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(101) University of New Mexico, Department of Biology

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(Research summary)

9. ABSTRACT

This project's general objectives were to develop methods for the large-scale production of malaria antigens, and to ascertain efficient means of immunizing mammals, utilizing rodent and primate testing systems. Specific objectives were: 1) antigen production: in vitro culture (sporozoite and related antigens, trophozoite antigens), in vivo culture (sporozoite and related antigens trophozoite antigens, attenuated malaria strains); 2) antigen investigation: rodent model system (sporozoite and related antigens, trophozoite antigens), primate model system (sporozoite antigens, trophozoite antigens); and 3) supportive investigation (ultrastructure studies, immunobiologic studies). Among the project's accomplishments were the production of a non-viable malaria antigen vaccine shown free of host antigen contamination. Although there yet are no methods for mass production of malaria antigen, the prospect of commercial production has been enhanced greatly. A suitable adjuvant for use with such a vaccine in humans is now available. Attempts to ascertain the need for a polyvalent vaccine were inconclusive. Results from studies on long-term immunity in Rhesus monkeys indicates that vaccine-induced immunity overrides the strain and variant antigenic differences which may exist as detected by sero-typing.

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REPORT SUMMARY

A. Project Title and Contract Number: Malaria Immunity and Vaccination AID/csd-3689

Principal Investigator: Paul H. Silverman

Contractor: The University of New Mexico

Mailing Address: Albuquerque, New Mexico 87131

Contract Period: June 30, 1972 - April 30, 1976

Period Covered by Report: Final Report - June 30, 1972 - April 30, 1976

Total A. I. D. funding of contract: $2,002,438

Total expenditures and obligations (estimated): $2,002,438

B. General Background

"The need for [a] malaria vaccine is becoming more urgent every year. The worldwide malaria eradication effort, which reached its peak in the mid-1960s, led to major reductions in the levels of malaria in 58 countries specifically aiming at eradication and led directly to country-wide eradication of malaria in 37 countries (21 already certified by WHO). However, by the late 1960s and early 1970s malaria recurred on a large scale in certain countries, largely due to administrative and operational problems including those brought on or aggravated by insecurity and political instability. These problems delayed the implementation of planned programs and allowed time for the development of technical obstacles, such as mosquito resistance to insecticides and parasite resistance to anti-malaria drugs. The emergence of technical problems in many areas will make it more difficult to repeat earlier successes in achieving a reduction of malaria in two to three years. With existing technology, progress will be slower and more costly. Protection of past gains will be more difficult.

"At the 28th World Health Assembly in May 1975, 31 country delegates referred to the serious worldwide problem. Several countries specifically expressed the hope a malaria vaccine might be developed. An effective vaccine would provide new methodology with great potential significance to anti-malaria programs.

"At the time AID support for malaria vaccine research was started in 1965, many immunologists and parasitologists felt that vaccination against malaria was impractical or impossible. However, AID and its Research Advisory Committee decided that in view of the seriousness of malaria recurrence, the diminishing effectiveness of current technology and potential benefits of immunization, the attempt should be made to support efforts to study malaria immunity and to explore the feasibility of immunization with an ultimate objective of developing a human vaccine. In supporting this research, AID has recognized that it is a high-risk-high-payoff program which, if successful, would have a tremendous beneficial impact throughout the world.

"In recent years more immunologists and parasitologists have granted the biological feasibility of immunization. Opinion at recent scientific meetings on malaria immunity is increasingly supportive of the view that practical immunization against human malaria may be largely a matter of time and effort and that recent advances warrant an increased effort to that end."

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The above introductory statement was prepared by AID personnel (1975) in support of the request for continued funding of the Malaria Immunity & Vaccination Program as a collaborative international network effort. This background statement provides an excellent, succinct introduction which identifies both the need for a malaria vaccine and also the skepticism expressed by "...many immunologists and parasitologists...that vaccination against malaria was impractical or impossible." The fact that the scientific community has shown increasing acceptance of the feasibility of a malaria vaccine, is due in no little part to the efforts of the malaria research group at The University of New Mexico. It is a fascinating irony that several of the more vociferous "skeptics" whose overt and covert hostility to the project at times resulted in negative, destructive pressures, are now guiding the network effort.

