REPORT ON SITE VISIT TO
MALARIA IMMUNOLOGY UNIT
INSTITUTO HAClONAL DE SALUS
BOGOTA, COLOMBIA

Visiting Team: Edgar Smith
               F. J. Lopez Antunano
               Peter G. Contacos

During Period: March 20 to March 23, 1979

Report Prepared by: Peter G. Contacos, Ph.D., M.D.

Conducted by: International Health Programs Staff
               American Public Health Association
               Office of Health, DS/HEA-M
               Agency for International Development

Under
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Task Order #24
REPORT ON SITE VISIT TO MALARIA IMMUNOLOGY UNIT
INSTITUTO NACIONAL DE SALUD, BOGOTA, COLOMBIA

The site visit group consisted of Mr. Edgar Smith (USAID), Dr. F.J. Lopez Antunano (PAHO) and Dr. Peter G. Contacos (APHA consultant).

INTRODUCTION

The Malaria Research Unit was finally funded by the Pan American Health Organization (PAHO) and the Agency for International Development (AID) in 1976 when the Instituto Nacional de Salud (INS) in Bogota, Colombia, decided to accept this project and established a Malaria Research Unit under the direction of Dr. Carlos Espinal. Funds for the project were provided from National (70%) and International (30%) sources.

In late 1977, in order to give functional and administrative support to the project, the INS created a Unit of Malaria Immunology and placed it in the Section of Microbiology and Immunology. Space for climatized animal facilities, insectary facilities, laboratories and an office for the Principal Investigator at the Institute were planned for and had been completed for the most part in early 1979. The adaptation of the Institute's field station at Armero in the Magdalena Valley into a field monkey center is an important aspect of this project.

Most of the activities over the last two years have been concerned with administration, renovations, new construction, acquisition of equipment and personnel recruitment. The full staff of 10 individuals required for the project were all on duty by the end of 1978. In spite of all the delays in providing the physical facilities, the group was able to be involved in some research activities.
Total international expenditures through December 1977 were $104,000.00 and in 1978 they were approximately $47,900.00 making a total of $151,000.00 through 1978. For 1978, the INS contribution to the project was 8,018,800 Colombian pesos or U.S. $222,500.00, quite a strong indication of their interest in this research project.

A new proposal for a 3 year contract has been submitted to AID. The proposed studies in the new contract relate well with the on-going contract at the University of New Mexico with close collaboration between the two groups.

**SCHEDULE FOR SITE VISIT**

- **Tuesday, March 20, 1979**
  - (1) Instituto Nacional de Salud (Bogota)
  - (2) Hospital Militar Central (Bogota)

- **Wednesday, March 21, 1979**
  - (1) Servicio Nacional de Malaria (SEM)
  - (2) Instituto Nacional de Salud

- **Thursday, March 22, 1979**
  - (1) The Experimental Station of the INS at Anermo, Colombia

- **Friday, March 23, 1979**
  - Depart Bogota, Colombia

**FINDINGS**

The first day was devoted to:

1. inspecting the facilities of the project specifically and of the institute in general,

2. meeting with the Director and the staff of the project for an intensive review of the work done in 1978 and

3. reviewing and discussing the proposed studies outlined in the new contract proposal for the period July, 1979 - June, 1982.

The project facilities at the INS consist of the Director's office, 2 very large laboratories, an adequate insectary complex, and an animal facility consisting of three large rooms, two of which are adequately provided with superior
caging for *Aotus* monkeys. Each of the three rooms has individual controls for varying and/or controlling climatic conditions. The animal facilities are exceptionally good and the Institute's veterinary officer is available at all times to insure for the general good health of the animals.

The laboratories are well equipped. In addition, there is keen interest between several sections of the INS for collaborative studies with the malaria project which provides for sharing of equipment thus precluding unnecessary duplication of equipment for the malaria project. The fact that the INS is well equipped is reflected in the relatively low budgetary requests for equipment for the first year of the three year proposal.

Review of the studies carried on during the past year included:

1. standardization of serologic methods for evaluating immune response to malaria antigens,
2. continuous *in vitro* culture of Colombian strains of *Plasmodium Falciparum*,
3. cryopreservation of malaria parasites,
4. immunopathologic studies with falciparum malaria patients,
5. *in vitro* drug sensitivity tests and
6. establishment of laboratory colonies of Colombian *Anopheles* mosquitoes.

