The Biomedical Research Portfolio of the Office of Health, Bureau for Science and Technology

Volume 1

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The Biomedical Research Portfolio
of the
Office of Health,
Bureau for Science and Technology
United States Agency for International Development

by

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SUMMARY

The projects within the Biomedical Research Portfolio of the Office of Health include investigation directed towards all phases of the control of human diseases. Original research is being conducted on major health problems of the developing world, making significant contributions to the prevention, diagnosis, and treatment of diseases which kill several millions annually, and deprive many more of healthy, productive livelihood. The continuing focus of the Agency is on technologies which are directed towards improving the survival and health of children throughout the developing world.

The biomedical research program of S&T/Health is currently providing direct support to over 60 research projects. Many additional research projects receive multilateral support through S&T/Health's contribution to the World Health Organization.

The Child Survival Action program is currently supporting 8 research projects, 6 in the area of immunization, and 2 dealing with the effects of vitamin A on the health of children.

The DIATECH program is funding 7 research groups working on the development of rapid and accurate diagnostic assays for serious diseases. Within DIATECH, there are 4 malaria grants, and 3 enteric infection grants.

The USAID malaria network continues to be a highly productive research organization. The work of the 16 member institutions came to fruition this November, as the first human trials of the synthetic anti-sporozoite vaccine were begun at the University of Maryland.

The support of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) made possible a large-scale field trial of a new, highly effective oral vaccine for cholera.

Funding for the Americares Foundation resulted in the establishment and evaluation of a nation-wide program for multidrug therapy for the treatment of leprosy in Venezuela.

The Vaccine Development and Health Research project is funding investigation for improved vaccines for pertussis, measles, typhoid, and rotaviral diarrhea. To prevent needle-transmitted diseases, a single-dose, non-reusable syringe has been developed and tested with excellent results. The International Institute for Infant Nutrition and Gastrointestinal Diseases at the Children's Hospital of Buffalo, New York is performing basic research on the mechanisms of acute and chronic diarrhea, and malnutrition.
The data collected in this Portfolio was provided by a direct poll of the principal investigators and implementing agencies. In so doing, the information is as up-to-date and accurate as possible.

Also included (as Appendices) are listings of the biomedical research activities funded by the Office of the Science Advisor, the Historically Black Colleges and Universities program, and the cooperative research agreements between the United States and India.
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APPENDIX I: USAID Malaria Research Network Support.

American Institute for Biological Sciences

Batelle Northwest Laboratories


APPENDIX V: Indo-U.S. Cooperation in Science and Technology.
**Project Title:**

Cooperative Agreement for Child Survival

**Project #:**

936-5951.01

**Project Goals:**

To improve the delivery, use, and effectiveness of Child Survival Technologies and program activities. This includes:

- Improve prevention and treatment of childhood diseases, with emphasis on immunization and vitamin A research.
- Improve quantitative/qualitative techniques for program evaluation/management.

**Funding:**

$4,706,000 for 5 years (for research and program activities).

**Project Manager:**

Pamela Johnson, Ph.D.
S&T/Health, (703) 235-8926.

**Implementing Agency:**

Institute for International Programs (IIP)
Johns Hopkins University
615 North Wolfe Street
Baltimore, Maryland 21205
Contact: Henry Mosley, M.D. (301) 955-7983
Sub-Grant Title:

Evaluation of Hepatitis B Immunization Strategies in Rwanda, East Africa.

Funding:

$38,190.00

Research Institution:

Johns Hopkins University
School of Hygiene and Public Health
615 North Wolfe Street
Baltimore, Maryland 21205

Principal Investigator:

B.F. Polk, M.D.

Specific Research Goals:

To evaluate the comparative immunogenicity of vaccine strategies to prevent Hepatitis B Virus (HBV) infections among infants in Rwanda, Africa.

Disease Incidence:

In Africa and Asia 10%-20% of the population carry HBV (are positive for HBsAg). Most adults (70-80%) have been exposed to the virus, and have antibody to the virus (anti-HBs). When infants are infected, 50% of them may become chronic carriers.

