vast mixture of biologic molecules to be found in cultures of P. falciparum are the immunogens, which may produce protection against malaria, as well as others which are benign, and yet others which are pathogenic and immunosuppressive. To produce an effective vaccine against malaria, it is imperative that the major antigenic molecules from P. falciparum cultures be identified and the immunoprotective ones be separated from these which are deleterious. In the process of doing the analysis, it would be essential to develop means of assaying for those unwanted elements to insure a means of determining the purity of the final product. Thus, analysis of the antigens from continuous cultures of P. falciparum is an important step in the development of a malaria vaccine.

During the past decade, studies on soluble plasmodial antigens have been conducted on rodent (Spira and Zuckerman, 1966; Hamburger and Zuckerman, 1977), avian (Smith et al., 1969), and human (Wilson et al., 1969; McAlister, 1972) malarial parasites. Much work has been done on antigens from P. falciparum using soluble antigens extracted from malaria infected human placentae and antisera collected primarily from West Africans immune to malaria (McGregor et al., 1968; McGregor and Wilson, 1971; Wilson and Williams, 1973; Wilson and Bartholomew, 1975; Wilson, McGregor and Hall, 1975; Wilson, 1980). Wilson, McGregor, and others in a series of reports have classified these soluble antigenic extracts according to their temperature sensitivities. The L-antigens (La^1, one antigen; La^2, several antigens; and Lb, three antigens) are labile to heating to 56°C; the R-antigens are stable at 56°C but not at 65°C, and the S-antigens are stable to heating at 100°C. The latter are not only serologically diverse but are different in successive parasitic episodes (Wilson et al., 1973). It

has also been shown that the S-antigens are often found in the sera of acutely infected individuals, but that these antigens disappear as the homologous antibody titers rise (Wilson et al., 1969). The L-antigens are not so readily demonstrable in sera of infected individuals but antibodies to them are (McGregor et al., 1968).

Using a short term culture technique, Wilson (1974) pulse labeled growing parasites with radioactive amino acids and determined autoradiographically that the La, Lb, and R-antigens contained the radio-label but that the S-antigen did not, suggesting that the former were of parasitic origin but the latter may have been of host origin. Although some of these soluble antigens have been characterized according to their temperature sensitivities and their physiochemical separation characteristics using Sephadex and DEAE cellulose chromatography, little is known about the roles these antigens play in the immunity or immunopathology of malaria (Wilson et al., 1973). Recently, Wilson (1980) has shown evidence that the heat-stable S-antigen may be useful for serotyping of P. falciparum.

4. General experimental design and time phased plan-of-work

Generally, soluble antigens, obtained either as exoantigens from the exhausted culture medium, or from extracts of specific parasite stages, will be used to immunize coccidia-free rabbits, guinea pigs or Saimiri monkeys. Antiparasite sera from these immunized animals will be used to identify parasite antigens using procedures outlined in Section C.5. These soluble antigens will be isolated and purified using physio-chemical methods such as column chromatography, DEAE ion-exchange chromatography, and preparative electrophoretic methods such as disc-gel electrophoresis and isoelectro-focusing. They will also be separated using immuno-electrophoretic methods such as dissecting specific antigen-antibody bands from crossed-electrophoresis gels. If applicable, then comparisons will be made between soluble antigens obtained from strains of P. falciparum isolated and maintained in vitro in my laboratory from different geographical regions. Presently, I have strains from Vietnam (FCR_1), Gambia (FCR_3, FCR_4), Nigeria (FCN_1), West Africa-location unknown (FCR_8), Kenya (FCR_7), The People's Republic of China (FCC_1, FCC_2) and Honduras (H_1). Accordingly, the antigenic diversity will be examined among widely dispersed strains, i.e., Africa, Central America and China as well as geographically associated strains, such the the two Gambian, Nigerian, Kenyan and Vietnam and the two Chinese strains. This work should be completed during the first year.

During the second year soluble parasite antigens, partially characterized during the first year will be tested to determine whether any may be possible vaccine candidates. This will be attempted by several means. Rabbit, guinea pig, and Saimiri monkey sera obtained from animals immunized with soluble parasite antigens will be tested for inhibitory antibodies in P. falciparum cultures. On the assumption that anti-plasmodial sera obtained from functionally immune individuals living in malarial endemic areas contains antibodies that can be used to identify "protective" antigens, human immune sera that shows in vitro parasite inhibition will be used to identify parasite antigens that may play a role in immunity to malaria. Likewise, purified or semipurified cultured parasite antigens will be used to neutralize inhibitory immune sera. Those antigens which react with human antimalarial sera will be further characterized by testing for cross-strain protection (in vitro inhibition of parasite strains from different geographic regions), comparative immunoelectrophoresis and polyacrylamide gel electrophoresis of radiolabeled parasite antigens precipitated by using immune sera.

During the third year, those soluble parasite antigens that have been isolated and partially purified will have their subcellular position determined by using ferritin or horse-radish peroxidase-linked immunoelectron microscopy. Immunoelectron microscopy will, in part, be conducted in collaboration with Dr. Susan Langreth, Uniformed Services University of the Health Sciences, Bethesda, Maryland. Stage specific parasite soluble extracts will be separated, localized, characterized and compared with "naturally elaborated" exoantigens. This latter study should help us to understand which exoantigens may play a biological role and thus are released into the culture medium as a reuslt of a biological process such as the rhoptry contents during erythrocyte penetration and merozoite surface coat, etc.

5. Specific research methodologies and techniques

a. Production of parasite antigens

Cultures of P. falciparum are currently being maintained in my laboratory by the continuous flow method (Trager and Jensen, 1976; Trager, 1979) and the petri dish-candle jar method (Jensen and Trager, 1977). The exhausted culture medium from these cultures will be the source of "naturally elaborated"* soluble antigens. For the most part, cultures of P. falciparum grown to obtain exoantigens will be cultured in RPMI 1640 medium supplemented with 5% New Zealand white rabbit serum. After preparation of the antigens, they will be used to immunize the same rabbit whose serum was used to supplement the cultures. There are several advantages to this approach in that it greatly simplifies the analysis of the antigenic mixture in that the rabbit should not produce antibodies against any of the culture medium constituents except host erythrocyte, parasite, or antigenically modified rabbit serum components. The identification of these three broad classes of antigen is the goal of this proposal. In general then, selected rabbits will be systematically bled for several weeks and their sera maintained at -20°C until used. Cultures of P. falciparum can be readily maintained at 20-25% parasitemias using any of our previously described methods of culture if the red cell suspension is maintained at 4%. During this culture period the parasitemia remains high, but the number of erythrocytes rapidly decreases. The exhausted culture medium (as determined from preliminary experiments) will be rich in soluble parasite (and erythrocyte) antigens. This medium is collected at 12 hr. intervals, centrifuged at 6,000 rpm to remove cellular elements (erythrocytes and extracellular parasite stages) and stored at -20°C until 2000 ml has been collected. Portions of the medium are then centrifuged at 120,000 x g for 2 hours to obtain a post-ribosomal soluble supernatant (PRSS). Experimental protocol determines the next steps at this point. If, as in my preliminary experiments, rabbit antiparasite antiserum to all antigenic components is desired, then the PRSS is concentrated 20:1 by ultrafiltration through a 5,000 m.w. cutoff membrane, lyophilized and stored at -70°C until used. Immunizations are done by reconstituting the freeze-dried antigen to 1/3 its drying volume and mixing with Freund's complete adjuvant and injecting it into the scapular region of the same rabbit whose serum was used to supplement the culture medium. The initial immunization will be followed, by booster injections in Freund's incomplete adjuvant. The resulting rabbit antiparasite antiserum will contain many different immunoglobulins corresponding to the mixture of antigens injected. Adsorption of the rabbit antiparasite sera with washed human erythrocytes results in antisera reactive only against the parasite antigens.

*In actuality, this exhausted culture medium will contain both biologically elaborated parasite exoantigens as well as antigens released by natural degradation of merozoites that fail to penetrate the erythrocytes.

b. Stage specific parasite antigens

To determine which developmental stages, trophozoites, schizonts or merozoites, contain soluble exoantigens that may be potential vaccine candidates, soluble antigens from three different stages of the erythrocytic cycle will be prepared as follows. Cultures of P. falciparum, grown in large quantity by the method of Jensen (1979b). When the parasitemias reach about 10% they will be treated with 5% aqueous sorbitol (Lambros and Vanderberg, 1979) to lyse all but the young ring stage trophozoites. This procedure reduces the parasitemia to about 2-3%, but effectively synchronizes the cultures. Approximately 48 hours after these newly synchronized parasites are returned to culture they will have increased to approximately 10-12% parasitemia and should be 80-90% trophozoite stage parasites. Culturing for a further 12-18 hours will allow these trophozoites to mature to multinucleated schizonts. Depending upon the stage that is to be analyzed, either trophozoite or schizont infected synchronized cultures will be harvested, washed in culture medium without serum, the cells disrupted by saponin lysis (Reese et al., 1979; Siddiqui, 1979) and the parasites collected, washed free of hemoglobin and disintegrated in a French pressure cell. The resulting mixture will then be centrifuged at 120,000 x g to obtain a crude trophozoite or schizont soluble antigen preparation. These preparations will then be fractionated and analyzed as were the exoantigen preparations described elsewhere.

To prepare merozoite soluble antigen fractions the following method has been devised (Trager and Jensen, unpublished). Cultures are grown in medium supplemented with rabbit serum to about 5-6% parasitemia, synchronized by treatment with 5% aqueous sorbitol according to the method of Lambros and Vanderberg (1979; Kilejian, 1980). After sorbitol treatment the parasitemia is reduced to about 2-3% ring stages. The ring stages are placed back into culture for about 40 hours at 37°C, at which time the parasitemia will have risen 10-12% and will be predominantely multinucleated schizonts. These parasites are removed from the culture vessels, washed twice by gentle centrifugation to remove accumulated erythrocyte ghosts and degenerate parasite debris, and resuspended to 10% cell suspension in screw capped centrifuge tubes in RPMI 1640 and 10% serum and red-cell extract 10% v/v that promotes normal merozoite maturation and release, and helps to maintain merozoite integrity until they are collected (Kilejian, 1980). The parasitized erythrocytes are kept in suspension by periodically inverting the tubes, thus preventing reinvasion of the uninfected erythrocytes by the freshly released merozoites. After 6 hours the uninfected erythrocytes are removed by gentle centrifugation, the erythrocyte ghosts are agglutinated by treatment with rabbit anti-human erythrocyte antiserum and removed by centrifugation leaving the final supernatant rich in merozoites, that appear, by electron microscopy, to be in excellent condition and relatively free of contaminating red cell membranes. This method produces merozoites in large quantity and in far better morphologic condition than any previously reported methods. Merozoites will be further purified using affinity chromatography by passing the merozoite-rich supernatant over a concanavalin A-Sepharose 4B column. The erythrocyte ghosts and membrane fragments should adhere to the lectin, allowing the merozoites to be eluted from the column. This last step is similar, in part, to the methods described by David et al., (1978) and Ambroise-Thomas and Billiault (1979). The final purity of the merozoite preparation can be readily determined using an indirect fluorescent antibody assay, where a small sample of the merozoite preparation is air-dried on a microscope slide, overlaid with rabbit anti-human erythrocyte antiserum and then with fluorescein labeled antirabbit immunoglobulin and observed using a fluorescent microscope. These merozoites will then be disintigrated in a French pressure cell, debris removed by ultracentrifugation and soluble antigens fractionated by physiochemical, and eletrophoretic procedures as will be the other soluble antigens examined in this study.