The details of these studies will not be dealt with here since they appear in the attached document (pages 3 & 4). The presentations were excellent and the discussions were stimulating. The entire staff impressed me personally in their ability to reason out and reply to questions posed. For example, when I was asked how a Unit on Malaria Immunology could justify doing drug sensitivity studies on strains of malaria, the reply was that they could exclude them in studies in *Aotus* monkeys which are not readily available for research even in Colombia. I was impressed with their awareness.
PROPOSED STUDIES

The main objectives of the proposed studies for the contract 1979-1982 are to establish a sizeable breeding colony of Aotus monkeys and to assess potential anti-malarial vaccines in monkeys obtained from this colony. The studies proposed for accomplishing these objectives, not necessarily in order of priority, are:

1. cryopreservation studies,
2. serology,
3. susceptibility of different subspecies of Aotus to P. falciparum infection,
4. breeding of Aotus monkeys,
5. immunization studies,
6. entomology and
7. field studies to determine the prevalence of malaria and the development of immune response in "non-immune" and "immune" individuals.

The details of these proposed studies can be found on pages 5-12. I would, however, like to comment on a few of the proposed studies.

SUSCEPTIBILITY OF DIFFERENT SUBSPECIES OF AOTUS TO P. FALCIPARUM INFECTION:

There are 4 subspecies of Aotus trivirgatus known to occur in Colombia. Aotus T. trivirgatus generally has low susceptibility to human malaria; A.T. Griseimembra has high susceptibility. Nothing is known about the susceptibility of A.T. Zonales or A.T. Lemurs to human malaria infection. Since owl monkeys, especially A.T. Griseimembra, are becoming less and less available for human malaria as well as other research, the significance of these studies to the over-all malaria vaccine program is obvious. I would have to give these studies a very high priority rating.
BREEDING OF AOTUS MONKEYS:

Aotus monkeys are becoming increasingly more difficult to obtain, even in Colombia because of the Government's efforts toward preservation of animal species. Thus, the breeding of Aotus monkeys, especially the most susceptible subspecies, A.T. Griseimembra, assumes special importance for the future progress of malaria vaccine development. In this connection, a breeding center for Aotus is being established in Armero which is located in the Magdalena River Valley. The field station which the site visit team visited is currently used by INS as a snake farm where venom is collected for antivenin production. The facility is excellent. It was formerly a large estate. The main house has plentiful space for living quarters, a surgery/dispensary and a large ready to use laboratory. The station has an outdoor insectary unit and a large open-air structure measuring at least 50 by 60 feet width would be ideal for Aotus monkeys. The plans are to capture Aotus from various parts of the country, determine their susceptibility to malaria, and, if indicated, breeding studies will be carried out with susceptible subspecies. Genetic studies on the subspecies will be collaboratively done with the Institute's Section on Genetics. As per attached correspondence between INS and the Dept. of Agriculture of Colombia, Aotus monkeys will be provided to the Unit of Malaria Immunology. Hopefully, monkeys will arrive within a few weeks. These studies also have a high priority rating.

IMMUNIZATION STUDIES:

In this connection, this project has its closest collaboration with one of the other network contractors, the University of New Mexico. Activities between
Colombia and New Mexico are to be closely integrated in an attempt to accelerate the development of a suitable antigenic preparation. In New Mexico, most studies will use "fresh" merozoite antigens. In Colombia, studies will center around the use of "frozen or lyophilized" preparations, again, a high priority rating because of the greater availability of Aotus monkeys as time progresses. This unit has the potential of assisting many contractors of the malaria vaccine network in vaccine testing.

FIELD STUDIES ON MALARIA PREVALENCE AND IMMUNE RESPONSE:

I mention these studies because they relate to the team visit to the Hospital Central Militar and to the Servicio de Eradicacion de Malaria. Dr. Hernan Torres, Chief of Internal Medicine, expressed his interest in the project for several reasons, foremost among them being that his service sees many emergency falciparum malaria cases especially from areas where drugs resistance is prevalent. He is keenly interested in collaborative studies with Dr. Espinal's unit on the general subject of immunology. Our visit with Dr. German Campos, Director of the Malaria Eradication Service, indicated a similar willingness to collaborate in longitudinal studies to define various epidemiologic malarial parameters. The importance of the above mentioned collaborative studies relates to the future aspects of the malaria vaccine research timetable.