Rationale:

HBV transmission in central Africa occurs mainly in early childhood. Incorporation of HBV into the routine vaccination schedule would be the most effective means of preventing this disease. However, the current cost of HBV vaccine is too great for routine use in LDC's. New low cost vaccination strategies would be of great benefit in preventing HBV infections.
Transmission of HBV is primarily through contact with blood and body fluids from infected individuals (either acute or chronic carriers). Person to person spread occurs principally among children in regions where the virus is endemic. Spread occurs by contamination of mucous membranes or small breaks in the skin by contact with secretions from playmates. Mothers can also transmit the infection to their infants during pregnancy or birth.

Immunization of children at birth can break the endemic pattern. Children who are immune cannot infect other children and will not transmit the disease to their offspring.

Low doses of HBV vaccine (Hepatovax, Merck) given intradermally (ID) have been shown to give high rates of seroconversion. A comparison of standard dose schedules with low dose schedules under field conditions is needed to determine the most cost-effective method to immunize the greatest number of people.

Techniques Used:

- Enzyme assays/probes (ELISA).

Ultimate Goal:

- Prophylaxis.

Research Description:

The infants will be vaccinated at birth, 1 month, and 4 months of age. They will be grouped as follows:

- A) Standard schedule of three 10 mg intramuscular (IM) doses
- B) Three 2 mg ID doses
- C) Three 2 mg IM doses.

Serum will be collected from cord blood, at 4 months (before the third vaccination), and at 5-6 months. The immune response will be measured by ELISA (kits from Abbott Laboratories).

Time for Completion:

1 year.

Progress:

- Project approval is pending submission of a revised protocol.
Collaborating Arrangements:

University Center for Public Health, Rwanda, Africa.

Sub-Grant Title:

The Early Immunization of Children with Inactivated Polio Vaccine (IPV).

Research Institution:

Johns Hopkins University
School of Hygiene and Public Health
615 North Wolfe Street
Baltimore, Md. 21205.

Principal Investigator:

A. Marshall McBean, M.D.

Funding:

$69,875.00

Specific Research Goals:

1) To vaccinate children at birth with IPV and determine the serologic response when they are given 1 or 2 additional doses.

2) To compare these responses with that of children immunized with 2 doses of IPV beginning at 2 months of age, and with children immunized with 3 doses of oral polio vaccine (OPV).

Disease Incidence:

In India, 5-10/1,000 children are paralyzed from polio before age three annually.

20%-35% of these cases are in children under 1 year old.

In India, this translates to about 500 cases of paralytic polio per day.
Rationale:

Successful immunization of the children in LDC's is hampered by low coverage rates, a high dropout rate, and low seroconversion rates (measures if the vaccine "took") in those who have been vaccinated. Reducing the number of doses of vaccine in an immunization schedule would be of great benefit.

Newly developed high potency (titer) IPV have been 100% effective in a 2 dose schedule given to 2 month old children in the USA and in India.

Newborn infants are able to respond to OPV during the first week of life. Those who do not respond serologically may have been primed for a rapid response to a second vaccination.

Thus it is reasonable to ask whether a 2-3 dose IPV schedule begun at birth can be as effective in inducing seroconversion as the IPV schedule begun at 8 weeks of age, or the OPV schedule begun at birth.

Techniques used:

Cell culture.

Ultimate Goal:

Prophylaxis.

Research Description:

Infants will be grouped as follows:

A) A dose of IPV at birth and at 4 months.
B) A dose of IPV at 2 months and at 4 months.
C) A dose of IPV at birth, at 2 months, and at 4 months.
D) A dose of OPV at birth, at 2 months, and at 4 months.

Serum will be prepared from the cord blood of all infants, and then at 6 months of age. The response to vaccination will be measured by the in vitro virus neutralization test (colorimetric metabolic inhibition).

Staffing:

M D. 3
Technician 1
Progress:

Project is pending country clearance.

Collaborating Arrangements:

Vellore Christian Medical College,
Vellore, India.

Time for Completion:

2 years.

Sub-Grant Title:

The Effect of Vaccination During Pregnancy on the Infant's Response to Tetanus, Polio, and Diphtheria Immunization.

Funding:

$51,318.00

Research Institution:

Johns Hopkins University
School of Hygiene and Public Health
615 North Wolfe Street
Baltimore, Md 21205.