C. Project Objectives

The general objectives identified in Contract No. AID/csd-3689 were twofold:

1. To develop methods for the large-scale production of malaria antigens, and

2. To ascertain efficient means of immunizing mammals, utilizing rodent and primate testing systems.

The specific objectives are outlined on pages 2-12 of Contract No. AID/csd-3689. In brief, the tasks to have been undertaken are listed below:

1. Antigen Production

   a. *In vitro* Culture
      Sporozoite and related antigens
      Trophozoite antigens

   b. *In vivo* Culture
      Sporozoite and related antigens
      Trophozoite antigens
      Attenuated malaria strains

2. Antigen Investigation

   a. Rodent Model System
      Sporozoite and related antigens
      Trophozoite antigens

b. Primate Model System
Trophozoite antigens
Sporozoite antigens

3. Supportive Investigation

a. Ultrastructure studies

b. Immunobiologic studies

Subcontracts, with AID approval, were let to the University of Illinois (1972-73), and to Rush-Presbyterian University (1974-75) for various parts of the overall tasks. As the work proceeded, certain of the objectives were abandoned (e.g., sporozoite antigen production and investigation) and new objectives or emphases were added (e.g., establishment of Aotus-P. falciparum system and enhanced biochemical exploration of nucleic acid antigens) in accordance with recommendation of RAC subcommittee consultants in February, 1975, or, the NAS 1974 Workshop recommendations. The specifics of each of these changes in objectives will be identified in the sections which deal with the various research areas. It should be emphasized that all of the activities carried out during the past three years and nine months fall within the various categories of tasks to be undertaken as outlined in the general and specific objectives of contract AID/csd-3689.

D. Accomplishments

The accomplishments achieved under Contract AID/csd-3689 will be presented in accordance with the outline of the tasks to be undertaken as listed under Project Objectives. Numerous tasks were pursued as basic prerequisites to to the performance of meaningful research activities. In this final report no attempt will be made to identify or comment on all of these routine activities except where the development of a new method or technique represents a significant new contribution to science. Publications are listed at the end of the narrative and referred to by numerical designation in the text.

IN VITRO CULTIVATION AND ANTIGEN PRODUCTION

Sporogonic forms

Pioneering efforts to develop methods for the in vitro cultivation of sporozoites as a source of antigen were carried out under the direction of Dr. Maria C. Rosales-Ronquillo-Sharp. These efforts led to the development of a new method for the aseptic rearing of Anopheles stephensi, a mosquito vector of malaria (12). Primary cultures and new cell lines were established from A. stephensi larval stages which are new to science (11, 13, 14). Utilizing the aseptic mosquito rearing technique, it was found possible to culture various organs for long-terms under antibiotic-free conditions (21). Young oocysts were observed to develop in the tissues of mosquito gut cultured under germ- and antibiotic-free conditions.

Several major findings include the determination that Plasmodium berghei ookinetes can undergo formation in vitro (13,23,24) and develop into young oocysts under conditions suitable for the cultivation of suitable host-cell lines. In addition to the mosquito cell lines examined as candidate host tissues, other established non-vector cell lines were examined. Several established non-vector cell lines which grew under pH and temperature conditions similar to the anopheline cell lines were tested. The fathead minnow line of epithelial cells appeared to demonstrate the capacity of supporting ookinete formation (16) and development to the young oocyst stage (17). These highly promising results have not been pursued since the decision to terminate the sporozoite work was made in early 1975.

Erythrocytic stages

Active work on the in vitro cultivation of the erythrocytic stages of rodent and primate malaria was undertaken beginning in late 1973 after the arrival of Dr. Clarence A. Speer. Most previous studies on in vitro cultivation of erythrocytic stages of malaria have been focused on the maintenance of the red blood cells. However, red blood cells are incapable of reproducing and the survival of parasites in erythrocytes has not been reported (until recently) to survive longer than 2-3 cycles.

For these reasons, it was decided to focus our efforts on the use of primary and established cultures of nucleated (eukaryotic) cells, which are capable of reproducing indefinitely in vitro, as potential host cells for malaria parasites. Encouragement for this approach was provided by the experience of other workers who were able to culture in vitro the obligate intracellular protozoan parasite, Eimeria, which is closely related to Plasmodia. Dr. Speer, who had trained with Prof. Datus Hammond, had considerable experience in this area of research.