GENERAL COMMENTS

Meetings with the Director of the Institute as well as with the Laboratory Director of the Institute indicated that they were both keenly interested in the project. Malaria is an important national problem for Colombia. In addition, they were pleased to become involved in the malaria vaccine research network because of its international aspects.
The esprit de corps and/or enthusiasm of Dr. Espinal and his staff, their ability to intelligently discuss the work they are doing, and their apparent keen interest in the overall objectives impressed me very much. This unit cannot help but be an asset to the malaria vaccine network.

RECOMMENDATIONS

The contract proposal for the period July, 1979 - June, 1982, entitled Assessment of Potential Antimalarial Vaccines should be approved. The majority of the proposed studies have a high priority in malaria vaccine research. The Institute is obviously very much interested in the project in that they have been providing 70% of the project's support. The project is located in a country where malaria is endemic, where falciparum malaria is still a problem, where Aotus monkeys which are susceptible to human malaria are indigenous. As the objectives of the overall malaria vaccine program are realized, having an on-going project in a country in which malaria is indigenous would indeed be desirable.

Under the training budgetary requirements, it is agreed that training in those laboratories responsible for developing specialized technologies related to malaria vaccines is important especially during the first year of the proposed project. However, provisions should also be made for on-site training by expert technologists so that problems may be solved on the spot with resources available at the Institute.
INDICATION OF HOST COUNTRY AND U.S. OFFICIALS CONTACTED:

1. Dr. Hernando Vidales
   Director
   Instituto Nacional de Salud

2. Dr. Alfredo Afanador
   Director of Laboratories, INS

3. Dr. Miguel Guzman
   Chief, Section of Diagnostica
   Investigacion y Referencia

4. Dr. Alberto Morales
   Chief, Department of Entomology

5. Dr. Paulina de Hoyos
   Department of Insect Genetics

6. Dr. Hernan Torres
   Chief of Internal Medicine
   Hospital Militar Central

7. Dr. German Campos G.
   Director
   Servicio Nacional de Malaria (SEM)

8. Dr. Jorge Rodriguez
   Chief, Epidemiology, SEM

9. Dr. Daniel Florez
   Chief, Technical Division, SEM

10. Dr. Oscar Beltran
    Assessor, CMS, SEM
APPENDIX I

ASSESSMENT OF POTENTIAL ANTIMALARIAL VACCINES

JULY 1979 - JUNE 1982

DR. CARLOS A. ESPINAL T. MD., PRINCIPAL INVESTIGATOR

INSTITUTO NACIONAL DE SALUD
BOGOTA, COLOMBIA
INTRODUCTION

EXPERIMENTAL PLANS

I. Cryopreservation studies

II. Serology

III. Susceptibility of different subspecies of *Aotus* to *P. falciparum* infection

IV. Breeding of *Aotus* monkeys

V. Immunization studies

VI. Entomology

VII. Field studies to determine the prevalence of malaria and the development (or course) of immune responses in 'non-immune' and 'immune' individuals

REFERENCES

BUDGET

BUDGETARY JUSTIFICATION
INTRODUCTION

In 1976 the Instituto Nacional de Salud (INS), Bogota, Colombia reached a decision to establish a Malaria Research Unit and, in February 1977, the Unit was funded under the direction of Dr. Carlos Espinal. Funds for this project were provided from national (about 70%) and international (about 30%) sources. The potential contributions of this Unit in the development of antimalaria vaccines are important for the following reasons:

1. Good back-up support for malaria personnel can be provided by the multi-disciplinary scientific expertise available at the Institute.
2. The Institute is located close to areas with continuous transmission of *P. vivax* and *P. falciparum*, including the transmission of chloroquine-resistant strains of *falciparum* malaria.
3. *Aotus trivirgatus griseimembra*, the best non-human primate host for the study of human malaria, is indigenous to Colombia.
4. Field stations of the Institute in different parts of the country permit convenient access to malarious areas and, in some instances, may serve as suitable holding and breeding colonies for *Aotus* monkeys.
5. Good working relationships with the Servicio de Eradicacion de Malaria (SEM) and the Hospital Militar Central have been established and both SEM and the Hospital Militar Central are providing support for malaria research activities at the Institute.
6. Notwithstanding its primary function as a national center, INS is interested in collaborating in research and training activities with institutions in other countries and in making important contributions
to global research efforts. It also has working arrangements with other institutions, such as the Naval Medical Research Institute Bethesda, Maryland, and the University of New Mexico, Albuquerque.