Principal Investigator:

A. Marshall McBean, M.D.

Specific Research Goals:

i. Evaluate the effect that vaccination of pregnant mothers with Diphtheria, tetanus, and polio has on their children's response to these diseases.

The presence or absence of IgM in cord blood, and the speed and magnitude of a "booster vaccination" given to the child in the first 3 days of life (of the same vaccines given to its pregnant mother) will be determined. Specific IgM antibodies in the cord blood, or a rapid antibody response to a "booster" vaccination indicates successful in utero immunization.
Disease Incidence:

In India, 5-10/1,000 children are paralyzed from polio before age 3 annually.

Of these cases, 20-35% are children under 1 year old.

This translates to about 500 cases of paralytic polio per day.

Rationale:

Serious problems exist in LDC's with respect to completing the entire series of vaccinations for children. Low coverage rates (10-36%) and low compliance (25% dropout rate) complicate generally low levels of immune responsiveness when compared to more developed countries. A new more potent Inactivated Polio Vaccine (IPV) may require only 2 immunizations, and thus have a better chance of being protective in a large number of children. If active immunization in utero can be achieved, it would reduce the necessary number of vaccinations at birth to a single dose of IPV, diphtheria toxoid, and tetanus toxoid. This would provide immunity through the 3-4 years when the child is at highest risk to paralytic polio, diphtheria, and tetanus.

Techniques Used:

Cell culture
Enzyme assays (ELISA)

Ultimate Goal:

Prophylaxis

Research Description:

Pregnant mothers will be vaccinated with IPV and diphtheria-tetanus (Td) in the 6th and 8th months of pregnancy. Serum will be collected pre-vaccination at the 6th month. Serum will be taken from cord blood at the time of delivery. Two groups of infants will be vaccinated at day 1: one group from mothers who received vaccination, the other from mothers who were not vaccinated.
At one month of age, all babies will have serum taken. The antibody responses will be measured as follows: ELISA assay for antibody to the tetanus or diphtheria toxin. The titer will be referenced to mouse paralysis (tetanus) or intradermal rabbit test (diphtheria). The polio assay will be for neutralizing antibody using the colorimetric assay.

**Staffing:**

M.D. 5  
Technician 2

**Time for Completion:**

1 year.

**Progress:**

Project is pending country clearance.

**Sub-Grant Title:**

Investigation of the Seroepidemiology of *Streptococcus* Pneumoniae Infections and the Immunogenicity of Pneumococcal Vaccines in India.

**Funding:**

$66,500.00

**Research Institution:**

Johns Hopkins University  
School of Hygiene and Public Health  
615 North Wolfe Street  
Baltimore, Maryland 21205.

**Principal Investigator:**

Mark C. Steinhoff, M.D.

**Specific Research Goals:**

1. Collect age specific seroepidemiology of *S. pneumoniae* in a population with significant rates of disease with the organism.
2. Determine the immunogenicity of protein-polysaccharide vaccines.

3. Determine the effects of age, sex, breast feeding, and nutritional status on the development of antibody.

Incidence of Disease:

Worldwide, there are 10.6 million deaths due to *S. pneumoniae* infections per year.

Of this total, 3.65 million are children under 5 years of age.

Rationale:

The National Academy of Science ranks *S. pneumoniae* vaccine development as the highest priority. No data is currently available on the immunogenicity of pneumococcal polysaccharide vaccines on young infants in LDC's. Investigators in Papua New Guinea suggest that young infants in LDC's may be responsive to polysaccharide vaccines (infants in the USA do not respond well to polysaccharide vaccines).

Techniques Used:

Radiolabelled reagents (RIA).

Ultimate Goal:

Prophylaxis.

Research Description:

Children will have blood drawn to evaluate the levels of existing antibody to pneumococcal polysaccharide. Fifty children at 12 months of age, and fifty children at 18 months of age will receive a single dose of 23 valent Pneumovax vaccine. These children will be tested for antibody to the vaccine 4-5 weeks post vaccination.

If a protein-polysaccharide vaccine is available (has IND status) it will be given at 2, 4, and 6 months of age along with routine DPT vaccination.

Nutritional status will be determined by height, weight, and arm circumference.
Staffing:

Ph.D. 1
M.D. 2

Progress:

The project is pending country clearances and protocol revision.