Preliminary experiments with primary cell cultures included cells prepared from hamster, mouse and monkey bone marrow, liver and macrophages. Various media were tested in conjunction with different cell types and included the addition of factors such as cyclic-AMP. (The details are recorded in recent progress reports and will appear in publication shortly [25,27].) As a result of these trials, it was found that P. berghei erythrocytic stages underwent replication in primary mouse and hamster bone marrow cells and produced extracellular merozoites which infected other cells. Because of this finding, which represented the first successful in vitro cultivation of mammalian plasmodium in nucleated cells, the work was extended to screen various established cell-line cultures in order to find a cell type capable of supporting growth of mammalian Plasmodium species.

A total of 14 established cell-lines were tested, each with at least 4 different culture media. The cell-lines originated from various sources including, human, monkey, mouse, hamster, bovine and fish. Although rodent, monkey and human malaria were used in various experiments as culture inocula, most of the efforts focused on P. berghei because it was most readily available. Eventually, it was found that P. berghei could be successfully cultivated in an established cell line, the mouse Leydig cell testicular tumor cultures (The full details have been presented in recent progress reports and also will be published shortly [27].) This major accomplishment provides stimulus and encouragement for the application of this approach to primate malarias.

Unfortunately, while at its most promising, the culture work at the University of New Mexico was terminated with the close of Contract AID/csd-3689.

ANTIGEN INVESTIGATION

Rhesus monkey-Plasmodium knowlesi system

This project has demonstrated that it is possible to vaccinate Rhesus monkeys against malaria with a lyophilized plasmodial blood stage antigen (8, 18, 19, 22). This is the only malaria antigen preparation available which retains its vaccine quality after lyophilization, an essential requisite for long-term storage in the tropics. The effectiveness of the vaccine has been repeatedly demonstrated and found to be free of observable side-effects (8). The vaccine has been shown to be highly effective over a four-year period, essentially, the life of a Rhesus monkey (7). The vaccination protection is effective against both homologous and heterologous variants of P. knowlesi.

An important characteristic of the malaria vaccine is a need for an adjuvant. This is related to the predominantly cell-mediated immune response which is characteristic of malaria as compared with the primarily antibody response of many microbial infections (28). This project has been able to identify an effective adjuvant suitable for use in humans (19).

In addition to the protection testing, a variety of other studies have been carried out to investigate the biochemical and ultrastructural characteristics of the malaria vaccine antigen (4, 9, 26). These studies have shown that the antigen is free of contamination with host materials and that it appears to consist largely of parasite membrane material. Other studies on the examination of various antigen components are summarized in a later section below.

Aotus monkey-Plasmodium falciparum system.

As part of the sub-contract to the Rush-Presbyterian Medical School and its Tropical Disease Laboratory at Stateville Prison, Joliet, Illinois, under Dr. Karl Rieckmann, an attempt was made to apply the methods developed in the P. knowlesi-Rhesus system to Aotus monkeys and human P. falciparum malaria. A single, inconclusive experiment was initiated which involved a total of six Aotus monkeys (3 vaccinated, 3 controls). Only about 25% of the standard vaccine dose was used and no immunological assays were run to determine the immune status of "vaccinated" animals.

Unfortunately, the decision by the Governor of the State of Illinois to close the medical volunteer prisoner program resulted in the premature termination of work under the sub-contract. In 1975, Dr. Rieckmann and the Aotus monkeys were moved to the University of New Mexico. No attempt to repeat this work has been made under Contract AID/csd-3689. Hopefully, this will be fully and properly pursued as part of the malaria vaccination program which has been funded by AID for 1976-79.

Rodent malaria system

The rodent has been used to investigate a variety of aspects of malaria vaccination and characterization of the antigen which would be difficult or impossible to pursue utilizing the expensive and time-consuming primate malaria system. The topics which were investigated focused particularly on chemical identification and on ultrastructural components of the standard French press antigen preparation.

Chemical analysis of the active vaccine antigen (as detailed in the 1975 annual report) revealed that for each milligram of protein, the antigen contained 70-100μg of nucleic acid, primarily RNA. Preliminary protection tests in mice with the nucleic acid fraction of P. berghei antigen revealed that as little as 1.6μg RNA conferred a highly significant increase in the life expectancy of infected A/J mice (1, 3). These preliminary data were presented to a group of AID consultants and RAC members during a brief visit to UNM in February, 1975. The visiting group recommended that the work on ribosome and nucleic acid antigen preparations "...appear promising and certainly should be extended."