c. Separation of soluble antigens according to their temperature sensitivities

The soluble antigens concentrated from the culture medium will be separated according to their relative stabilities at various temperatures (Wilson et al., 1969). Rabbits will be immunized with antigen preparations that have been treated in 56°C water bath, and thus, should contain only R- and S-antigens; and antigens treated at 65°C, containing only S-antigens. By comparing various combinations of temperature-treated antigen preparations and rabbit antisera to these preparations, the R- and S-antigens will be identified and isolated. Thus, antigens from P. falciparum cultures will be classified according to the published accounts so that correlations can be made with those soluble parasite antigens derived from human placental homogenates.

d. Isolation of specific parasite antigens and subcellular localization

Preparations of parasite soluble antigens, whether from exhausted culture medium or from specific stage parasite extracts will be fractionated using standard procedures for column chromatography, ion exchange chromatography, and preparative electrophoretic procedures such as disc-gel electrophoresis and preparative column electrofocusing (Kirkland, 1971; Cooper, 1977; Weir, 1978). These isolated antigens will then be used to immunize the same rabbits whose serum was used to supplement the parasite cultures. Analysis of antigenic purity and other immunological characterizations will be conducted using double diffusion methods (Ouchterlony 1958) and one and two dimensional immunoelectrophoresis (Weeks, 1973), as well as in vitro parasite development inhibition assay. In this latter method, antiparasite immune serum obtained from rabbits or Saimiri monkeys will be added to cultures of P. falciparum and compared to serum obtained from the same animals before immunization according to the methods of Campell et al. (1979), Reese et al. (1979), Green et al. (1980) and Kilejian (1980).

To obtain small quantities of highly purified antigens, separations will be made using two dimensional immunoelectrophoresis (crossed electrophoresis) according to the methods of Weeks (1973) with subsequent immunizations of rabbits with the specific antigen (Vaitukaitis et al., 1979) followed by immunoadsorption-affinity chromatography (Koch and Nielsen, 1975; Afchain et al., 1978). These highly purified parasite antigens will then be used to produce the specific rabbit antiparasite immunoglobulin that will then be used to determine the morphologic position of the analogous antigens within the parasite. Purity of antiparasite immunoglobulin will be determined using immunoelectrophoresis. It is proposed that the immuno-electron microscopy be done in collaboration with Dr. Susan Langreth, my former colleague at the Rockfeller University, who is presently working at the Uniformed Services University, Health Services, Bethesda, Maryland. Dr. Langreth has agreed to consult and collaborate on this portion of the project. Localization of parasite antigens will be carried out by treating culture infected erythrocytes and free parasites with the specific antiserum and then with ferritin tagged or enzyme-linked antirabbit immunoglobulin with subsequent ultrastructural examination according to the methods of Sternberger (1973) and Langreth et al. (1979). The purified antigens and corresponding specific antiparasite antisera will be tested against serum collected from functionally immune individuals (sections h, i). Specific antiparasite antisera will also be used to compare P. falciparum strains from different geographic regions (section e). Purified (or semi-purified, as the case may be) parasite antigens that show promise as possible "protective" antigens by virtue of their morphologic position (merozoite surface or apical complex associated antigens), by the in vitro parasite development inhibition assay, or by cross-reactivity with serum from immune individuals should then be tested in Aotus monkeys, but these studies are beyond the scope of this proposal.

e. Immune human serum comparisons with cultured antigens

By the middle of the second year of this project we will have purified and partially characterized several antigens from cultured parasites. Purified or semipurified, as the case may be, cultured parasite antigens will be examined using serum obtained from functionally immune African adults. This serum will be obtained from malaria clinics in Sudan where I will be working off and on during the course of this project. Parasites will be grown in culture medium containing tritiated isoleucine and soluble antigens separated as described above. These parasite soluble antigens will be isolated by precipitating them as immune complexes using antimalarial sera that is inhibitory to P. falciparum developing in vitro. These antigens will be characterized using polyacrylamide gel electrophoresis/autoradiographic methods as outlined by Kilejian, 1980. In an attempt to determine the validity of the in vitro parasite inhibition assays using the rabbit, guinea pig and Saimiri monkey sera, soluble parasite antigens will be used to neutralize the inhibitory action of human antimalarial sera. Naturally, those antigens that induce production of inhibitory antisera in experimental animals and also neutralize the inhibitory properties of selected human antimalarial sera will probably be good vaccine candidates.

D. FACILITIES AND RESOURCES

1. Assessment of institutional facilities and resources

The research will be conducted in the Department of Microbiology and Public Health at Michigan State University in Dr. James B. Jensen's laboratory in Giltner Hall, and, in part, the M.S.U. Parasitology Laboratory in the Ministry of Health, Khartoum, Sudan.

My M.S.U.-U.S.A. laboratory consists of 1,000 square feet of space equipped with 1 Steri-gard tissue culture hood, 2 water jacketed and 1 low temperature forced-air incubators, complete system apparati for continuous flow cultivation of P. falciparum, 1-electrophoresis power supply, apparati for polyacrylamide slab gel and disc-gel electrophoresis, 1 ultra low temperature freezer, 1 -20°C freezer, 1 Beckman model L4 ultracentrifuge, 1 Amicon ultrafiltration cell, 2 liquid N_2 storage refrigerators, 1 Nikon Optiphot research microscope with fluorescence and automatic photographic capabilities, 2 Nikon Labophot light microscopes, Corning model 125 digital pH meter, 1 Gilson model FC-80 microfraction collector, low speed International model UV centrifuge, 1 Beckman model LS 7500 scintillation counter, 1 Labconco lyophilizer, and miscellaneous lab equipment such as magnetic stirrers, hot plates, glassware, pipettes, candle jars (desiccators), etc. Available in the Department of Microbiology and Public Health are complete electron microscopic preparation facilities and a Philips 300 electron microscope.

In collaboration with several other MSU parasitologists, I have developed a fully equipped research facility in Khartoum, Sudan under the sponsorship of NIH. This laboratory contains 2,500 sq. ft. in the Central Laboratory of the Ministry of Health. It has complete animal care facilities, a laminar flow tissue culture hood, Beckman low speed and high speed centrifuges, both -20°C and -70°C freezers, refrigerators, liquid N_2 storage tanks and specimen transporters, a Hitachi Model 60 Spectrophotometer, complete tissue culture facilities, 2 Nikon Labophot light microscopes equipped for fluorescense microscopy, 2 Beckman scintillation counters for beta and gamma emissions.

2. Personnel qualifications and collaborating contacts

Dr. James B. Jensen, the principal investigator, has recently moved to ichigan State University from the laboratory of Dr. William Trager at the Rockefeller niversity. While at the Rockefeller University Drs. Jensen and Trager pioneered the irst continuous cultivation of P. falciparum. Dr. Jensen has had previous experience ith ultrastructural techinques, having published several papers (see C.V.) on pccidian penetration and ultrastructural development as well as collaborating with Langreth on malarial parasite fine structure studies.

At Michigan State University, two widely recognized immunoparasitologists, Drs. effrey F. Williams and Felipe Kierszenbaum of the Department of Microbiology and ublic Health have both consented to act as non-salaried consultants to this project. t. Kierszenbaum has published extensively on, and is presently engaged in studies of ost immune responses to Trypanosoma cruzi. Dr. Williams is an internationally cognized authority on immuno-parasitology, particularly with regard to host immuno-gical responses to larval parasites. Drs. Kierszenbaum's and Williams' C.V.s are cluded with this proposal.

In Khartoum, Dr. Abdulla A. Dafalla, an immunoparasitologist in the Faculty of dicine, University of Khartoum, who spent his sabbatical leave working in my boratory at MSU has agreed to assist with studies in Sudan. Dr. Dafalla's research terest is in the area of human immune responses to P. falciparum. He came to my boratory to learn techniques for in vitro cultivation of P. falciparum so that ese cultures can be used to assess the immune status of individuals in Sudan. Dafalla is an MD-Ph.D. from the London School of Tropical Medicine.

In addition to the above named consultants and collaborators, Dr. Susan Langreth the Uniformed Services University, Health Services, Bethesda, Maryland has agreed collaborate on the immuno-electron microscopy portions of the proposed research. is expected that Dr. Jensen will be able to continue his close working relation-ip with other members of the USAID malaria research network - especially Drs. eodore Green of the University of Missouri, William Trager, Rockefeller University, rl Reichmann, University of New Mexico, and Carlos A. Espinal, Bogotá, Colombia.

3. Other research contracts and grants received

-Search for strains of Plasmodium falciparum resistance to 4 aminoquinoline in Sudan using in vitro culture techniques
Sponsored by U.S. Public Health Service-National Institutes of Health
Funding $151,688
Duration 5 years, 1980-1984

-Erythrocyte requirements and serum replacement for continuous cultures of Plasmodium falciparum
Sponsored by The World Health Organization
Funding $ 40,700 (First year)
Duration 3 years, 1980-1983

-The development of a model for antigenic analysis of cultured Plasmodium falciparum
Sponsored by The United States Agency for International Development
Funding $34,840
Duration 1 year, April 1980-March 1981

E. APPENDIX

Relevant Preliminary Experiments, An Interim Report of USAID Project

Our laboratory has recently begun to analyze soluble antigens from exhausted culture medium used to grow P. falciparum. Initially, sera from New Zealand white rabbits were collected weekly and stored at -20°C until approximately 100 ml from each rabbit had been obtained. Rabbit serum can supplant the human serum requirement for culturing P. falciparum (Sax et al., 1978) when used at a 10% v/v/concentration. Using the petri dish-candle jar technique for culturing P. falciparum (Jensen and Trager, 1977), parasites were grown to 20% parasitemia in medium supplemented with the rabbit serum. The cultures were maintained at high parasitemias for several days by changing the medium every 12 hours. The purpose of this procedure was to enrich on the culture medium with soluble parasite antigens produced by the rapidly growing culture. The rationale behind this procedure was that three major antigens could conceivably accumulate in the medium a) the merozoite surface coat material which is sloughed from the parasite as it enters the erythrocyte (Bannister et al., 1977; Aikawa et al., 1978; Langreth, Nguyen-Dinh, and Trager, 1978); b) the antigenic surface alterations of the infected erythrocyte (the "knobs") which are shed from the erythrocyte membrane as the parasite matures (Langreth et al., 1978); and c) rhoptry contents which are presumably discharged during penetration of the erythrocyte (Bannister et al., 1977; Kilejian, 1976). Kilejian has presented evidence that a histidine-rich protein (HRP) from P. lophurae may be associated with the rhoptries, and may play a functional role in entry of the merozoite into the erythrocyte (Kilejian, 1976). Furthermore, the HRP is quite immunogenic, producing protection in duckling against an invariably fatal infection of P. lophurae (Kilejian, 1978). Plasmodium falciparum may also possess a similar protein (Kilejian and Jensen, 1977).