Time for Completion:

1 year.

Sub-Grant Title:

Immunogenicity of the Edmonston-Zagreb (EZ) and Schwarz Measles Vaccines.

Funding:

Pending.

Research Institution:

Department of International Health
School of Hygiene and Public Health
Johns Hopkins University
615 North Wolfe Street
Baltimore, Maryland 21205

Principal Investigator:

Neal Halsey, M.D., Associate Professor.

Specific Research Goals:

1. To compare the immunogenicity of two Edmonston Zagreb strain (EZ-Y and EZ-M) with the Schwarz Strain measles vaccine in 5 to 6 and 9 to 12 month old infants.

2. To compare the rates of adverse reactions associated with the vaccines.

Incidence of Disease:

Measles contributes to the deaths of 2 million children per year.
**Rationale:**

More children die of measles and its aftermath than all other vaccine-preventable diseases combined. It is thus the single most important cause of vaccine preventable morbidity and mortality in developing countries. Infants under 1 year are at greatest risk of measles-induced complications and death. Case fatality ratios range from 12% to 24%. Malnutrition, poverty, overcrowding and cultural beliefs are important risk factors.

Current measles vaccines (Moraten and Schwarz) when administered in LDC's give satisfactory results (high seroconversion rates) only in children over 9-12 months of age. This leaves a very substantial portion of the infant population not protected from the disease.

The Edmonston-Zagreb (EZ) vaccine is a live, attenuated measles vaccine, it is stable, and has been tested over 7-16 years and 20 million doses. A recent review suggests that the EZ vaccine may protect children as young as 6 months.

The inability to immunize infants under 9 months of age in LDC's is a major deterrent to the control of measles transmission. The EZ strain has shown 90% seroconversion of small numbers of 5-6 month old infants.

**Techniques Used:**

Cell culture.

**Ultimate Goal:**

Prophylaxis.

**Research Description:**

Children in the 5-6 and 9-12 month age groups will receive either the EZ-Y or EZ-M or the Schwarz measles vaccine. (EZ-Y and EZ-M are the same strain of virus, but made in Yugoslavia and Mexico, respectively). Twelve months post vaccination, a serum sample will be taken and analyzed by hemagglutination-inhibition assay for antibody to the virus. Seroconversion is measured as:

A) the presence of any detectable antibody in those originally negative;

B) a 2-fold rise in titer of a previously positive person;

and C) antibody present when the maternal antibody would not normally be present in the population tested.
Staffing:

M.D. 2
Technician 4
Computer programmer 1

Time for Completion of Project:

2 years.

Progress:

Project is waiting for site selection and revision of protocol.

Sub-Grant Title:

Evaluation of the Effectiveness of Low-Dose Intramuscular (IM) and Intradermal (ID) Hepatitis b Vaccine in Healthy Neonates.

Funding:

$80,733.00.00

Research Institution:

Department of International Health
Johns Hopkins University
School of Hygiene and Public Health
615 North Wolfe Street
Baltimore, Maryland 21205

Principal Investigator:

Robert Polk, M.D.

Research Goals:

To determine the feasibility of low-cost immunization for infants who are at increased risk of disease from hepatitis b virus (HBV) infections.

To compare the immune response to HBV in infants given:

- 3 (three) 2 microgram doses ID
- 3 (three) 2 microgram doses IM
- 3 (three) 10 microgram doses IM
All will be injected within 6 days of birth, at 2 months of age, and at 4 months of age.

Incidence of Disease:

In Southeast Asia, China, and the Amazon Basin, essentially all the population is infected with HBV at birth or in childhood. Of these some 5-20% become carriers. 90% of children born to mothers who have a HBV infection (are HBsAg+) will become carriers.

Rationale:

Hepatitis B Virus (HBV) is a major cause of morbidity and mortality worldwide. The severity of infections ranges from inapparent to acute fulminating hepatic necrosis. About 25% of carriers of HBV progress to chronic cirrhosis. Liver cancer in carriers is about 12-300X the rate of non-carriers.

A safe highly immunogenic vaccine is available. A series of three 10 microgram intramuscular (IM) injections produces immunity in essentially 100% of healthy infants and children. Unfortunately the dose is about $50 per child.