In response to the written recommendation and oral advice to acquire formal relations with a biochemical consultant, an arrangement with Dr. Michael Bleyman of the University of North Carolina Department of Biochemistry was effected to utilize his services during the remaining time available under AID/csd-3689. This has proved to be a highly fruitful relationship. The preliminary data on the immunogenicity of nucleic acid constituents of P. berghei antigen have been confirmed and extended. The active RNA has been shown to be derived from P. berghei ribosomes (1). This is the first report on the immunogenicity of malaria nucleic acids.

In addition, it was found that the protective effect in rodents can be transferred by RNA extracted from lymph nodes of rats immune to P. berghei infection (2).

On the basis of these results and because of the previous reports of numerous workers on the antigenic variation of mammalian malaria during the erythrocytic cycle, it was postulated that mimicry of host antigens by parasites resides in the ability of the parasite to acquire genetic information from the host by means of sharing of post-transcriptional mechanisms. In order to pursue the relevance of this postulate, Dr. Bleyman undertook to analyze the genomic homology and transcriptional and translational relations between P. berghei and several rodent hosts (mouse, rat, hamster, guinea pig and agouti). The following determinations and techniques were carried out: 1) DNA content of P. berghei and estimates of the haploid coding length; 2) DNA/RNA hybridization techniques applied to host and parasite material; 3) analysis of mRNA in infected reticulocytes; 4) characterization of ribosomal RNA from host and parasite; 5) annealing experiments between plasmodial and rodent tRNA, mRNA and DNA; and, 6) characterization of ribosomal protein.

The results ruled out adventitious contamination and showed persistence of host informational macromolecules within the parasite. These data strongly support the proposed co-utilization of mammalian information by the parasite from the host material as shared informational macromolecules (5).

The full ramifications of these results are difficult to estimate, but clearly they have major immunological and therapeutic implications for most protozoan and metazoan parasitic infections of international concern (i.e. trypanasomiasis, leishmaniasis, schistosomiasis, filariasis, etc.). Unfortunately, this work has been terminated with the end of Contract AID/csd-3689. Presentations at national meetings and publication of the results will be carried out in the coming months, although the funds with which to do this are limited or no longer available.

Sporozoite antigen studies in rodents

At the time of the transfer of the Malaria Immunity and Vaccination project from the University of Illinois to the University of New Mexico in July, 1972, a sub-contract was placed with the University of Illinois. This subcontract, under the direction of Dr. Nelda Alger was charged with the responsibility of undertaking "...critical studies on quantitative and qualitative aspects of sporozoite immunization...needed for the establishment of a suitable rodent model system". In addition, services in the area of "ultrastructural evaluation" with the electron microscope were to be provided to the University of New Mexico by the University of Illinois.

After visits to the University of Illinois by Dr. Silverman in September, 1972 and February, 1973 in the company of Mr. Edgar Smith, Dr. Michael Rechcigl, Professor John Scanlon (AID consultant) and Dr. Robert Schenkel (Associate Director of the Malaria Project, UNM), and on the basis of the first six months report (January, 1973), it was concluded not to renew the sub-contract which was due to terminate in June, 1973. The decision was conveyed to Dr. Alger and the University of Illinois in February, 1973. The work on sporozoite immunity, however, was to be continued at the University of New Mexico and was to be based on the progress and experiences of the Illinois sub-contract group.

As the prime AID contractor responsible for certifying an appropriate "level of effort" judged to be minimally adequate for payment to a sub-contractor, the University of New Mexico was faced with a difficult task by the lack of adequate information from the University of Illinois. Requests were made to the University of Illinois in July, October and December of 1973 asking for the relevant data and materials. These data were also needed in order to allow work to proceed at the University of New Mexico without wasteful replication of effort.

After protracted delays by the University of Illinois, the relevant documents and materials were sent to the University of New Mexico on September 19, 1975. After careful review and discussion, the conclusion was reached that the sub-contractor apparently carried out activities during 1972-73 related to the tasks identified in the SCOPE OF WORK section of the sub-contract.

SUPPORTIVE INVESTIGATIONS

Ultrastructural studies

As a result of the inadequacies of the electron microscope services originally planned to be available through the sub-contract with the University of Illinois, a new electron microscope facility was provided by the University of New Mexico. Also the services of a scanning electron microscope (SEM) were made

available to the project at the UNM School of Medicine. These studies have proven to be of considerable value in assessing the in vitro cultivation work, in the ultrastructural analysis of malaria antigen and the evaluation of the hypersensitivity skin reactions in immunized and control monkeys (8, 9, 23, 24, 26, 27).