The exhausted culture medium was thus collected every 12 hours, centrifuged to remove the intact cells, and stored frozen at -20°C until enough was accumulated to spin in the ultracentrifuge at 120,000 x g for 2 hours to remove all particulate matter. The supernatant fluid was then concentrated by ultrafiltration to 1/20th its volume, lyophilized and stored at -70°C until used. Just prior to using, the soluble antigen preparation was reconstituted to 1/3 its drying volume, emulsified in Freund's complete adjuvant and injected subcutaneously (scapular) and intramuscularly (groin) into the same rabbit whose serum had been used in growing the parasite cultures. The rationale in this procedure was that the rabbit should not produce antibodies against the serum components of the preparation, but parasite-erythrocyte elements only. After 2 booster injections of the antigen preparation, emulsified in Freund's incomplete adjuvant, spaced at 14-day intervals, the rabbit was bled to obtain the anti-parasite antiserum.

Antiserum was raised in rabbits against exoantigens from exhausted cultured medium of strain FCC_1 (from the People's Republic of China) and African FCR_3 (Jensen and Trager, 1978) line K+ which produces the erythrocyte surface alterations (knobs) and line $K\bar{c}$, a clone of FCR_3 which does not produce knobs. Preliminary results using Ouchterlony double diffusion in agar plates has revealed two soluble parasite exoantigens common to both the Chinese FCC_1 and the African FCR_3 strains, two apparently strain specific exoantigens and three erythrocyte membrane antigens which can be demonstrated by comparing antisera before and after adsorption with the same erythrocytes used to culture the parasites. One of the common exoantigens seen between FCC_1 and FCR_3K+ is lacking in line $FCR_3K\bar{c}$, suggesting that this antigen may be associated with the production of knobs. The rabbit antiparasite sera gave 1:1280 indirect fluorescent antibody titers.

In other experiments I have found that unfractionated soluble antigen preparations from cultured P. falciparum produce a marked hyperpyrexia in rabbits while control preparations containing the same erythrocytes and serum, sans parasites does not. In these experiments rabbits first received a small i.v. injection of an extract made from erythrocytes, serum and medium used to culture the parasites. Rectal temperature remained normal for 3 hours. The rabbits then received a similar i.v. injection prepared from P. falciparum cultured in the same erythrocytes and medium used in the control. Within 30 minutes the rabbits began to hyperventilate, had shivers and their temperatures rose from the normal of 38°C to 41°C (100°F to 106°F) and remained elevated for 3-4 hours.

From the results of these investigations, and others, the rabbit may be a useful model in analysis of soluble antigens from cultured P. falciparum. However, we have recently discovered that sera from our nonimmunized rabbits show an unaccountable indirect fluorescent antibody titer (up to 1:320) when tested against cultured schizonts. All of these rabbits were, however, infected with various species of Eimeria, a sporozoan closely related to Plasmodia. We are presently investigating the use of coccidia-free rabbits, guinea pigs, as well as the squirrel monkey, Saimiri sciureus as possible models for antigenic anaylsis. Other laboratories are using Aotus monkeys, but certainly, a more readily available model must be found to preserve these scarce and valuable animals for definitive testing of previously screened vaccine candidates.

Soluble parasite antigens, collected from exhausted culture medium or from the soluble portion of freeze-thaw preparations of cultured schizonts were used in a lymphocyte blast-transformation assay using lymphocytes obtained from Dr. A. A. Dafalla, an African scientist doing research in the laboratory. Dr. Dafalla, a Sudanese immunoparasitologist who lives in a malaria hyperendemic area is "functionally" immune to P. falciparum in that he rarely gets malaria. He had a mild (typical) case of falciparum malaria 6 weeks before he came to my laboratory. We used peripherial blood lymphocytes from Dr. Dafalla in a blast-transformation assay. Dr. Jensen's lymphocytes were used as a nonimmune control. Surprisingly Dr. Dafalla appeared to be immunosuppressed with regard to response of his lymphocytes to concanavalin A and Dr. Jensen's lymphocytes gave a higher response to the malaria parasite soluble antigens (Table 1). Assuming that Dr. Jensen may not have been a good control for this study due to the fact that he has been in Africa on several occasions and might possibly have had at one time a subclinical infection of falciparum malaria, the experiment was repeated as outlined below. Erythrocytes and serum was collected from three graduate students and FCR_3 parasites were grown in the erythrocytes of each student respectively. Schizonts were concentrated using gelatin floatation and the soluble parasite antigens extracted by freeze-thawing the schizonts and the particulate materials removed by centrifugation. Each student's peripherial blood lymphocytes were then incubated with the parasite extract collected from parasites cultured in the students' own erythrocytes. In this way the test system contained no antigens other than the parasites'. As can be seen from data in Table 2 crude soluble schizont preparations produce nonspecific blast-transformations in perpherial blood lymphocytes of nonimmune individuals. One of the students (T.C.) had been previously infected with the FCR_3 strain of P. falciparum. As can be seen from Table 2 he was immunosuppressed for concanavalin A and didn't respond as well as could be expected to the malaria parasite antigens. These results suggest that lymphocyte blast-transformation assays may not be a good index of immunity and that some soluble antigen(s) produce nonspecific lymphocyte stimulation and therefore may be deleterious elements that should be identified and removed from vaccine preparations.

Table 1. Stimulation Index

$$\left[\frac{\text{trt. cpm - background (med)}}{\text{control (ConAo) - background}}\right]$$

72 hrs

	JBJ cpm ± std. error	SI	AAD cpm ± std. error	SI
Med.	389 ± 90		133 ± 36	
ConA 0 μg/ml	624 ± 63	1.0	482 ± 59	1.0
ConA 2	2276 ± 117	8.03	935 ± 98	2.30
ConA 5	6629 ± 178	26.55	3266 ± 247	8.98
†Sol FCC_1 5 μl/ml	920 ± 47	2.26	513 ± 81	1.09
10	738 ± 102	1.68	560 ± 87	1.22
25	461 ± 26	.31	441 ± 42	.88
FCR_3 TC 5	950 ± 152	2.39	869 ± 108	2.12
25	1112 ± 81	3.08	677 ± 51	1.56
Sol RBC 10	345 ± 34	--	524 ± 51	1.12
25	85 ± 32	*	133 ± 8	*

120 hrs

Med.	169 ± 24		105 ± 23	
ConA 0	2397 ± 794	1.0	947 ± 269	1.00
2	33322 ± 1589	14.88	9703 ± 430	11.40
5	35250 ± 2964	15.75	47920 ± 1486	56.79
Sol FCC_1 5 μl/ml	3356 ± 457	1.43	1040 ± 88	1.11
10	2654 ± 462	1.12	1354 ± 243	1.48
25	1026 ± 118	.38	907 ± 290	.95
FCR_3 TC 5	12610 ± 779	5.58	6183 ± 1506	7.22
25	14014 ± 1934	6.21	4817 ± 1134	5.60
Sol RBC 10	3264 ± 779	1.39	1513 ± 262	1.67
25	185 ± 33	*	149 ± 6	*

*Significant drop in viability.

†Exoantigens from culture medium of *P. falciparum* from The People's Republic of China, Strain FCC_1.

Table 2.

72 hrs

	Al G. (A) cpm ± std. error	SI	Tom C. (B) cpm ± std. error	SI	Joe C. (C) cpm ± std. error	SI
Med. background	54 ± 9		229 ± 37		118 ± 27	
ConA 0 μg/ml control	378 ± 65	1.0	1370 ± 396	1.0	1428 ± 114	1.0
ConA 2 μg/ml	1136 ± 234	3.34	2258 ± 60	1.78	2762 ± 74	2.02
ConA 5 μg/ml	3202 ± 333	9.72	7456 ± 300	6.33	14875 ± 959	11.26
FCR_{3TC} 5 μg/ml (Schizont)	708 ± 91	2.02	893 ± 189	.58	2125 ± 125	1.53
FCR_{3TC} 10 μl/ml	609 ± 122	1.71	4728 ± 313	3.94	3067 ± 268	2.25
FCR_{3TC} 25 μl/ml	761 ± 41	2.18	3664 ± 198	3.01	3048 ± 208	2.24
Sol RBC 5	383 ± 75	1.02	777 ± 57	.48	1483 ± 156	1.04
Sol RBC 10	304 ± 10	0.77	566 ± 40	.30	957 ± 22	.64
Sol RBC 25	362 ± 86	0.95	795 ± 54	.50	793 ± 47	.52

120 hrs

Med.	84 ± 11		740 ± 148		113 ± 6	
ConA 0 μg/ml	1070 ± 322	1.0	14405 ± 2935	1.0	10411 ± 1538	1.0
ConA 2	14369 ± 1647	14.49	24789 ± 798	1.76	33562 ± 1880	3.25
ConA 5	23042 ± 3421	23.28	24538 ± 2718	1.74	49228 ± 3412	4.77
FCR_{3TC} 5 μl/ml	4483 ± 1314	4.46	5769 ± 954	.37	19634 ± 1437	1.90
FCR_{3TC} 10	5249 ± 1225	5.24	30188 ± 1999	2.15	22618 ± 1391	2.19
FCR_{3TC} 25	5260 ± 1610	5.25	34812 ± 1880	2.49	24118 ± 1637	2.33
Sol RBC 5	1957 ± 474	1.90	8570 ± 1483	.57	7120 ± 1835	.68
Sol RBC 10	874 ± 28	.80	6027 ± 2195	.39	7507 ± 1780	.72
Sol RBC 25	1440 ± 853	1.38	4360 ± 256	.26	2827 ± 571	.26

F. BUDGET INFORMATION AND ESTIMATES

1. Estimated total funding requested of A.I.D.

First year: $118,434

Second year: 90,399

Third year: 99,419

2. Financial contribution or cost-sharing arrangements

Dr. J. B. Jensen's salary is to be paid by Michigan State University

3. Detailed budget for first year and total project

a. Salaries

Personnel	Title	% time	1st yr	2nd yr	3rd yr	Total
1) James B. Jensen	Principal Investigator	30%				
2) (To be named)	Research Associate Immunologist	100%	15,000	16,050	17,174	48,224
3) Michael Boland	Research Assistant, Technician	100%	14,000	14,980	16,029	45,009
4) (To be named)	Graduate Research Assistant	50%	6,500	6,955	7,442	20,897

b. Consultants

1) Jeffrey F. Williams, Professor, MSU 2%

2) Felipe Kierszenbaum, Associate Professor, MSU 2%

3) Abdalla A. Dafalla, Professor, Faculty of Medicine, University Kartoum, Sudan 5%

c. Fringe benefits (19% - for items 2 and 3)	5,510	5,896	6,309	17,715
Salaries Subtotal	41,010	43,881	46,954	131,845
d. Travel	850	1,000	2,400	4,250*

*Travel funds for the third year are for the purpose of collaboration between Dr. Jensen's laboratory and Dr. S. Langreth of USUHS, Bethesda, Maryland, as outlined in the proposal.