International funds were provided to (a) assist in the establishment of the Unit, and in the training of personnel, (b) study immune mechanisms in simian and human hosts, and (c) determine the efficacy and side-effects of malaria immunogens in monkeys.

The Unit is now fully operational. It has two laboratories, extensive primate facilities, and access to a mosquito insectary in the Entomology Department. The staff consists of Dr. Carlos Espinal T., MD. (Director), Maria del Pilar Carillo A. (Entomology, National Univ.) Leon Enrique S. (Biology, National Univ.), Marco Fidel Suarez A. (Biology, National Univ.), Margarita Groot S., M.S. (Zoology, Rutgers Univ.), Patricia Guerra M. (Bacteriology, Javeriana Univ.), Edith M. de Monsalve (Bacteriology, Javeriana Univ.), Patricia Olaya G. (Microbiology Javeriana Univ.), Patricia de la Vega A. (Biology, Los Andes Univ.).

Dr. Espinal is thoroughly familiar with current research activities in all aspects of malaria immunology, having received three years training at New York University (Sporozoite Immunology) and the University of New Mexico (Merozoite Immunology). In addition, he has attended and participated in numerous seminars and meetings dealing with this subject matter, and is in close contact with other investigators working in this field. Three other members of his staff have spent several weeks working at U.S. malaria research centers, including the University of New Mexico (Dr. Rieckmann), the Naval Medical
Research Institute (Dr. Beaudoin) and the Center for Disease Control (Dr. Collins). The training and exchange of scientific personnel at U.S. institutions is regarded as an important component of this research program in Bogota.

The other two main objectives of the program (b & c) have only been partially realised due to the extended period of time required to complete the animal facilities. These facilities have now been completed and monkeys are being acquired for breeding and immunological studies.

Investigations that have been conducted during the past two years include:

1. Standardization of ELISA (Enzyme linked immunosorbent assay) technique for detecting and quantifying \textit{P. falciparum} and \textit{P. vivax} antibodies. This technique was standardized using serum obtained from acute malaria patients, and is an important parameter which will be used to evaluate the immune response to malaria antigens.

2. Continuous culture of Colombian strains of \textit{P. falciparum} using the Trager-Jensen candle jar method. At the moment, particular attention is being given to the cryopreservation of parasites to be used in establishing successful cultures. This work is necessary to obtain optimal survival of stabilates obtained in the endemic areas during transit to the INS laboratory in Bogota. The marked production of gametocytes observed in many of these cultures requires further investigation.
3. Immunopathological studies with *Plasmodium falciparum* malaria patients have been initiated in collaboration with Dr. Torres, Chief of Medicine at the Hospital Militar Central in Bogota. The main objective of this collaboration work at present is to standardize techniques for measuring immune complex deposits in the kidneys which can later be used to detect any adverse pathological side effects after immunization.

4. *In vitro* drug-susceptibility tests for characterizing various strains of *P. falciparum*. The results thus far indicate a high level of resistance to chloroquine in the Vista Hermosa area of Colombia.

5. Establishment and maintenance of *Anopheles albimanus* obtained from the northern part of Colombia during the past 12 months and establishment of a laboratory colony of *A. darlingi* from the llanos have been accomplished or are in progress. The ultimate objectives are the infection of mosquitoes from gametocytes obtained from culture, patients or *Aotus*, and the sporozoite transmission of falciparum infections to *Aotus* monkeys. The ability to challenge immunized monkeys with sporozoites would provide the best means for determining the efficacy of potential malaria vaccines.
EXPERIMENTAL PLANS

The main objective of the proposed studies is to establish a sizeable breeding colony of Aotus monkeys and to assess potential antimalarial vaccines in monkeys obtained from this colony. In addition, supportive studies are planned with malaria-infected patients to improve our understanding of the development of immunity to malaria.