These studies confirmed that the P. berghei stages cultivated in vitro in a eukaryotic cell line (Leydig cells) were undergoing normal schizogony. These data give support to the view that a continuous cultivatable cell line may offer a potential production system for malaria vaccine antigen.

The ultrastructural analysis of an active immunizing preparation of P. knowlesi antigen indicates that the vaccine consists mostly of parasite membranes in association with a fine granular material (ribosomes?). The vaccine material was shown to be free of contamination with host cell membrane.

Electron microscopic studies of biopsy material taken from skin-test sites injected with malaria vaccine antigen, aided in confirming that the cellular response in vaccine immunity was typical of a delayed hypersensitivity reaction (8). The EM information provided additional support for the view that a major component of the immunity to malaria is of the cell mediated type. This view is in contrast to the previously expressed opinion that the major component of malaria immunity was antibody mediated. It is increasingly apparent that both components are involved, most likely on some interactive basis. The nature of the mechanism remains to be fully elucidated. Recent in vitro work in our laboratory indicates that opsonizing antibody may be related to the degree of phagocytosis of parasites by peritoneal macrophages in rodent malaria. These techniques will need to be explored further to ascertain the relation to protective immunity in vivo.

Immunobiologic studies

A variety of immunological assays have been under investigation with the goal of developing laboratory and field techniques which can provide indices which reflect the immune status of malaria host populations. These indices will also be essential in establishing the efficacy of vaccination procedures. Work in this project, as well as by other investigators, has demonstrated that standard serological assays do not yield data which correlate with the immune status of hosts and can not be used for predictive evaluation of vaccination procedures. The range of standard serological assays examined have included the merozoite inhibition test. All of these tests have been abandoned because of their lack of utility for our purposes. The fact that cell-mediated immune factors are largely involved in malaria immunity, indicates that cell mediated immune correlates are more likely to be relevant than assays which use solely serum. Indeed, we have found that

two cell-mediated correlate tests have yielded highly significant results. These tests are: 1) the lymphocyte transformation test (8); and, 2) the delayed hypersensitivity skin test (8). In addition, a highly sensitive solid-phase radioimmunoassay test which detects small amounts of IgG has provided correlative data and may lead to a useful laboratory and field test (29). Hopefully, these immunological assays will be pursued as part of the future activities of the AID Malaria Immunity and Vaccination effort.

SUMMARY

In the internal AID document (1975) entitled "Proposed Strategy for Expediting Research and Development for a Malaria Vaccine", prepared by AID personnel, there is listed on pages 2 & 3:

"A. Problems Which Must Be Solved:

"1. The production of a purified vaccine with no side effects which is long-lasting and gives consistently high protection.

"2. Development of methods for mass production of antigen for such a vaccine.

"3. Selection of a suitable adjuvant for use with such a vaccine in humans.

"4. Determination of the feasibility and need for a polyvalent vaccine both in terms of blood stage and sporozoite antigens and in terms of species of malaria.

"5. In connection with these four major problems there are numerous other determinations which must be made such as:

"a. The degree of strain and variant specificity of the antigen.

"b. The establishment of standards of comparison for use in testing antigens.

"c. Purification and characterization of the antigen.

"d. Development of a reliable serological or other test for determining the effectiveness of vaccination and its duration.

"e. Delineation of the parameters of post-challenge anemia in test animals.

"f. Determination of the number of strains of malaria in a given area.

"g. Development of a method of assessing strain differences within a single species of malaria."

It is particularly instructive to review the accomplishments of the UNM Malaria Immunity and Vaccination project against the list of "Problems which must be solved".

A. 1. We have produced a nonviable malaria antigen vaccine that has been shown by biochemical, ultrastructural and immunological criteria to be free of host antigen contamination.* The UNM antigen can be lyophilized and stored for long periods in a freeze dried state with little or no loss of activity as determined by immunological assays. It produces no side effects and induces protection in the Rhesus monkey which is essentially life-long to the highly virulent infection with homologous or heterologous variants of Plasmodium knowlesi.

* It is of interest to note that tests in our laboratory with the "merozoite vaccine" of Mitchell et al. have indicated strong auto-immune reactions due to antigen contamination with host RBC's.