	1st yr	2nd yr	3rd yr	Total
e. Non-expendable equipment				
-High speed refrigerated centrifuge and accessories	8,000			
-Top loading analytical balance	3,000			
-Clinical-hematocrit centrifuge	600			
-Chromatographic peristaltic pump	1,000			
-Bausch and Lomb Spectronic 21 spectrophotometer	2,500			
-Low temperature incubator	1,800			
-35 mm camera, with 50 mm f3.5 macrolens	600			
-Chromatography columns	700			
-Electrophoresis power supply	1,000			
-Liquid N_2 Dewar flask	400			
-Amicon ultrafiltration cells (2)	650			
-Water bath	500			
-Refrigerated fraction collector	3,600			
-UV absorbance monitor	2,600			
-Thermister electronic thermometer and probe	300			
-Ultrasonic disembrator	1,300			
-1,000x Nikon glycerin immersion UV fluorescence objective	1,200			
-Beckman microfuge B and accessories	1,000			
-Slab gel electrophoresis apparatus	1,000			
-Office filing cabinets	500			
	32,250	-0-	-0-	32,250
f. Expendable supplies and equipment				
-Laboratory glassware				
-Tissue culture plastic ware				
-Culture media				
-HEPES				
-Photographic and graphic supplies				
-Chemical, chromotographic reagents				
-Pasteur pipettes				
-Serologic pipettes				
-Reference materials, (books, journals, etc.)				
-Telephone				
-Xerox	10,000	11,000	12,100	33,100
Other				
-Human blood, 60 units/yr @$40/unit	2,400	2,640	2,904	7,944
-Laboratory animal purchases and maintenance	5,000	5,500	6,050	16,550
-Glassblowing of special culture vessels	1,800	-0-	-0-	1,800
	19,200	19,140	21,054	59,394
g. Publication costs	500	550	605	1,655
Total direct costs	93,810			229,394
Less equipment	32,250			
	61,560	64,571	71,013	197,144
h. Indirect costs (40% of direct cost except equipment)	24,624	25,828	28,406	78,858
GRAND TOTAL	118,434	90,399	99,419	308,252

Justification for non-expendable equipment

-High speed refrigerated centrifuge and accessories are needed to conduct gradient separations, prepare the red-cell extract and reclaim merozoites and erythrocyte membrane fragments. My low speed centrifuge will not go over 2,500 rpm and for some applications such as red-cell extract preparation 20,000 rpm are needed.

-Toploading analytical balance is needed to prepare culture media, buffers for chromatography and electrophoresis and other reagents. My present balance will not hold more than 100 gms and then only with difficulty. Buffers and balanced salt solutions etc. need to be made in large quantities.

-Clinical-hematocrit centrifuge to be used to determine hematocrits in experimental infections in Saimiri monkeys, to determine red cell volumes in experimental cultures and general laboratory centrifugations such as washing blood, etc.

-Chromatographic peristaltic pump to fill liquid chromatography columns, to produce buffer gradients for ion exchange chromatography, etc.

-Bausch and Lomb Spectronic 21 spectrophotometer to determine total protein concentrations, contaminating hemoglobin levels, nucleic acid bands, etc. in antigen preparations and to determine levels of culture medium glucose, erythrocyte ATP, etc. during antigen production.

-Low temperature, forced air 37°C incubator for the large scale parasite production.

-35 mm camera with 50 mm f 3.5 macrolens to photograph acrylamide gels, graphic productions for manuscript and orally presented papers and to record results such as immunoelectrophoretic and double diffusion gels, etc.

-Chromatography columns to separate protein fractions in order to partially purify antigens from contaminating serum and erythrocyte (hemoglobin) components.

-Electrophoresis power supply. The one I presently have (a Heathkit) I received second-hand. It is not very stable and should be replaced with a more reliable unit. To be used for immunoelectrophoretic and polyacrylamide preparative and analytical procedures.

-Liquid N_2 Dewar flask to transport liquid N_2 from the Physics Department to my laboratory. Liquid N_2 is used to maintain my parasite stabilates.

-Amicon ultrafiltration cells (2). I need a large (400 ml) and small (20 ml) volume cell to concentrate antigens from the culture medium and to reclaim proteins from buffer dilutents after liquid chromatographic fractionation.

-Water bath to prewarm culture media, inactivate sera, etc.

-Refrigerated fraction collector to separate protein components found in exhausted culture medium and other liquid chromatographic procedures. This unit can also be used to refrigerate electrophoretic apparati during immunoelectrophoresis.

-UV absorbance monitor. An essential accessory to the fraction collector-liquid chromatography system.

-Themister electronic thermometer and probe, to monitor body temperature changes in immunized animals to determine pyrogenicity of antigen preparations.

-Ultrasonic disembrator to break up parasites to release soluble antigens.

-1,000X Nikon glycerin immersion UV fluorescence objective for indirect fluorescent antibody assays. I already have a Nikon fluorescent microscope but need a 1,000X glycerine immersion objective for it.

-Beckman microfuge B and accessories. Separations of small quantities of freeze-thawed schizonts, merozoites, etc. from the soluble supernatants at high speed requires a centrifuge which handles small quantities. Also a valuable tool for preparations of small parasite samples for electron microscopy.

-Slab gel electrophoresis apparatus for analysis and preparations of proteins from antigen preparations.

-Office filing cabinets not supplied by the University. I need at least three file cabinets to file data, references and miscellaneous papers.

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Assurance for Protection of Human Subjects

This proposal requires the use of human blood, which will be purchased from established blood banking services. At no time will this proposal deal with the direct use of human subjects. Assurance for protection of interests of human subjects will be the responsibility of the Blood Bank Medical Personnel. The names of donors will not be known by the investigators of this proposal. Human blood to be used in this proposal will be purchased from blood banks at the normal market rates.

James B. Jensen
Principal Investigator

October 2, 1979

TO: James B. Jensen
Microbiology and Public Health

FROM: Dr. Robert K. Ringer, Chairman
All-Univeristy Committee on Animal Facilities and Care

RE: Supplementary Information Sheet for Research Proposals Involving Use of Birds or Mammals

I wish to notify you that the aspects of your research proposal summarized on your Supplementary Information Sheet were approved by the All-University Committee on Animal Facilities and Care. The title of the proposal, date of approval, and number assigned to the Supplementary Information Sheet by the Committee are given below. You may wish to retain this information for future use. In the course of your research, if there are any substantive changes from the information reported on the Supplementary Information Sheet, such changes should be reported promptly to the All-University Committee.

Title of Proposal: Antigenic Analysis of *Plasmodium falciparum*

Date of approval: September 28, 1979

Number assigned to Supplementary Information Sheet: 717

MICHIGAN STATE UNIVERSITY

UNIVERSITY COMMITTEE ON RESEARCH INVOLVING
HUMAN SUBJECTS (UCRIHS)
238 ADMINISTRATION BUILDING
(517) 355-2186

EAST LANSING • MICHIGAN • 48824

September 11, 1979

Dr. James B. Jensen
Giltner Hall
Department of Microbiology
and Public Health

Subject: Proposal entitled "Antigenic Analysis of Cultured *Plasmodium falciparum*"

Dear Dr. Jensen:

The above referenced project was recently submitted for review to the UCRIHS.

We are pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and the Committee, therefore, approved this project at its meeting on September 10, 1979.

Projects involving the use of human subjects must be reviewed at least annually. If you plan to continue this project beyond one year, please make provisions for obtaining appropriate UCRIHS approval prior to the anniversary date noted above.

Thank you for bringing this project to our attention. If we can be of any future help, please do not hesitate to let us know.

Sincerely,

Henry E. Bredeck
Chairman, UCRIHS

HEB/jms

CURRICULUM VITAE

JAMES B. JENSEN

Department of Microbiology and Public Health
Michigan State University
East Lansing, Michigan 48824
(517) 353-5105

Personal

Social Security [redacted]
[redacted], [redacted]
Married, four children
Home Address: [redacted]
Home Phone: [redacted]

Education

Undergraduate: Brigham Young University (1965-1970)
B.S., Zoology (1970)

Graduate: Brigham Young University (1970-1972)
M.S., Zoology (1972)
Utah State University (1972-1974)
Auburn University, Alabama (1974-1975)
Ph.D. (1976)

Academic and Research Experience

1979-	Assistant Professor of Parasitology, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824
1976-1979	Assistant professor of Parasitology, The Rockefeller University, New York, N.Y. 10021
1978	World Health Organization Consultant of Malaria, University College Medical School, University of Ibadan, Ibadan, Nigeria
1977-	Visiting Assistant Professor of Tropical Medicine and Parasitology, Cornell Medical College, Cornell University, New York, New York 10021
1975-1976	Research Associate, Department of Parasitology, The Rockefeller University
1974-1975	Graduate Student, Ph.D. (Under Dr. S. A Edgar) Departments of Zoology and Poultry Science, Auburn University, Auburn, Alabama 36830
1972-1974	Graduate Student, Ph.D. (Under the late Dr. D. M. Hammond) Department of Zoology, Utah State University, Logan, Utah
1970-1972	Graduate Student, M.S. (Under Dr. F. L. Andersen) Department of Zoology, Brigham Young University, Provo, Utah 84602
1965-1970	Student, B.S. Department of Zoology, Brigham Young University, Provo, Utah 84603
1962-1964	Missionary for the Church of Jesus Christ of Latter-Day Saints, Great Lakes Mission, Ft. Wayne, Indiana
1961-1962	Student, School of Forestry, Washington State University, Pullman, Washington

Teaching Experience

1977-1979	Visiting Assistant Professor of Tropical Medicine, Cornell Medical College, Cornell University, New York
1977-1979	Parasitism and Symbiosis, team taught, The Rockefeller University, New York
1973-1974	Teaching Assistant, Department of Zoology, Utah State University, Logan, Utah - Introduction to Biology - Parasitology - Biological Electron Microscopy
1971-1972	Teaching Assistant, Department of Zoology, Brigham Young University, Provo, Utah - Introduction to Zoology

Honors

1973 - Best student paper, Rocky Mountain Conference of Parasitologists

1974 - D. M. Hammond Memorial Award
Best student paper, Rocky Mountain Conference of Parasitologists

1974-1975 - Coccivac Predoctorate Fellowship, Auburn University

1979 - Outstanding Young Man of America, United States Junior Chamber of Commerce

Honorary and Professional Affiliations

Society of Protozoologists
American Society of Parasitologists
American Society of Tropical Medicine and Hygiene
Rocky Mountain Conference of Parasitologists
Sigma Xi, full member, 1976, The Rockefeller University Chapter
The New York Society of Tropical Medicine

General Areas of Research Experience

In vitro cultivation of coccidia
Ultrastructural studies on schizogony and gametogony of *Isospora* and *Eimeria* species of coccidia; and penetration of the sporozoites of *Isospora* and *Eimeria* into cultured cells.
Chemotherapy of bovine coccidiosis
Physiology of excystation of coccidia
In vitro cultivation of *Plasmodium falciparum*
Antigen preparation from *Plasmodium* cultures

Master's Thesis: The effects of different gases on excystation of occcidian oocysts

Doctorate Dissertation: The penetration of coccidian sporozoites into cultured cells

1976-1980 Continuing research on cultivation of malaria parasites, and isolation of a possible antigen from these cultures, for use as a vaccine to malaria

Publications

1. Jensen, J. B. and D. M. Hammond. 1975. Ultrastructure of the invasion of Eimeria magna sporozoites into cultured cells. J. Protozool. 22:411-415.

2. Jensen, J. B. and S. A. Edgar. 1976. Effects of antiphagocytic agents on penetration of Eimeria magna sporozoites into cultured cells. J. Parasitol. 62:203-206.