I. Cryopreservation studies. The transport and preservation of P. falciparum-infected blood is a necessary step in the adaptation of a parasite isolate to an in vitro culture system. Methods must be developed to regularly transport infected blood from malarious areas to the laboratory in Bogota, and from there to other collaborating institutions in the AID network including the University of New Mexico. Since a standardized method for preserving strains during shipment is not available, special attention will be given to this problem from the outset of the project. A standard method of cryopreservation will also be important in monitoring antigenic changes which may develop in culture-adapted strains. This is particularly relevant for the merozoite preparations which are to be evaluated as immunizing agents and which will also be used as antigens for the various serological assays. Various preservation and transport procedures will be evaluated for practicality as well as for efficacy. Since cultivation is the final disposition of the isolates, efficacy will be described in terms of the relative success of culturing samples of the same isolate treated in different ways. Parasite
density will be determined after the first 95 hours in culture. Cultures of the isolates will be monitored for a period of 3 months to indicate the efficacy of a given approach for adapting the parasite to culture.

II. Serology. The development of a malaria vaccine will be facilitated by the availability of a serologic assay which could examine the immune response during the vaccination studies in Aotus monkeys. This aspect of the work will be particularly important in monitoring the purity of candidate vaccines and the host reaction to their administration. Therefore, the assay must be of sufficiently high sensitivity and broad specificity to detect all antigens present in the immunizing preparations. A serologic test appropriate for wide scale use would be exceedingly helpful once suitable candidate vaccines become available for field trials. Serologic data generated during the experimental phase of the vaccine development program will provide the basis of the serologic testing during the longitudinal program and later during field trials.

The enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence will be examined for their suitability during the immunization studies in Aotus monkeys. They will also be performed on patients with P. falciparum infections from the Central Military Hospital in Bogota. The sensitivity and specificity of both tests will be determined following exposure to the immunizing antigen. Four different antigen preparations, will be used in the assays. They will include schizonts and merozoites both from cryopreserved
material of the original isolate and from fresh material from continuous cultures maintained in Bogota and New Mexico.

The GARI assay\(^3\) will be used to complement the serologic studies of the antibody response in infected patients, in immunized monkeys and serum specimens. Sera will be forwarded to New Mexico, where the test will actually be performed.

III. Susceptibility of different subspecies of Aotus to P. falciparum infection. The primate most often used in chemotherapeutic and immunological studies of malaria is \textit{Aotus trivirgatus griseimembra}. Its distribution extends from the north of Colombia through the valleys of the Sinu River, San Jorge River, through Bajo Cauca and the Magdalena valley\(^4\). The subspecies is reported to be in danger of extinction due to uncontrolled exportation and destruction of its natural habitat. In 1966, Ycung \textit{et al}\(^5\) established the susceptibility of \textit{Aotus trivirgatus trivirgatus} from Panama to several local strains of \textit{Plasmodium vivax}. In 1967, Geiman and his group\(^6\) successfully infected \textit{Aotus} monkeys from the north of Colombia with African strains of \textit{P. falciparum}. Since then many chemotherapeutic studies have been performed using the \textit{Aotus trivirgatus griseimembra-P. falciparum} or \textit{P. vivax} model\(^7\). Preliminary observations show that once a strain of \textit{P. falciparum} is adapted by passage in \textit{A. t. griseimembra}, the infection can readily be transferred to \textit{A. t. trivirgatus}\(^8\).

Other authors describe the low susceptibility of \textit{A. t. trivirgatus} from the Amazon to experimental falciparum infections\(^7\). Two other subspecies of \textit{Aotus} are found in Colombia: \textit{A. t. zonales} and \textit{A. t. lemurus}. Both subspecies have a wide distribution in the central and eastern
parts of the country, however, there is no information as to their potential value for malaria studies. Determination of the susceptibility to infection with *P. falciparum* of the various subspecies of Colombian *Aotus* may establish other models with similar characteristics to that of *A. t. griseimembra*.