2. Although methods for mass production of malaria antigen are not yet in hand, the prospect of commercial production has been greatly enhanced by our finding that mammalian malaria can be cultured in vitro in nucleated cells which are capable of continuous cultivation.

3. Our work demonstrates that "...a suitable adjuvant for use with such a vaccine in humans" is available. The components of the adjuvant which we described in 1975 (19) have all been cleared for use in human vaccination.

4. Our attempts were inconclusive to ascertain the need for a polyvalent vaccine. However, it would appear that an effective erythrocytic stage antigen might well suffice as an adequate prophylactic vaccine.

5. a. The results from our studies on long-term immunity in Rhesus monkeys indicate that our vaccine-induced immunity over-rides the strain and variant antigenic differences which may exist as detected by sero-typing. Work in other laboratories indicate that antigenic cross reactivity in P. falciparum is very broad and immunity to one geographic strain confers resistance to others widely removed (e.g. Asian vs. African).

b. This group has sought cooperative agreement on the establishment of standards for use in the comparison of malaria antigens. This remains an important accomplishment to achieve.

c. The antigen used in our vaccine has been more fully characterized and described by biochemical and ultrastructural means than any other malaria vaccine yet reported by other workers.

d. We have reported on the only immunological assays so far shown to correlate with resistance and susceptibility. These tests include

the 1) lymphocyte transformation test, 2) the delayed hypersensitivity skin test, and 3) the solid-phase radioimmunoassay.

e. The work reported from this project represents the only data which has consistently followed post-challenge hematologic events in test animals. We hope that all future work will adopt the parameters for such studies as reported in our various publications.

f. and g. The relevance and necessity of extensive work on strain (antigenic) differences seems to be based largely on experimental results obtained with an artificial system (P. knowlesi in Rhesus monkeys). Nevertheless, the value of doing such work might be substantiated if experience in more natural systems (Aotus monkeys and P. falciparum) supports this concern.

E. Dissemination and Utilization of Research Results

PUBLICATIONS

AID/csd-3689 1972-1976

Articles

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Abstracts

30. Barr, M. L., J. Heidrich and P. H. Silverman (1974). Vaccination of mice with ribosomal material extracted from Plasmodium berghei. Fourth International Convocation on Immunology, Grand Island, New York.

31. Barr, M. L., J. Heidrich and P. H. Silverman (1974). Vaccination of mice with ribosomal material extracted from Plasmodium berghei Proceedings of the 2nd International Congress on Immunology, Brighton, England.

32. Barr, M. L., J. Heidrich and P. H. Silverman (1974). Protection of mice with ribosomes from Plasmodium berghei and the RNA from immune lymph nodes and spleens against a lethal challenge. Second International Congress of Immunology. Brighton, England.

33. Barr, M. L., E. J. Cabrera, R. H. Schenkel and P. H. Silverman (1975). Protective effect of subcellular fractions of P. berghei against a lethal challenge of P. berghei in mice. 50th Anniversary Meeting of the American Society of Parasitologists, New Orleans, La.

34. Barr, M. L., E. J. Cabrera and J. E. Heidrich (1976). Immune lymph node RNA as a protective malarial immunogen against Plasmodium berghei. Proceedings of the 60th Annual Meeting of the Federation of American Society for Experimental Biologists, Anaheim, California.

35. Beckwith, R., R. H. Schenkel and P. H. Silverman (1973). Qualitative analysis of phospholipids isolated from non-viable Plasmodium antigen. Proceedings of the Rocky Mountain Conference of Parasitologists, Provo, Utah.

36. Cabrera, E. J., C. A. Speer and P. H. Silverman (1974). Immunological assays as indicators of protection in animals vaccinated with non-viable plasmodial antigens. Third International Congress of Parasitology. Vol. 2, Section D6, P. 1126.

37. Cabrera, E. J. (1975). Skin hypersensitivity in Rhesus monkeys vaccinated against Plasmodium knowlesi. Joint Meeting of the Southern California and Rocky Mountain Conference of Parasitologists, Las Vegas, Nevada.

38. Cabrera, E. J., R. H. Schenkel, M. L. Barr and P. H. Silverman (1975). Lymphocyte reactivity during Plasmodium knowlesi infection in Rhesus monkeys (Macaca mulatta). 50th Anniversary Meeting of the American Society of Parasitologists, New Orleans, Louisiana.