3. Jensen, J. B. and S. A. Edgar. 1976. Possible secretory function of the rhoptries of Eimeria magna during penetration of cultured cells. J. Parasitol. 62:988-992.

4. Jensen, J. B., P. A. Nyberg, S. D. Burton and W. R. Jolley. 1976. The effects of selected gases on excystation of coccidian oocysts. J. Parasitol 62:195-198.

5. Jolley, W. R., S. D. Burton, P. A. Nyberg and J. B. Jensen. 1976. Formatio of sulfhydryl groups in the walls of Eimeria magna and E. tenella oocysts subjected to in vitro excystation. J. Parasitol. 62:199-202.

6. Miner, M. L. and J. B. Jensen. 1976. Decoquinate in the control of experimentally induced coccidiosis of calves. Am. J. Vet. Res. 37:1043-1045

7 Trager, W. and J. B. Jensen. 1976. Human malaria parasites in continuous culture. Science 193:673-675.

8 Wheat, B. E., J. B. Jensen, J. V. Ernst and B. Chobotar. 1976. Ultrastructure of macrogametogenesis of Eimeria mivati. Z. Parasitenk. 50:125-136.

9. Jensen, J. B. and S. A. Edgar. 1977. Penetration of sporozoites of Isospora canis into cultured cells, an ultrastructural study. J. Protozool. 25:169-173.

10. Jensen, J. B. and W. Trager. 1977. Plasmodium falciparum in culture: Use of outdated erythrocytes and description of candle jar method. J. Parasitol. 63:883-886.

11. Kilejian, A. and J. B. Jensen. 1977. A histidine-rich protein from Plasmodium falciparum and its interaction with membranes. Bull WHO 55:191-197.

12. Trager, W. and J. B. Jensen. 1977. Cultivation of erythrocytic stages of Plasmodium falciparum. Bull WHO 55:363-365.

13. Jensen, J. B. 1978. Concentration of trophozoite- and Schizont-infected erythrocytes of Plasmodium falciparum from continuous cultures. Am. J. Trop. Med. Hyg. 27:1274-1276.

14. Jensen, J. B. and W. Trager. 1978. Plasmodium falciparum in culture: Isolation of new strains and observation of gametocytogenesis. Am. J. Trop. Med. Hyg. 27:743-746.

15. Jensen, J. B. and W. Trager. 1978. Some recent advances in the cultivation of Plasmodium falciparum. Israel J. Med. Sci. 14:563-570.

16. Langreth, S. G., J. B. Jensen, R. T. Reese and W. Trager. 1978. Fine structure of human malaria in vitro J. Protozool. 25:433-452.

17. Reese, R. T., W. Trager, J. B. Jensen, D. A. Miller, and R. Tantravahi. 1978. Immunization against malaria with antigen from Plasmodium falciparum cultured in vitro. Proc. Natl. Acad. Sci. USA 75:5665-5668.

18. Trager, W. and J. B. Jensen. 1978. Cultivation of malarial parasites, a review. Nature 273:621-622.

19. Jensen, J. B. 1979. Observations on gametocytogenesis in Plasmodium falciparum from continuous cultures. J. Protozool. 26:129-132.

20. Jensen, J. B. 1979. Some aspects of serum requirements for continuous cultivation of Plasmodium falciparum. Bull. WHO Suppl. 57:27-31.

21. Jensen, J. B., W. Trager, and R. L. Beaudoin. 1979. Techniques for in vitro cultivation of erythrocytic and exoerythrocytic stages of malarial parasites, pp. 255-265. In Practical applications of in vitro techniques, K. Maramorosch and H. Hirumi (eds.). Academic Press, New York.

22. Jensen, J. B., W. Trager, and J. Doherty. 1979. A semi-automated apparatus for the continuous cultivation of Plasmodium falciparum. Exp. Parasitol. 48:36-41.

23. Trager, W., and J. B. Jensen. 1979. Cultivation of erythrocytic and exoerythrocytic stages. In Malaria in man and experimental animals, J. P. Kreier (ed.). Academic Press, New York. (In press).

CURRICULUM VITAE

Felipe Kierszenbaum, Ph. D.

PRESENT POSITION	Associate Professor Department of Microbiology and Public Health Michigan State University East Lansing, Michigan 48824
EDUCATION	1963. M. S. Biochemistry (with Honor) University of Buenos Aires 1966. Ph. D. Immunology (Distinguished Mention) University of Buenos Aires
POSTDOCTORAL TRAINING	1966-1969. Research Associate Department of Biochemistry Scripps Clinic and Research Foundation La Jolla, California
PROFESSIONAL CAREER	Please, see page 2. For Research Support see page 3.
SOCIETY MEMBERSHIP	American Association of Immunologists American Society for Microbiology The Reticuloendothelial Society American Society of Tropical Medicine and Hygiene American Society of Parasitologists Society of Protozoologists
EDITORIAL SERVICE	1978-1980. Associate Editor, The Journal of Immunology. 1980-1983 Associate Editor, Advances in Parasitic Diseases
CURRENT RESEARCH	Cellular Immunology Immunoparasitology
CURRENT TEACHING	Immunology, Medical Microbiology, Parasitology. Medical, graduate and undergraduate students.
PERSONAL	[redacted] Married, 2 children U. S. Citizen Ethnic group: Hispanic

Felipe Kierszenbaum, Ph. D.

Professional Career

	A. RESEARCH	B. TEACHING
1977 to date	Associate Professor Dept. of Microbiology & Public Health Michigan State University	
1975-1977	Visiting Investigator Dept. of Pathology Yale University Sch. of Medicine	
1974-1975	Senior Research Scientist Dept. of Experimental Immunobiology Wellcome Research Laboratories Beckenham, Kent, Great Britain	
1969-1974	Head, Immunochemistry Section	Head, Teaching Work (Equivalent to Assistant Professor) Dept. of Microbiology, Parasitology and Immunology Univ. of Buenos Aires Sch. of Medicine
1966-1969	Research Associate and N.I.H. Postdoctoral Fellow Dept. of Biochemistry Scripps Clinic & Research Fndn. La Jolla, California (Under Dr. Walter B. Dandliker)	
1963-1966	Graduate Student	Teaching Assistant Dept. of Microbiology, Parasitology & Immunology Univ. of Buenos Aires Sch. of Medicine (Mentor: Dr. Armando S. Parodi, deceased)
960-1963		Teaching Assistant Analytical Chemistry and Biochemistry Sch. of Pharmacy & Biochemistry Univ. of Buenos Aires

C. FELLOWSHIPS: 1967-1969. National Inst. of Health (Postdoctoral)
1972. World Health Organization (Special Fellowship)

D. OTHER: Sep.-Nov. 1974: Visiting Professor at the World Health Organization Research and Training Center for Immunology, Geneva, Switzerland.

BIBLIOGRAPHY

DR. F. KIERSZENBAUM

1. Doctoral thesis: Immunologic Properties of Bacterial Lipopolysaccharides University of Buenos Aires, 1966. Distinguished Mention. Director of research: Dr. Armando S. Parodi (deceased).

2. Grinstein, S., Kierszenbaum, F. and Ferraresi, R.W. 1966. Action adjuvante du lipopolysaccharide d'Escherichia coli et sa fraction lipide sur la production d'anticorps. Revue d' Immunologie 30: 141-150.

3. Frigerio, M.J., Kierszenbaum, F. and Nejamkis, M.R. 1966. Nonspecific resistance action invoked by bacterial lipopolysaccharides against tuberculous infection. Int. Arch. Allergy Appl. Immunol. 32: 91-97.

4. Kierszenbaum, F., Nota, N.R., Marra, R.B., and Besuschio, S.C. 1966. Cell participation in the stimulation of the immune response by Escherichia coli lipopolysaccharide. Int. Arch. Allergy Appl. Immunol. 32: 91-97.

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11. Kierszenbaum, F., Budzko, D.B., and Parodi, A.S. 1970. Alterations in the enzymatic activity of plasma of guinea pigs infected with Junin virus. Archiv Ges. Virusforsch. 30: 217-223.

12. Levison, S.A., Portmann, A.J., Kierszenbaum, F. and Dandliker, W.B. 1971. Kinetic behavior of antihapten antibody of restricted heterogeneity by stopped flow polarization kinetics. Biochem. Biophys. Res. Commun. 43: 258-266.

13. Kierszenbaum, F. 1972. Antagonistic effects of bacterial endotoxin and phytohemagglutinin upon the primary immune response. Int. Arch. Allergy. Appl. Immunol. 43: 161-171.

14. Kierszenbaum, F. and Saavedra, L.E. 1972. The effect of bacterial endotoxin on the infection of mice with Trypanosoma cruzi. J. Protozool. 19: 655-657.

15. Budzko, D. B. and Kierszenbaum, F. 1973. Endotoxin-induced protection against Junin virus infection. Arch. Ges. Virusforsch. 41: 203-210.

16. Kierszenbaum, F. and Budzko, D.B. 1973. Immunological properties of a trypanosomal lipopolysaccharide and its effects on the infection with Trypanosoma cruzi. Immunol. Commun. 2: 265-276.

17. Participants of the World Health Organization Meeting on the Immunology of Chagas' Disease. Proceedings of the meeting. Bull. World Health Org. 1974. 50: 459-472.

18. Kierszenbaum, F., Knecht, E., Budzko, D.B. and Pizzimenti, M.C. 1974. Phagocytosis: a defense mechanism against infection with Trypanosoma cruzi. J. Immunol. 112: 1839-1844.

19. Budzko, D.B. and Kierszenbaum, F. 1974. Isolation of Trypanosoma cruzi from blood. J. Parasit. 60: 1037-1038.

20. Budzko, D.B., Pizzimenti, M.C. and Kierszenbaum, F. 1975. Effects of complement depletion in experimental Chagas' disease. Immune lysis of virulent blood forms of Trypanosoma cruzi. Infect. Immun. 11: 86-91.

21. Kierszenbaum, F. 1975. Estudios sobre resistencia contra la enfermedad de Chagas experimental. Medicina (Buenos Aires) 35: 371-372.

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23. Kierszenbaum, F. 1975. Enhancement of resistance and suppression of immunization against experimental Trypanosoma cruzi infection by Corynebacterium parvum. Infect. Immun. 12: 1227-1229.

24. Kierszenbaum, F., Ivanyi, J. and Budzko, D.B. 1976. Mechanisms of natural resistance to trypanosomal infection. Role of complement in avian resistance to Trypanosoma cruzi infection. Immunology 30: 1-6.

25. Kierszenbaum, F. 1976. Cross-reactivity of lytic antibodies against blood forms of Trypanosoma cruzi. J. Parasit. 62: 134-135.

26. Kierszenbaum, F. and Howard, J.G. 1976. Mechanisms of resistance against experimental Trypanosoma cruzi infection. The importance of antibodies and the antibody-forming capacity in the Biozzi high and low responder mice. J. Immunol. 116: 1208-1211.

27. Kierszenbaum, F. and Weinman, D. 1977. Antibody-independent activation of the alternative complement pathway in normal serum by parasitic cells. Immunology 32: 245-249.