Intradermal (ID) injection of human diploid rabies vaccines or influenzae vaccine or booster doses of typhoid vaccine (or others) have been shown to be as effective as larger doses given by the IM route. A low dose ID HBV schedule may be safer, cheaper, and just as effective as the higher dose IM schedule. Evidence exists that as little as 1.25 micrograms of HBV given IM is immunogenic. Doses of 2.5-5.0 micrograms given subcutaneously (SC) have protected infants born to mothers who carry the virus (HBsAg+).

Techniques Used:

Radiolabelled reagents (RIA)
Enzyme assays (ELISA)

Ultimate Goal:

Prophylaxis.
Research Description:

Neonates will be vaccinated at three dosing levels of vaccine. They will be examined for local reactions post vaccination, and checked for seroconversion at 4 and 6 months post vaccination by ELISA (seroconversion indicates if the vaccine "took"). Infants with a poor antibody response will be revaccinated. If possible, additional samples will be taken at 18 months post vaccination.

Staffing:

M.D. 3  
Technician 1  
Computer programmer 1

Time for Completion:

18 months.

Progress:

Project has recently started in Baltimore, Maryland.

Sub-Grant Title:

Assessing the Impact of Vitamin A Supplementation on the Reduction of Incidence, Severity, and Rate of Episodes of Morbidity Among Children Aged 6 Years and Under in a Southeast Asian Country.

Funding:

$515,000.35

Research Institution:

Department of International Health  
School of Hygiene and Public Health  
Johns Hopkins University  
615 North Wolfe Street  
Baltimore, Maryland 21205

Principal Investigitor:

Michele Forman, Ph.D.
Specific Research Goals:

1. To determine the effect of vitamin A supplementation (200,000 I.U.) three times yearly on the morbidity of children under 6 years of age. This will be contrasted with those who get the RDA of vitamin A three times per year.

2. Serum retinol levels will be determined.

3. The rates of first and multiple episodes of diarrhea caused by selected organisms will be recorded.

4. The nutritional status of each child will be assessed.

5. The effect of any other covariates (education, income, family size) will also be evaluated.

Incidence of Disease:

In Asia alone, 500,000 children per year are judged to be deficient in vitamin A, or have manifestations of xerophthalmia. Malnourished children are 4 times as likely to die when they are vitamin A deficient, than if they were malnourished but with normal vitamin A levels. Vitamin A deficiency is also associated with growth retardation. Additionally, children deficient in vitamin A also have an increased risk of diarrheal and respiratory diseases.

Rationale:

Vitamin A deficiency is associated with both blindness and increased mortality. Vitamin A deficiency blindness ranges from mild nightblindness to severe ulceration and scarring of the eye (generally called xerophthalmia). At least 80% of xerophthalmia is associated with low vitamin A levels in the blood.

Vitamin A is a fat soluble vitamin essential for a wide variety of normal physiological functions, including vision, immune response, and growth. Dietary sources include liver and organ foods, milk, dark green leafy vegetables, and yellow fruits. In previous studies, 95% of the families in Peru were classified as having a very poor consumption of vitamin A.

In animals, vitamin A has been shown to enhance the activity of the immune system. In humans vitamin A supplementation has been shown to reduce mortality by 35% after supplementation with 200,000 I.U. 3 times per year.
Techniques Used:

Enzyme Assays (ELISA)
Analytic separations (HPLC)

Ultimate Goal:

Prophylaxis.

Research Description:

The study will be performed in the Albay province of the Philippines. Here vitamin A deficiency is endemic (20% of all children are nightblind). Children between the ages of 6 months and 5 years will be divided into 3 groups.

Group 1 will receive 200,000 I.U. of vitamin A three times yearly for two years.

Group 2 will receive 2,500 I.U. (the RDA) of vitamin A three times yearly for two years.

Group 3 will receive no treatment.

The children will be observed for morbid changes (diseases) over the two year period. In cases of diarrhea, sentinel organisms will be assayed for in the diarrheal fluid. The organisms are: Rotavirus, Shigella, and enterotoxogenic E. coli (ETEC), representing viral, invasive, and toxic infections. Blood levels of retinol will be determined. Impression cytology, a new method of determining the condition of the covering of the eye in cases of xerophthalmia, will be performed to assess conjunctival involvement. Acute respiratory infections will be recorded.