The susceptibility of *Aotus* to malaria will at first be studied using a chloroquine-sensitive strain of *Plasmodium falciparum* adapted to monkeys, thereby permitting effective control of the virulence and pathogenesis of the infection. Initially, two *A. t. griseimembra* will be infected, serving as parasite donors for the other subspecies of *Aotus* (the two donor monkeys will subsequently be treated with a dose of chloroquine to obtain a radical cure). Infected *Aotus* will be monitored daily by thick and thin blood smears to determine parasite density. Levels of parasitemia will not be allowed to exceed 20-30% by treating the monkeys with chloroquine. Susceptibility of the different subspecies of *Aotus* to *P. falciparum* will be ascertained by comparing the course of infection in the test animals with that observed in *A. t. griseimembra*.

IV. Breeding of *Aotus* monkeys. *Aotus trivirgatus griseimembra* from the north of Colombia is currently the most appropriate subspecies to use in malaria studies. If untreated, well adapted *P. falciparum* strains often produce a fatal parasitemia in this monkey. Furthermore, the monkey's immune response to the infection may be similar to that observed in man (Rieckmann, 1977). Due to agricultural development and the concomitant destruction of the monkey's natural habitat in
9. Certain areas, this particular subspecies has become increasingly difficult to obtain. For this reason, the breeding of Aotus monkeys, especially of the A. t. griseimembra subspecies, assumes special importance for future progress in development of a malaria vaccine. A breeding center for Aotus will be established in Armero, an established field station of the INS, in the Magdalena River valley. Aotus trivirgatus from various areas of the country will be captured, their susceptibility to malaria determined, and if appropriate, breeding studies will be carried out with those subspecies which are susceptible. Classification to subspecies will follow morphological criteria described by Hernandez-Camacho.

Selection of breeding pairs will be based on compatibility, and suitable pairs will be caged together. Genetic studies, including karyotypic analysis, will be made in order to determine the relationship between the pair's reproductive success and their karyotypes. The INS has immediate plans for the initiation of reproductive studies of Aotus in large environmental cages which approximate the natural habitat.

V. Immunization studies. Activities between New Mexico and Colombia will be closely integrated in order to accelerate the development of a suitable antigenic preparation. In New Mexico most studies will use "fresh" merozoite antigens because they appear to be as effective as other developmental stages in inducing protection against malaria. In Colombia, efficacy studies will center around the use of frozen or lyophilized preparations that will be assessed under various environmental conditions. It is also proposed to explore various regimens and dosage schedules to achieve maximum
protection with the lowest quantity of antigen and the least number of inoculations. Serologic assays will also be performed throughout the immunization experiments and, based on the results obtained, modifications may be made in the immunization schedule, e.g. time intervals between immunizations, doses of antigen, etc. Serum biochemistry determinations and histopathological examinations will be accomplished on immunized monkeys in order to observe the development of systemic or local side effects after immunization. Studies will also be conducted to determine the effects of vaccination on Aotus monkeys with chronic infections of malaria. This type of study is urgently needed since candidates for vaccination in the human population can be expected to have had extensive prior experience with the parasite at the time of vaccination.

Adverse side effects following intramuscular administration of Freund's adjuvant has rendered it unacceptable for human use and an appropriate non-toxic substitute is required for the development of an effective merozoite vaccine. Selected adjuvants will be used as immunopotentiators during immunization experiments in the P. falciparum-Aotus system.

VI. Entomology. The ability to challenge immunized monkeys with sporozoites would provide the best means for determining the efficacy of potential malaria vaccines. To accomplish this, however, it is important to be able to routinely induce falciparum infections in Aotus monkeys following sporozoite inoculation by mosquitoes. For this reason the Malaria Immunology Unit in collaboration with the Entomology Department is in the process of adapting several Colombian species of anophelines for laboratory rearing with the
objective of establishing the entire life cycle of *P. falciparum*. Preliminary observations from the Center for Disease Control, Atlanta, indicate the potential for establishing the complete life cycle in *Aotus* monkeys including the infection of mosquitoes, and the transmission of the infection to monkeys by the mosquitoes.

During the adaptation of *P. falciparum* to the culture system, certain isolates show a marked increase in the level of gametocytes, accompanied by the disappearance of asexual stages. Similar observations have been made at New Mexico with isolates originating in Colombia. The conditions of gametocyte production and maturation in vitro will be studied by varying (1) the source of the parasite isolate (non-immune in contrast to immune donors), (2) concentration of serum (3) the pH level, and (4) the O₂/CO₂ concentrations. Different methods for concentrating gametocytes and for infecting the mosquitoes will be studied in order to provide a reliable means for inducing infections in *Aotus* monkeys.