28. Kierszenbaum, F. and Budzko, D. B. 1977. Cytotoxic effects of normal sera on lymphoid cells. I. Antibody-independent killing of heterologous thymocytes by guinea pig, rabbit and human sera: role of the alternative pathway of complement activation. Cell. Immunol. 29: 137-146.

29. Budzko, D. B. and Kierszenbaum, F. 1977. Cytotoxic effects of normal sera on lymphoic cells. II. Requirements for inhibition of nonspecific serum cytotoxicity by agarose. Transplantation 23: 337-342.

30. Kierszenbaum, F. and Waksman, B. H. 1977. Kinetics of the potentiation of DNA synthesis by Lymphocyte Activating Factor. In Regulatory Mechanisms in Lymphocyte Activation, pp. 704-706. Ed. by D. O. Lucas, Academic Press, Inc., New York.

31. Kierszenbaum, F. and Waksman, B. H. 1977. Mechanism of action of Lymphocyte Activating Factor (LAF). I. Association of LAF with early DNA synthesis. Immunology 33: 663-669.

32. Budzko, D. B. and Kierszenbaum, F. and Waksman, B. H. 1977. Cytotoxic effects of normal sera on lymphoic cells. III. Selective killing of mouse T cells by normal guinea pig serum. J. Immunol. 119: 381-386.

33. Budzko, D. B. and Kierszenbaum, F. 1978. Complement, serum. In McGraw-Hill Yearbook of Science and Technology, pp. 129-130. McGraw-Hill Book Co. New York.

34. Kierszenbaum, F. and Budzko, D. B. 1978. Identification and characterizatio of a new subset of T lymphocytes. J. Immunol. 121: 166-171.

35. Kierszenbaum, F. and Waksman, B. H. 1978. Mechanisms of action of Lymphocyte Activating Factor (LAF). II. LAF effect is resistant to elevation of cyclic AMP. Immunochemistry 15: 807-811.

36. Katz, S. P., Kierszenbaum, F. and Waksman, B. H. 1978. Mechanism of action of Lymphocyte Activating Factor. III. Evidence that LAF acts on stimulated lymphocytes by raising cyclic GMP in G_1. J. Immunol. 121: 2386-2391.

37. Kierszenbaum, F. and Pienkowski, M. M. 1979. Thymus-dependent control of host defense mechanisms against Trypanosoma cruzi infection. Infect. Immun. 24: 117-120.

38. Kierszenbaum, F. and Ferraresi, R. W. 1979. Enhancement of resistance against Trypanosoma cruzi infection by the immunoregulatory agent muramyl dipeptide. Infect. Immun. 25: 273-278.

39. Kierszenbaum, F. and Budzko, D. B. 1979. The guinea pig complement-activating surface marker (GPCA): functional evidence of its characteristic presence on murine T lymphocytes. In The Molecular Basis of Immune Cell Function, pp. 462-465. Ed. by J. G. Kaplan, Elsevier-North-Holland Biomedical Press, Amsterdam.

40. Kierszenbaum, F. 1979. Antibody-dependent killing of bloodstream forms of Trypanosoma cruzi by human peripheral blood leukocytes. Amer. J. Trop. Med. Hyg. 28: 965-968.

41. Kierszenbaum, F. 1979. Immunization against experimental Trypanosoma cruzi infection: inherent difficulties of a uniform comparative evaluation of antigenic preparations. Tropenmed. Parasit. 30: 287-288.

42. Kierszenbaum, F. and Budzko, D. B. 1979. Changes occurring on the surface of mouse T cells during concanavalin A-induced lymphoblastic transformation. Immunology 38: 827-834.

43. Kierszenbaum, F., Budzko, D. B. and Miller, H. C. 1980. Selective removal of T cell function from mouse lymphocyte suspensions by treatment with normal guinea pig serum. J. Immunol. 124: 1599-1602.

44. Kierszenbaum, F. and Hayes, M. M. 1980. Mechanisms of resistance against experimental Trypanosoma cruzi infection. Requirements for cellular destruction of circulating forms of T. cruzi in human and murine in vitro systems. Immunology 40: 61-66.

45. Kierszenbaum, F. and Hayes, M. M. 1980. Evaluation of lymphocyte responsiveness to polyclonal activators during acute and chronic experimental Trypanosoma cruzi infection. Am. J. Trop. Med. Hyg. 29: 708-710.

46. Kierszenbaum, F. 1980. Protection of congenitally athymic mice against Trypanosoma cruzi infection by passive antibody transfer. J. Parasit. in press.

47. Kierszenbaum, F. and Budzko, D. B. 198 . The guinea pig complement-activating lymphocyte surface component (GPCA): a mouse T cell marker distinct from Thy-1. Immunology, in press.

48. Kierszenbaum, F., Ackerman, S. J. and Gleich, G. J. 198 . Destruction of bloodstream forms of Trypanosoma cruzi by eosinophil granule major basic protein. Submitted.

49. Kierszenbaum, F. 198 . Immunologic properties of rat lymphocytes bearing the guinea pig complement-activating marker (GPCA). Functional properties of peripheral GPCA-positive lymphocytes. Submitted.

50. Hayes, M. M. and Kierszenbaum, F. 198 . Experimental Chagas' disease: kinetics of lymphocyte responses and immunologic control of the transitio from acute to chronic Trypanosoma cruzi infection. Submitted.

51.

12/19/79

CURRICULUM VITAE

JEFFREY F. WILLIAMS

Personal:

Born: [redacted]
Home Address: [redacted] 517-355-3335(O)
Soc. Sec. No: [redacted]
Citizenship: British subject. U.S. Immigrant status.

Education:

Primary:	Public Schools of Bristol, England.	
Secondary:	Queen Elizabeth's Hospital, Bristol, England.	
Undergrad:	1960-64	School of Veterinary Medicine, University of Bristol.
	1964	Bachelor of Veterinary Science (equiv. to DVM) University of Bristol. Awarded with Honors. Distinctions awarded in Parasitology and in Public Health.
	1964	Admitted to the Royal College of Veterinary Surgeons, London.
Graduate:	1964-68	Graduate School of Arts and Sciences University of Pennsylvania.
	1968	Doctor of Philosophy in the Department of Parasitology, University of Pennsylvania. Advisor: Professor E. J. L. Soulsby Thesis: "Immunologic Studies on Experimental Ascariasis."

Professional Experience:

June-Sept. 1964	Veterinarian in private small animal practice, England.
Sept. 1964-June 1966	Assistant Instructor in Parasitology, University of Pennsylvania. Teaching in Veterinary Parasitology; research on ascariasis.
June 1966-June 1968	Research Fellow in Parasitology, University of Pennsylvania. Teaching course in Veterinary Protozoology.
July 1968-Dec. 1970	Parasitologist, Centro Panamericano de Zoonosis, Pan American Health Organization, Buenos Aires, Argentina. Field and laboratory work on helminthic zoonoses of South America, especially echinococcosis. Training postdoctoral fellows from Latin American countries. Participant in First WHO/FAO International Seminar on Hydatid Disease, October, 1970. Consultant on hydatid disease control to Public Health Authorities of Peru, Chile, Uruguay and Argentina.

Professional Experience (cont.):

Jan. 1971-June 1974	Assistant Professor, Department of Microbiology and Public Health, Michigan State University. Primary responsibility for the teaching of Veterinary Parasitology; team leader in Veterinary Epidemiology and Public Health 1974; teaching of graduate courses on aspects of the pathology, epidemiology and immunology of parasitism and regular contributions in graduate course MPH 827 Immunochemistry. Coordinator, integrated veterinary medicine course on alimentary system.
June 1974-June 1977	Associate Professor, Department of Microbiology and Public Health, Michigan State University.
July 1977-Present	Professor, Department of Microbiology and Public Health, Michigan State University.
Sept. 1977-Nov. 1979	Assistant Dean for Research and Graduate Education, College of Veterinary Medicine, Michigan State University. Administrative responsibility for funded research programs in the College of Veterinary Medicine; liaison with Michigan Agricultural Experiment Station; management of Veterinary Research Farm; representative of College of Veterinary Medicine to MSU Research Advisory Council and All-University Research Grant Program Committee.
Nov. 1979-Present	Professor, Department of Microbiology and Public Health and Director of the NIH Sudan Project.

Honors and Grants:

1964-1968	Fulbright Scholarship for Graduate Study in the USA.
1966-1968	Pennsylvania Plan Scholarship for Research in Medical Sciences, University of Pennsylvania.
1972-1975	Principal Investigator, NIH Grant No. AI-10842 (yrs. 1-3). Immunology of Larval Tapeworm Infection (total award: $81,000).
1975-1978	Principal Investigator, NIH Grant No. AI-10842 (yrs. 4-6). Immunology of Larval Tapeworm Infection (total award: $281,540).
1978-1981	Principal Investigator, NIH Grant No. AI-10842 (yrs. 7-9). Immunology of Larval Tapeworm Infection ($280,000 direct costs).
1978-1980	Co-Principal Investigator, Michigan Department of Agriculture to study PBB Recontamination (total award: $200,000).
1979	Principal Investigator, USDA, Project No. 1340. Influence of Gastrointestinal Helminths on Hormonal Status of Dairy Cattle (total award: $10,000).
1979	Henry Baldwin Ward Medal, American Society of Parasitologists. Received August 1, 1979 at the 54th Annual Meeting of the American Society of Parasitologists, Minneapolis, Minnesota.
1979	Phi Zeta Honorary Lectureship. College of Veterinary Medicine, University of Missouri, Columbia, Missouri.

Honors and Grants (cont.):

1979-1984 Principal Investigator, NIH Training Grant No. AI-07203. Experimental Parasitology (total award: $219,739).

1979-1984 Principal Investigator, NIH ICIDR Program Project Grant No. AI-16312. Collaborative Research on Parasitic Diseases in Sudan (total award: $1,515,000).

Professional Societies:

Fellow of the Royal Society of Tropical Medicine and Hygiene.
Member of the Royal College of Veterinary Surgeons.
Member of the British Society of Immunology.
Member of the Phi Zeta Veterinary Association.
Member of the American Society of Parasitology.
Member of the American Society of Tropical Medicine and Hygiene.
Member of the World Association for the Advancement of Veterinary Parasitology.
Member of the Conference of Research Workers on Animal Disease.
Member of the American Association of Veterinary Parasitologists.
Member of the Michigan Veterinary Medical Association.
Member of the American Association of Sheep and Goat Practitioners.
Program Chairman of the American Association of Veterinary Parasitologists Annual Meeting, July 22 to 24, 1979, Seattle, Washington.
Vice-President of the American Association of Veterinary Parasitologists, 1979-1981.

Extension Services:

Member of the Medical Advisory Group to Detroit Zoo, Royal Oak, Michigan.
Lecturer on parasitic diseases of sheep in MSU extension courses on sheep diseases 1973, 1974.
Lecturer on parasitic diseases of goats at MSU -- Farmer's Week 1976, 1977, 1978.

Academic Committee Assignments -- 1972 to Present:

Departmental:

1972-73 Member, Faculty Advisory Committee, Department of Microbiology and Public Health (elected by voting faculty).
1973-74 Chairman, Faculty Advisory Committee, Department of MPH and member of Departmental Administrative Committee (elected).
1974-77 Member, Graduate Committee, Department of MPH (Chairman of the Committee 1976-77, elected).
1975-77 Member, Selection Committee for a Chairman, Department of MPH.
1979-80 Member, Five Year Planning Committee, Department of MPH.