Staffing:

Ph.D. 1
M.D. 5
Technician 2
Computer programmer 1

Time for Completion:

2 years.
Progress:

The project was scheduled to begin in August 1986 in the Philippines. However, difficulties in obtaining country clearance have delayed the start-up. The Institute for International Programs is considering an alternative site in Indonesia.
Project Title:
Diagnostic Technologies for Community Health (DIATECH).

Project #:
936-5935.

Funding for Entire Project:
$7.25 million for 5 years (9/85-9/90).

Project Goals:
To improve the health status of less-developed country populations through the development, adaptation and transfer of simple, cost-effective diagnostic technologies.

Implementing Agency:
Program for Appropriate Technology (PATH)
Canal Place
4 Nickerson Street
Seattle, Washington 98109
Contact: Dr. Karen Auditore-Hargreaves or Mr. William Farrand (206) 285-3500

Project Manager:
Thomas Bender, M.D.
S&T/Health (703) 235-9823

Grant Title:
Development and Evaluation of DNA Probe Technology for the Diagnosis of Malaria in Volunteers.

Grant #:
860300045

Funding:
$68,147.00

Research Institution:
University of Maryland
Baltimore, Maryland.
Principal Investigator:
James B. Kaper, Ph.D.

Specific Research Goals:

Five DNA probes will be evaluated for the diagnosis of malaria caused by *Plasmodium falciparum*. Four of the probes will be cloned fragments of *P. falciparum* DNA, the fifth will be a synthetic probe (composed of a 21 base-pair oligonucleotide sequence).

The results of the DNA probe study will be compared with results obtained using thick blood smears and ELISA assays. This will determine if parasitemia can be detected earlier in the disease with probes than by other methods.

Incidence of Disease:

There are 100-200 million persons infected with malaria.

1 million children under the age of 5 die from malaria annually.

The total worldwide mortality from malaria is approximately 5 million.

Rationale:

Nucleic acid probes (both DNA and RNA) represent an important new technology in the diagnosis of infectious diseases. In one study using nucleic acid probes for the diagnosis of malaria, parasitemia levels of 0.0001% were detected in 10 microliter blood samples. The DNA probe technique is particularly attractive in terms of its sensitivity and its capacity to screen large numbers of blood samples.

The probe functions by the binding (hybridization) of the specific DNA sequences of the probe with matching DNA sequences of the parasite. The probe "lights-up" the sample by virtue of a radioactive label (32P) attached to it.

Techniques Used:

- Cell culture
- Radiolabeled reagents
- Recombinant DNA/splicing
- Enzyme assays (ELISA)
Ultimate Goal: Diagnosis.

Research Description:

INITIAL STUDIES:

DNA will be extracted from *P. falciparum* and applied (spotted) to filter membranes in various concentrations, both as purified material and reconstituted into blood.

Blood samples will have various amounts of intact parasites added, and the efficiency of the DNA extraction process will be determined.

Actual clinical samples will be tested in conjunction with volunteer vaccine trials.

Regarding the DNA probe, the probe concentration, and the time and temperature of hybridization will be examined. The labeling of the probe will also be examined. The methods used for labeling with 32P will include nick translation, end-labeling, and primer extension. This is done because different probes may have different optimal labeling requirements.

Different sample extraction techniques and different kinds of filter membranes (nitrocellulose, Whatman 541, Zeta-probe, etc.) will be examined.

STUDIES USING CLINICAL SPECIMENS:

When optimal conditions for each probe have been developed, five different probes will be evaluated using blood samples from volunteers experimentally infected with *P. falciparum*. Blood samples will be drawn from each volunteer at 12-hour intervals.

For the first study, only those samples for which clear-cut parasitemia is evident (as determined by thick smears) will be tested with the probes, using fresh, unfrozen samples. The results of the DNA probe analyses will be compared to the results of microscopic examination using thick smears and ELISA on the same samples.

For the second study, fresh, unfrozen blood samples will be examined over the total duration of the study, not just those samples where parasitemia is evident. This includes the pre-parasitemia stage, the parasitemia stage, and the post treatment (with chloroquine) stage.
Staffing:

Ph.D.  1
Technician  1

Time for Completion:

One year.