39. Cabrera, E. J., M. L. Barr and P. H. Silverman (1976). Long-term studies on Rhesus monkeys vaccinated against malaria. Proceedings of the 60th Annual Meeting of the Federation of American Societies for Experimental Biology. Anaheim, California.

40. Heidrich, J., E. J. Cabrera, M. L. Barr, R. H. Schenkel and P. H. Silverman (1976). Hemazoin - A malarial pigment. Proceedings of the Rocky Mountain Conference of Parasitologists. Albuquerque, New Mexico.

41. Heidrich, J. E., R. H. Schenkel, M. L. Barr and E. J. Cabrera (1976). Effects of incubation of ^{51}CR labeled RBC with malarial hemazoin. Proceedings of the 60th Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, California.

42. Jakstys, B. P., N. E. Alger, J. A. Harant and P. H. Silverman (1972). Ultrastructural analysis of P. berghei sporozoite antigen. Proceedings of the Society of Protozoologists 25th Annual Meeting, Minneapolis, Minnesota, 15.

43. Jost, R. (1975). Lymphocyte transformation studies in Rhesus monkeys vaccinated against Plasmodium knowlesi. Proceedings of the Southern California and Rocky Mountain Conference of Parasitologists, Joint Annual Meeting, Las Vegas, Nevada.

44. Rosales-Ronquillo, M. C. and P. H. Silverman (1973). In vitro ookinete development of Plasmodium berghei. Annual Meeting of the Society of Parasitologists, Toronto, Canada.

45. Rosales-Ronquillo, M. C., C. A. Speer and P. H. Silverman (1973). Mode of locomotion of ookinete cultivated in vitro. Annual Meeting of American Society of Protozoologists, Houston, Texas.

46. Rosales-Ronquillo, M. C., G. Nienaber and P. H. Silverman (1973). Mass rearing of Anopheles stephensi using a commercial "fish food", Tetramin. Southwest Conference of Entomological Society of America, San Antonio, Texas.

47. Rosales-Ronquillo, M. C. and P. H. Silverman (1974). In vitro cultivation of ookinetes in vector and non-vector cell lines. 3rd International Congress of Parasitology, Munich, Germany.

48. Rosales-Ronquillo-Sharp and P. H. Silverman 1975). Application of tissue culture to problems in malariology. IV International Conference on Invertebrate Tissue Culture, Mont Gabriel, Quebec, Canada.

49. Schenkel, R. H., G. L. Simpson and P. H. Silverman (1972). Protection of Rhesus monkeys against Plasmodium knowlesi by vaccination with antigen. Proceedings of the 47th Annual Meeting of American Society of Parasitologists. P. 47, No. 101.

50. Schenkel, R. H., E. J. Cabrera, C. A. Speer and P. H. Silverman (1974). Vaccination of Rhesus monkeys against Plasmodium knowlesi with lyophilized antigen. Proceedings of the 49th Annual Meeting of the American Society of Parasitologists. p. 25, No. 27.

51. Schenkel, R. H., E. J. Cabrera, C. A. Speer and P. H. Silverman (1974). Vaccination of Rhesus monkeys against Plasmodium knowlesi with lyophilized antigen. Proceedings of the 2nd International Congress on Immunology, Brighton, England.

52. Speer, C. A., M. C. Rosales-Ronquillo and P. H. Silverman (1973). Scanning electron microscopy of primary mosquito cell cultures used in cultivating Plasmodium berghei. Journal of Protozoology (Suppl). 20:62.

53. Speer, C. A. and P. H. Silverman (1974). Attempts to obtain growth of Plasmodium berghei in various mammalian cell cultures. Proceedings of the Third International Congress of Parasitology, Vol. 1, Section A2, p. 32.

54. Speer, C. A. and P. H. Silverman (1975). Cultivation of the malaria parasite, Plasmodium berghei, in Leydig cell tumor cultures. 26th Annual Meeting of the Tissue Culture Association, Inc., Montreal, Canada.

55. Stewart, T., R. H. Schenkel, M. L. Barr, E. J. Cabrera and P. H. Silverman (1975). A solid phase radioimmunoassay for detection of antimalarial antibody. 50th Annual Meeting of the American Society of Parasitologists., New Orleans, Louisiana.

MEETINGS ATTENDED

American Institute of Biological Sciences, Minneapolis, Minnesota, August 27-30, 1972.