VII. Field studies to determine the prevalence of malaria and the development (or course) of immune responses in 'non-immune' and 'immune' individuals. Plans are already under way to obtain regular malariometric and immunological data from non-immune and partially-immune military recruits who are assigned to malarious areas for a period of 18-24 months. This study is being planned in collaboration with Dr. Torres, Chief of Internal Medicine, Hospital Central Militar, Bogota. Recruits from non-endemic areas with no previous exposure to malaria are at a high risk when they are transferred to malarious areas. Dr. Torres is planning to conduct
a carefully controlled study with some of these recruits to determine which one of three prophylactic regimens is the most effective in reducing malaria morbidity. It is anticipated that these drug regimens may not be entirely effective in suppressing patent parasitemia, thus resulting in development of a level of immunity to falciparum malaria in these individuals. Detailed and frequent recording of the level of parasitemia and symptoms as well as measurement of immune responses by the ELISA and GARI assays would provide extremely useful information concerning the development of immunity in drug-suppressed infections, and, this in turn, should be of value in assessing immune responses following vaccination with blood stage antigens.

Sporadic field surveys concerning the prevalence of malaria have already been carried out in the Vista Hermosa area of Colombia. In the future, detailed longitudinal studies are planned in collaboration with the Servicio de Eradicacion de Malaria, to define various epidemiological malaria parameters over a period of several years. This would indicate the extent of seasonal and annual variability in the transmission of malaria, and provide the necessary background information for field trials with potential vaccines which could be carried out in the area.
REFERENCES


COLOMBIAN COMPONENT

1979-1980

SALARIES AND WAGES

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FRINGE BENEFITS

CONSULTANT FEES (1)

2,500

3,500

EQUIPMENT

High security environmental chamber and cages for mosquitoes. (2)

9,000

33,000

2 Dissecting microscopes

9,500

MATERIALS AND SERVICE

Laboratory supplies (including freight).

30,000

41,000

30,000

4,000
TRAVEL

As part of the AID network, close collaboration and frequent on-site contact must be maintained for the most efficient flow of data and information:

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PUBLICATION COSTS

500

INDIRECT COSTS

12,810

TOTAL

155,300
BUDGETARY JUSTIFICATION

(1). Consultant fees: Consultants from the AID network, other U.S. agencies, and the Pan American Health Organization (PAHO) (in particular Dr. Francisco Lopez Antoniano, PAHO Director of Malaria Research) will be needed to assist with the drug sensitivity testing and the longitudinal study in the Vista Hermosa region. Substantial consultant fees have been requested as part of the budget because of the scope of the proposed research suggests the need for technical assistance on a regular basis.

(2). High Security environmental chamber: Several species of anopheline mosquitoes will be adapted for maintenance in the laboratory. Since these species will be used to harbor Plasmodium falciparum, a parasite infective to humans, high security containment of the adult mosquitoes is essential. In addition, since the different species of Anopheles must be maintained separately, a high security environmental chamber will be necessary.

(3). Centrifuge and rotors: Many aspects of the project require the availability of parasites free from the bulk of contaminating debris and extraneous microorganisms. Current methods of preparing the parasites in acceptable condition are based on high speed centrifugation of suspensions placed on density gradients if high yields are needed, and column separation procedures if very clean preparations are needed and yield is not important. The first requirement can only be filled by availability on site of a Sorvall RC-2B high speed centrifuge with swing out head and appropriate rotor.
(4). Refrigerator: The primate colony at INS will require a standard household-type refrigerator for short time storage of perishable food items, blood samples, and drugs.

(5). Primate cages: An isolation area is needed to provide facilities for holding animals separate from the main colony 1) before introducing them into the main colony and 2) when they have been subsequently infected with pathogens accidentally transmitted in the laboratory with the potential of spreading to other animals in the colony (i.e. infections with *Pseudomonas* or *Strongyloides*). Additional cages will be required if breeding *Aotus* in captivity is to be accomplished. It is now well established that successful mating is dependent on appropriate caging of the breeding pair. The cages must be large enough to promote mating conditions and for long term survival of offspring who require constant attention and care from both parents during the lengthy period following parturition.