Curriculum Vitae -- Jeffrey F. Williams -- 4

Academic Committee Assignments -- 1972 to Present (cont.):

College:

1972-74	College of Veterinary Medicine, Graduate Research Committee (elected).
1974-75	Member, Selection Committee for a Dean in the College of Veterinary Medicine, Departmental representative (elected).
1974-76	Member, Honor Board (elected by students).
1976-77	Member, Dean's Committee for Self-Evaluating the Veterinary College (appointed by the Dean).
1976-77	Chairman, Committee on Student Performance (appointed).

University:

1974-75	Member, University Educational Policies Committee (appointed by College of Veterinary Medicine, College Advisory Council).
1978-	Member, MSU Title XII Committee.

Graduate Student Advisory and Examining Committees:

Major Professor -- Department of MPH:

R. W. Leid	Ph.D.	1971-73
A. J. Musoke	Ph.D.	1973-75
A. Ansari	M.S.	1973-75
S. J. Hustead	M.S.	1974-76
J. Picone	M.S.	1974-78
M. Calkins	M.S.	1975-present
A. Zajac	M.S.	1975-78
B. Hammerberg	Ph.D.	1975-77
J. Ayuya	M.S.	1976-78
M. Signs	M.S.	1977-present
R. Cook	Ph.D.	1974-79,(Research Advisor, degree granted in Department of Pathology)
P. Engelkirk	Ph.D.	1977-present
M. Ballard	M.S.	1978-present
D. Blaies	M.S.	1978-present

Committee Member -- Department of MPH:

A. J. Musoke	M.S.	1971-73
J. Soni	Ph.D.	1971-73
C. Skov	Ph.D.	1971-74
L. Luong	M.S.	1974-75
D. Liu	Ph.D.	1973-76
L. Baum	Ph.D.	1973-76
S. Thoongsuwan	Ph.D.	1974-76
W. Freimuth	M.S.	1974-76
C. Stone	M.S.	1975-76

Graduate Student Advisory and Examining Committees (cont.):

Committee Member -- Other Departments:

S. Srihakim	M.S.	(Pathology)	1971-72
C. Bink	M.S.	(Pathology)	1973-74
B. Yamini	Ph.D.	(Pathology)	1973-76
J. Wamakoya	M.S.	(Large An Med)	1973-75
H. Lewandowski	Ph.D.	(Entomology)	1974-77
B. Hammerberg	M.S.	(Biochemistry)	1973-74
N. Johnson	Ph.D.	(Dairy Sci)	1975-78
H. Kaski	M.S.	(Entomology)	1976-77
J. Rogers	M.S.	(Entomology	1977-present
C. Carmichael	Ph.D.	(Zoology)	1977-present

Postdoctoral Fellows Supervised

George A. Conder	September, 1979 - present
Gary L. Mills	August, 1979 - present
Richard M. Sandeman	November, 1980 - present

Editorial and Reviewing Services:

Jan. 1977-present	Elected member of the editorial board, Journal of Parasitology (official journal of the American Society for Parasitology).
Jan. 1971-present	Ad hoc manuscript reviewer for Experimental Parasitology, Journal of the American Veterinary Medical Association, Canadian Journal of Comparative Medicine.
1976- 79	Grant reviewer for Pan American Health Organization, National Science Foundation.

Foreign Languages:

Fluent in Spanish, good working knowledge of French and Portuguese.
Read Spanish, French, Portuguese, some Italian.

Outside Interests:

Mixed farming.
Husbandry and management of sheep and goats.

Curriculum Vitae -- Jeffrey F. Williams -- 6

Publications:

1. Schryver, H., W. Medway, and J. F. Williams. 1967. The stomach *Braunina cordiformis* in the Atlantic Bottlenose Dolphi. J. Amer. Vet. Med. Assoc. 151: 884-886.

2. Splitter, E. J., E. J. L. Soulsby, J. F. Williams, and E. L. Jeska. 1967. Gel-diffusion and hemagglutination studies with *Trypanosoma theileri*. Exp. Parasitol. 20: 160-166.

3. Jeska, E. L., J. F. Williams, and D. F. Cox. 1969. *Ascaris suum*: Larval returns in rabbits, guinea pigs and mice after low-dose exposure to eggs. Exp. Parasitol. 26: 187-192.

4. Williams, J. F., and E. J. L. Soulsby. 1970. Antigenic analysis of migratory stages of *Ascaris suum*. I. Comparison of eggs, larvae and adults. Exp. Parasitol. 27 150-162.

5. Williams, J. F. and E. J. L. Soulsby. 1970. Antigenic analysis of migratory stages of *Ascaris suum*. II. Host components. Exp. Parasitol. 27: 362-367.

6. Williams, J. F., and H. Lopez-Adaros. 1970. Algunas consideraciones acerca de la indicencia y distribucion actual de la hidatidosis. Archiv. Int. Hidatodosis. 24: 291-296.

7. Williams, J. F., and C. W. Colli. 1970. Primary cystic infection with *Echinococcus granulosus* and *Taenia hydatigena* in *Meriones unguiculatus* J. Parasitol. 56: 509-521.

8. Williams, J. F., and A. Trejos. 1970. The influence of gelatin capsules upon the activity of bunamidine hydrochloride against *Echinococcus granulosus* in dogs. Res. Vet. Sci. 2: 392-394.

9. Williams, J. F., and U. Prezioso. 1970. Latex agglutination test for hydatid disease using Boerner slides. J. Parasitol. 56: 1253-1255.

10. Williams, J. F., H. Lopez-Adaros, and A. Trejos. 1971. Current Prevalence and distribution of hydatidosis with special reference to the Americas. Amer. J. Trop. Med. Hyg. 20: 224-236.

11. Oriol, R., J. F. Williams, M. Perez-Esandi, and C. Oriol. 1971. Purification of lipoprotein antigens of *Echinococcus granulosus* from sheep hydatid fluid. Amer. J. Trop. Med. Hyg. 20: 569-574.

12. Williams, J. F., M. Perez-Esandi, and R. Oriol. 1971. Evaluation of purified lipoprotein antigens of *Echinococcus granulosus* in the immunodiagnosis of human infection. Amer. J. Trop. Med. Hyg. 20: 575-579.

Publications (cont.):

13. Williams, J. F., and M. Perez-Esandi. 1971. Reaginic antibodies in dogs infected with *Echinococcus granulosus*. Immunology. 20: 451-455.

14. Williams, J. F., and U. Prezioso. 1971. La Prueba de hemaglutinacion indirecta para hidatidosis empleando celulas tratadas con glutaraldehido. Rev. Inst. Med. Trop. Sao Paulo. 13: 333-335.

15. Williams, J. F. 1971. Immunological responses of dogs to tapeworm infection. Gaines Vet. Symp. 21: 35-40.

16. Williams, J. F. 1972. An evaluation of the Casoni test in human hydatidosis using an antigen solution of low nitrogen concentration. Trans. Royal Soc. Trop. Med. Hyg. 66: 160-164.

17. Colli, C. W., and J. F. Williams. 1972. Influence of temperature on the infectivity of eggs of *Echinococcus granulosus* in laboratory rodents. J. Parasitol. 58: 422-426.

18. Williams, J. F., and C. W. Colli. 1972. Influence of ionizing irradiation on the infectivity of eggs of *Echinococcus granulosus* in laboratory rodents. J. Parasitol. 58: 427-430.

19. Varela-Diaz, V. M., M. A. Gemmell, and J. F. Williams, 1972. *Taenia hydatigena* and *T. ovis*: Antigen Sharing. XII. Immunological responses of the mammalian host against tapeworm infections. Exp. Parasitol. 32: 96-101.

20. Schantz, P. M., J. F. Williams, and C. Riva Posse. 1973. Epidemiology of hydatid disease in southern Argentina. Amer. J. Trop. Med. Hyg. 22: 629-641.

21. Williams, J. F., and C. W. Colli, R. W. Leid, and R. McArthur. 1973. The effects of bunamidine hydrochloride on the infectivity of taeniid ova. J. Parasitol. 59: 1141-1144.

22. Leid, R. W., and J. F. Williams. 1974. The immunological response of the rat to infection with *Taenia taeniaeformis*. I. Immunoglobulin classes involved in passive transfer of resistance. Immunology. 27: 195-208.

23. Leid, R. W., and J. F. Williams. 1974. The immunological response of the rat to infection with *Taenia taeniaeformis*. II. Characterization of reaginic antibody and an allergen associated with the larval stage. Immunology. 27: 209-225.

24. Varela-Díaz, V. M., J. F. Williams, E. Coltorti, and C. S. F. Williams. 1974. Survival of cysts of *Echinococcus granulosus* after transplant into homologous and heterologous hosts. J. Parasitol. 60: 608-612.

Curriculum Vitae -- Jeffrey F. Williams -- 8

Publications (cont.):

25. Musoke, A. J., and J. F. Williams. 1975. Immunoglobulins associated with passive transfer of resistance to *Taenia taeniaeformis* in the mouse. Immunology. 28: 97-102.

26. Anteson, R. K., and J. F. Williams. 1975. Selective depletion of hemolymph proteins of *Biomphalaria glabrata* infected with *Schistosoma mansoni*. J. Parasitol. 61: 149-151.

27. Leid, R. W., and J. F. Williams. 1975. Reaginic antibody response in rabbits infected with *Taenia pisiformis*. Int. J. Parasitol. 5: 203-2(

28. Dade, A. W., J. F. Williams, D. L. Whitenack, and C. S. F. Williams. 197! An epizootic and cerebral nematodiasis in rabbits due to *Ascaris columnaris*. Lab. Anim. Science. 25: 65-69.

29. Williams, J. F., J. Westheimer, and W. R. Banman. 1975. *Mesocestoides* infection in the dog. J. Amer. Vet. Med. Assoc. 166: 996-998.

30. Musoke, A. J., J. F. Williams, R. W. Leid, and C. S. F. Williams. 1975. The immunological response of the rat to infection with *Taenia taeniaeformis*. IV. Immunoglobulins involved in passive transfer of resistance from mothers to offspring. Immunology. 29: 845-854.

31. Dade, A. W., and J. F. Williams. 1975. Hepatic and peritoneal invasion by adult ascarids *(Toxocara canis)* in a dog. Vet. Med./Small Anim. Clin. August: 947-949.

32. Musoke, A. J., and J. F. Williams. 1975. The immunological response of the rat to infection with *Taenia taeniaeformis*. V. Sequence of appear ance of protective immunoglobulins and the mechanism of action of 7Sγ2a antibodies. Immunology. 29: 855-866.

33. Williams, J. F., and A. W. Dade. 1976. *Dirofilaria immitis* infection in a wolverine. J. Parasitol. 62: 174-175.

34. Hammerberg, B., A. J. Musoke, S. T. Hustead, and J. F. Williams. 1976. Anti-complementary substances associated with taeniid metacestodes, p. 233-240. *In* E. J. L. Soulsby (ed.), Pathophysiology of helminth infections. Academic Press, Inc., New York.

35. Musoke, A. J., and J. F. Williams. 1976. Immunological response of the rat to infection with *Taenia taeniaeformis*: Protective antibody response to implanted parasites. Int. J. Parasitol. 6: 265-269.