Paul H. Silverman (Chaired Symposium of the American Society of Parasitologists.)

American Society of Parasitologists and American Society of Tropical Medicine and Hygiene, Combined Annual Meetings. Miami, Florida, November 3-5, 1972.

Robert H. Schenkel*
Edelberto J. Cabrera

Midwinter Conference on Immunology, San Francisco, California, January 6-9, 1973.

Paul H. Silverman

Southwest Conference of the Entomological Society of America, San Antonio, Texas, February 11-13, 1973.

Gregory Nienaber* (46)
Charles O. Gaines

Midwest Regional Conference on Developmental Biology, St. Louis, Missouri, April 11-14, 1973.

M. C. Rosales-Ronquillo

Rocky Mountain Conference of Parasitologists, Provo, Utah, May 4-5, 1973.

Edelberto J. Cabrera
Robert H. Schenkel
Paul H. Silverman
Raana Beckwith* (35)

48th Annual Meeting of American Society of Parasitologists, Toronto, Canada, June 26-30, 1973.

M. C. Rosales-Ronquillo* (44)
Paul H. Silverman

Annual Meeting of the Society of Protozoologists, Houston, Texas, December 27-29, 1973.

C. A. Speer* (52)
M. C. Rosales-Ronquillo* (45)
M. L. Barr

Cancer Immunotherapy Conference, Denver, Colorado, February 21, 1974.

Edelberto J. Cabrera

4th International Convocation on Immunology, Grand Island, New York, June 2-4, 1974.

M. L. Barr* (30)
J. L. Heidrich

Workshop on Problems Related to an Anti-Malarial Vaccine. National Academy of Science, Washington, D. C., June 24, 25, 26, 1974.

P. H. Silverman, R. H. Schenkel, M. C. Rosales-Ronquillo and C. A. Speer. (Review of activities of the Malaria Immunity and Vaccination Program were presented by attendees.)

2nd International Congress on Immunology, Brighton, England, July 22-26, 1974.

M. L. Barr* (31)
R. H. Schenkel* (51)

American Society of Parasitologists Annual Meeting, Kansas City, Missouri August 4-9, 1974.

R. H. Schenkel* (50)

3rd International Congress of Parasitology, Munich, Germany, August 25-31, 1974.

E. J. Cabrera* (36)
M. C. Rosales-Ronquillo* (47)
C. A. Speer* (53)

59th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 13-16, 1975.

M. L. Barr
J. L. Heidrich

Southern California and Rocky Mountain Conference of Parasitologists, Joint Annual Meeting, Las Vegas, Nevada, May 3, 1975.

E. J. Cabrera* (37)
R. Jost* (43)
R. H. Schenkel
C. A. Speer

IV International Conference on Invertebrate Tissue Culture, Mont Gabriel, Quebec, Canada, June 5-8, 1975.

M. C. Rosales-Ronquillo-Sharp* (48)

50th Anniversary Meeting of the American Society of Parasitologists and the American Society of Tropical Medicine and Hygiene, Joint Meeting, New Orleans Louisiana, November 6-14, 1975.

K. H. Rieckmann
P. H. Silverman
T. Stewart* (55)
M. L. Barr* (33)
E. J. Cabrera*(38)

60th Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim,California, April 11-16, 1976.

M. L. Barr* (34)
E. J. Cabrera* (39)
J. Heidrich* (41)

Rocky Mountain Conference of Parasitologists, Albuquerque, New Mexico, May 6,7,8, 1976.

J. Heidrich* (40)
P. Silverman (gave current status of the malaria vaccination program at UNM)
E. J. Cabrera (chaired section on Immunology and Physiology)

* Presented paper -- see Abstract section of Publications for title. Abstract No.( )

FINANCIAL REPORT

July 1, 1972 through April 30, 1976

AID/csd-3689

Category	Total Obligated Budget	Estimated Expenditures*
Salaries and Wages	$ 801,622	$ 808,290
Fringe Benefits	123,780	104,336
Consultants	3,208	4,975
Travel & Transportation	25,784	24,198
Equipment	97,916	94,758
Materials and Supplies	354,610	363,490
Sub-contracts:		
Rush-Stateville	73,651	77,928
Univ. of Illinois	148,477	148,395
Other Direct Costs (Publications)	2,412	1,412
Overhead	370,978	374,656
	$2,002,438	$2,002,438

* Final official report from the Comptroller's office will follow.