(6). Microscopes and optical equipment: The optical microscopes available to the unit are presently in full use. Expansion of the program to accommodate the work being proposed will significantly increase the demand on the available microscopes to the point that their availability will become limiting. The proposed work will require an additional compound microscope with phase optics which will be used to quantify parasites, to monitor parasitemias, or prepare vaccine doses, as well as in hemocytometry of infected and control monkeys. In addition, one compound microscope and two dissecting microscopes are required for use in the high security insectary.
(7). **Training:** Since the program in Colombia will rely heavily on specialized technology being generated in the U.S., it is essential that the personnel have hands-on experience in those laboratories responsible for developing that technology: NMRI, CEC, and the University of New Mexico. In addition, several institutions offer courses on latest available techniques and technologies: WHO and NIH.
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Bogotá, Marzo 15, 1977

Doctor
PRIMITIVO ERICERO MORENO
Gerente General del Instituto Nacional de Recursos Renovables y del Ambiente
INDEPENA
CIUDAD

Estimado doctor Ericero:

En mi condición de Director del Instituto Nacional de Salud, establecimiento público autónomo el Ministerio de Salud, creado por el Decreto-Ley 470 de 1968 y reorganizado por el Decreto-Ley 671 de 1975, siento el necesario no permitir exponer la siguiente:

La institución que legalmente representa tiene como objetivo la promoción, coordinación y realización de la investigación en el campo de la salud, la bio-medicina y la medicina preventiva, así como la aplicación de la tecnología para fines de diagnóstico, preservación de la salud y vigilancia epidemiológica y, en general, el ejercicio de las funciones necesarias para el mejoramiento de la salud.

Dentro de los programas de medicina preventiva se han venido realizando, entre otros, investigaciones en el campo de la inmunología de la materia, con la cooperación de la Organización Mundial para la Salud y otros organismos internacionales, con fines a la obtención de una vacuna contra el paludismo. Para el desarrollo del programa el Instituto necesita proveer del género Acton triflingus en una cantidad anual de 150-200 ejemplares.

Con el objeto de obtener tales primates, se permite solicitar de usted el aportamiento de uno lindio por el término de un (1) año renovable, que permita la captura de los animales, en algunas zonas del país conocidas con el lapsus.

Para su información le comunico que el Instituto está contando con instalaciones en la Estación Experimental del Campo de Armero (Tolima), a fin de establecer un criadero y reposición de las animales que hubieran fallecido durante el periodo de la investigación.
en estos casos el Instituto practicará las autopsias y protocolos correspondientes para su envío al laboratorio.

Anticipo a usted mis agradecimientos por la colaboración que tenga o bien prestar a esta Institución con el otorgamiento de la licencia en referencia y me suelo muy agradecido.

[Signature]

HERNANDO VIDEALES NEIRA
Director

[Stamp] INSTITUTO NACIONAL DE SALUD
A LOS FUNCIONARIOS DEL INSTITUTO NACIONAL DE LOS RECURSOS NATURALES RENOVABLES Y DEL AMBIENTE (INDERENA) Y A QUIEN PUEDA INTERESAR .-

Por la presente hago constar que el portador de la presente es funcionario del Instituto Nacional de Salud (INAS) o del Servicio de Erradicación de la Malaria (SEM) del Ministerio de Salud y se halla autorizado para transportar con destino a la División de Fauna Terrestre de INDERENA (Bogotá), ejemplares vivos o muertos de la especie de mono denominada "Mico de noche", "Mono de noche", "tutamono", "sorbehumo", "marta", "martica" o "marteja" (*Aotus trivirgatus*), que serán destinados a las investigaciones biológicas y médicas que en cooperación viene adelantando el Instituto Nacional de Salud y el INDERENA.

Por lo tanto antepicio todos nuestros agradecimientos.

Atentamente,

[Signature]

JORGE E. HERNÁNDEZ CAMACHO
Jefe División Fauna Terrestre
Instituto Nacional de los Recursos Naturales Renovables y del Ambiente - Inderena.

Bogotá, D.E., Marzo 14 de 1979