36. Williams, J. F., and R. Oriol. 1976. Immunological response to experimental echinococcosis in *Meriones unguiculatus*. J. Parasitol. 62: 563-568.

Publications (cont.):

37. Williams, J. F., and K. K. Keahey. 1976. Sudden death associated with treatment of three dogs with bunamidine hydrochloride. J. Amer. Vet. Med. Assoc. 168: 689-690.

38. Ansari, A., and J. F. Williams. 1976. The eosinophilic response of the rat to infection with *Taenia taeniaeformis*. J. Parasitol. 62: 728-736

39. Ansari, A., J. F. Williams, and A. J. Musoke. 1976. Antibody-mediated secondary eosinophilic response to *Taenia taeniaeformis* in the rat. J. Parasitol. 62: 737-740.

40. Williams, J. F., A. W. Dade, and R. Benne. 1976. Posterior paralysis associated with anthelmintic treatment of sheep. J. Amer. Vet. Med. Assoc. 169: 1307-1309.

41. Hammerberg, B., A. J. Musoke, and J. F. Williams. 1977. Activation of complement by hydatid cyst fluid of *Echinococcus granulosus*. J. Parasitol. 63: 327-331.

42. Hustead, S. T., and J. F. Williams. 1977. Permeability studies of taeniid metacestodes. I. Uptake of proteins by larval stages of *Taenia taeniaeformis, Taenia crassiceps,* and *Echinococcus granulosus*. J. Parasitol. 63: 314-321.

43. Hustead, S. T., and J. F. Williams. 1977. Permeability studies of taeniid metacestodes. II. Antibody-mediated effects on membrane permeability in larvae of *Taenia taeniaeformis* and *Taenia crassiceps*. J. Parasitol. 63: 322-326.

44. Williams, J. F., C. S. F. Williams, M. Signs, and L. Hokama. 1977. Evaluation of a polycarbonate filter for the detection of microfilaremia in dogs in Central Michigan. J. Amer. Vet. Med. Assoc. 170: 714-716.

45. Hammerberg, B., A. J. Musoke, J. F. Williams, and R. W. Leid. 1977. Absorption of homologous immunoglobulins by the suckling rat. Lab. Anim. Sci. 27: 50-53.

46. Williams, J. F. 1977. Parasitic diseases of the respiratory tract. *In* R. Kirk (ed.), Current Veterinary Therapy. W. B. Saunders, Philadelphia. 270-276.

7. Dade, A. W., J. F. Williams, A. L. Trapp, and W. H. Ball. 1977. Cerebral nematodiasis in captive nutria. J.A.V.M.A. 171: 885-886.

8. Musoke, A. J., H. W. Cox, and J. F. Williams. 1977. Experimental infection with *Plasmodium chabaudi* in rats: Antigen and antibody associated with anemia and glomerulonephritis of acute infection. J. Parasitol. 63: 1081-1088.

Curriculum Vitae -- Jeffrey F. Williams -- 10

Publications (cont.):

49. Musoke, A. J., J. F. Williams, and R. W. Leid. 1978. Immunological response of the rat ot infection with *Taenia taeniaeformis*. VI. The role of immediate hypersensitivity in resistance to reinfection. Immunology. 34: 565-570.

50. Hammerberg, B., and J. F. Williams. 1978. Interaction between *Taenia taeniaeformis* and the complement system. J. Immunol. 120: 1033-1037.

51. Hammerberg, B., and J. F. Williams. 1978. Physicochemical characterization of complement-interacting factors from *Taenia taeniaeformis*. J. Immunol. 120: 1039-1045.

52. Cook, R. W., and J. F. Williams. 1978. Surgical removal of the pyloric antrum in weanling rats. Lab. Animal Sci. 28: 437-439.

53. Williams, J. F., and C. S. F. Williams. 1978. Psoroptic ear mites in dairy goats. J.A.V.M.A. 173: 1582-1583.

54. Leid, R. W., and J. F. Williams. 1979. Helminth parasites and the host inflammatory system. *In* Chapter 7 Chemical Zoology, B. Scheer and M. Sorkin (eds.). Academic Press. Vol. XI: 229-271.

55. Ayuya, J. M., and J. F. Williams. 1979. Immunological response of the rat to infection with *Taenia taeniaeformis*. VII. Oral and parenteral immunization with parasite antigens. Immunology. 36: 825-834.

56. Williams, J. F. 1979. Recent advances in the immunology of cestode infections. J. Parasitol. 65: 337-349.

57. Hammerberg, B., C. Dangler, and J. F. Williams. 1980. *Taenia taeniaeformis:* Chemical composition of parasite factors affecting coagulation and complement cascades. J. Parasitol. 66: 569-576.

58. Zajac, A., and J. F. Williams. 1980. Infection with *Schistosomatium douthitti* (Fam: Schistosomatidae) in the Meadow Vole, *Microtus pennsylvanicus* in Michigan. J. Parasitol. 66: 366-367.

59. Engelkirk, P., J. F. Williams, R. W. Leid, and G. Schmidt. 1980. Zoonotic Onchocerciasis. *In* Parasitic Zoonoses. (In press).

60. Picone, J., J. F. Williams, R. W. Leid, and L. D. Fay. 1980. Polycystic Liver in White-Tailed Deer. J. Wildlife Diseases. (In press).

61. Dubey, J. P., S. P. Sharma, C. W. G. Lopes, J. F. Williams, C. S. F. Williams, and S. E. Weisbrode. 1980. Caprine Toxoplasmosis: Abortion, Clinical Signs, and Distribution of *Toxoplasma* in Tissues of Goats fed *Toxoplasma gondii* Oocysts. Amer. J. Vet. Res. 41:1072-1076.

Curriculum Vitae -- Jeffrey F. Williams -- 11

Publications (cont.):

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Attachment B
PAGE 51

ENVIRONMENTAL THRESHOLD DETERMINATION

TO: AA/DS, Mr. Sander Levin

THRU: DS/PPU, Mr. John Gunning

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: _____
1-29-78

Antigenic Analysis of Cultured Plasmodium falciparum (New) - Michigan State University. Duration of project, 3 years; total estimated cost, $308,251.

Project Summary and Background: This project was proposed by a young researcher who contributed to early breakthroughs in malaria research and who now has established his own laboratory at Michigan State University. Initially it was believed that the merozoite stage of development of malaria was the stage that contained antigens likely to be protective against the malaria parasite. However, recent research results have suggested that analyses of all parasite antigens should be conducted before it can be determined which one is the best candidate for a vaccine. The results from the research in this proposal should extend understanding of the antigenic composition of cultured malaria parasites and particularly the soluble exoantigens. This knowledge will facilitate the development of the malaria vaccine by identifying antigens that may elicit a protective immune response.

RAC Discussion: Malaria research in the AID network is now following three parallel tracks. This project represents one of those tracks, namely, looking at soluble exoantigens which are released into the supernatant fluid of in-vitro cultures of P. falciparum. It adds a new dimension to the overall research effort. The RAC reviewers felt that the proposal was in line with the recently revised AID strategy and was in an area considered as high priority. It was suggested that the researcher use a strain of malaria that is specific to the site of the field tests, that the research be narrowly focused on its main objective and that budget items, particularly laboratory equipment be examined carefully.

RAC Recommendation: That the project be approved with two provisos: (1) the research be focused on the study of exoantigens and (2) the budgetary and other items be reexamined by AID staff in light of RAC's discussion.

Related Research Projects Funded by AID: This project will become part of an AID malaria research program currently consisting of ten projects carried out by a network of nine 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:

1. Large Scale Cultures of Human Malaria (Plasmodium falciparum) and their Use for Immunization and for Antigenic Analysis of the Parasites (Rockefeller University)

2. Sporozoite-Induced Immunity in Simian Malaria (New York University)

3. Preparation and Evaluation of Merozoite Antigen in the Immunoprophylaxis of Malaria (University of New Mexico)

4. Continuous In-vitro Cultivation of Various Strains of *Plasmodium falciparum* at High Parasitemia Production and Purification of Antigen and Vaccination Studies (University of Hawaii)

5. *In-vitro* Cultivation of Human Malaria (New York University)

6. *In-vitro* Cultivation Malaria Parasites (Gorgas Memorial Institute of Tropical and Preventive Medicine)

7. Assessment of Potential Antimalaria Vaccines (Instituto Nacional de Salud, Bogota, Colombia)

8. Ultrastructural and Cytochemical Analysis of Malaria Antigens (Uniformed Services University of the Health Sciences)

9. *In-vitro* Cell Culture of the Erythrocytic Stage of Malarial Parasite (Biomedical Institute)

10. Isolation and Evaluation of Malaria Immunogens (University of Missouri)

ACTION MEMORANDUM FOR THE ADMINISTRATOR

MAR 27

THRU: ES

FROM: AA/DS, Stephen C. Joseph, M.D. (Acting)

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

Discussion: We are submitting for your approval five research projects following the recommendations of the RAC which reviewed the project proposals at its meeting on March 3-4. Two proposals are for new projects and three proposals are for extension of ongoing activities.

All five projects are included within the program activities described in the FY 81 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). Information on individual projects together with RAC recommendations is appended at TAB B.

In brief, two projects were endorsed as proposed and the remaining three projects were recommended for approval subject to specified modifications and conditions. We agree with the RAC recommendations and shall incorporate them into the relevant Project Authorization (PAFs) for my signature if you give your approval. In the case of the International Fertility Research Program, the proposed project funding exceeds my $10 million authorization limit. If you concur with its implementation you are requested to sign the PAF for the project (TAB C).

The recommendation relating to a sixth project "Program for Applied Research on Fertility Regulation" (PARFR) is a follow-up of the November RAC meeting. It is included for your information only since the project was approved by A/AID on January 2, 1981.

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In addition to the above projects, RAC also reviewed preliminary proposals for "Forest Surveys" and "Man and the Biosphere" projects. The RAC comments on these proposals are included with other project summaries under TAB B. Inasmuch as the two proposals are still in the developmental stage, we are not asking you for your approval of these projects at this time.

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

The approval of the five 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).

Recommendations: 1. That you approve the implementation of five RAC endorsed projects listed at TAB A along the lines of the RAC recommendations.

APPROVED: M. Parker *
DISAPPROVED:
DATE: 9 MAY 1981

2. That you sign the attached Project Authorization (PAF) for the International Fertility Research Program (IFRP). - See Tab 5 for Project Statement (??)

Attachments:

TAB A - List of projects and funds proposed for implementation
TAB B - Project summaries with RAC recommendations
TAB C - Project authorization for the International Fertility Research Program project

Clearances:
GC:JRBolton JRB Date 4/24/81
GC/THFA:ARichstein Date
A/AID:HMinners Date 16 Apr 81
AA/PPC:CPaolillo CP Date 4/24/81
DS/DAA/FN:EBabb Date 3-25-81
DS/DAA/DT:FTugwell Date 3/24/81
DS/PO:BChapnick Date 3/20/81
AAA/PPC/PDPR:JErikssonfor Date 4/20/81

DS/PO/RES:MRechcigl:lws/cev:3/16/81:X59011

* with the understanding that the International Fertility Research Program will be implemented in accordance with the policy set forth in Dr. Joseph's memorandum

[redacted]
M. cleared - JRB 5/6/81