Time Before Field Use:

2 years (approximately).

Progress:

Not Available, project is just starting.

Grant Title:

A Monoclonal Antibody "Antigen Capture" Assay for Diagnosis of Malaria.

Grant #:  
860700121

Funding:  
$135,872.00

Research Institution:

Georgetown University
Washington, D.C.

Principal Investigator:

Diane Wallace Taylor, Ph.D.

Specific Research Goals:

Develop and evaluate a two-site, monoclonal antibody-based assay for the detection of P. falciparum antigens in serum and urine.
Incidence of Disease:

Yearly, 1 million children under the age of 5 die from malaria. Yearly, there are 5 million total deaths from malaria. An estimated 100-200 million persons are infected.

Rationale:

A two-sited monoclonal antibody (MAb) capture assay detected the heat-stable antigen (Ag7H8) in the blood and urine of mice infected with *P. falciparum*. A similar antigen was detected in the serum of Africans with acute falciparum malaria. This should be of value for species-specific diagnosis of *P. falciparum* infections. A large panel of sera/plasma is available for screening for this antigen. Many of these samples are from children living in Nigeria, Ghana, and The Gambia, many with known levels of parasitemia.

A two-sited MAb assay is possible because the 7H8 antigen has two epitopes which are recognized by the same MAb.

Techniques Used:

Hybridomas
Cell culture
Recombinant DNA/splicing
Southern Blotting
Enzyme assays/probes

Ultimate Goal:

Diagnosis.

Research Description:

1. Ensure the stability of the hybridoma cell lines and the production of MAb's.

2. Develop the two-sited antigen-capture assay:
   a. Purify the MAb and couple them to enzymes.
   b. Identify the best solid-phase support system.
   c. Determine the best amount of MAb to use, and the optimal time of adsorption.
   d. Determine the best blocking agent.
   e. Determine the best enzyme-substrate combination.
   f. Determine the optimal incubation times.
   g. Determine the best combination of primary (capture) and secondary (enzyme-labeled) MAb to use.
   h. Determine the sensitivity of the assay.
i. Determine the specificity of the two-sited assay.
j. Optimize the amount of human plasma/blood to use.
k. Adapt the assay for analyzing urine.
l. Evaluate storage methods.
m. Compare this assay with other malarial antigen-detection assays.
n. Compare the 2-sited capture assay with a direct binding assay.

3. Screen serum, blood, and urine from known positive and negative individuals.

**Staffing:**

Ph.D. 1
Technician 1

**Time for Completion:**

2 years.

**Time Before Field Use**

2 years (approximately)

**Progress:**

Not Available

**Grant Title:**

Non Radioactive Ultrasensitive Detection of Amplified Plasmodium falciparum Genes.

**Grant #:**

860500061

**Funding:**

$44,986.00

**Research Institution:**

University of Southern California
Los Angeles, California.
Principal Investigator:

W. John Martin, M.D., Ph.D.

Specific Research Goals:

Development of an ultrasensitive nonradioisotopic assay for the detection of nanogram quantities of P. falciparum in human blood. This technique should allow the selective amplification of a unique sequence of P. falciparum DNA by about 100,000-fold. The reaction will be quantified by measuring the incorporation of labeled (biotinylated) nucleotides into the reaction product using an indicator (avidin-peroxidase).

Incidence of Disease:

See previous malaria projects.

Rationale:

Specific oligonucleotides (short DNA base sequences) corresponding to the flanking regions of a specific gene can act as primers for the synthesis of that specific gene. When this reaction is repeatedly cycled using DNA polymerase, it is possible to amplify the specific gene over 200,000 fold within one hour. The synthesis of the gene can be quantitated using non-isotopic methods.

Techniques Used:

Radiolabeled reagents
Recombinant DNA/splicing
Enzyme assays/probes
Southern blotting

Ultimate Goal:

Diagnosis.

Research Description:

STEP 1. Synthesis of the oligonucleotide probes.

(This step has been completed.)

STEP 2. Purification of the synthesized probes. This step has been partially completed.
STEP 3. Collection of blood from *P. vivax* and *P. falciparum* infected patients diagnosed at the LAC-USC Medical Center. Over 100 patients with *P. vivax* and 5-10 with *P. falciparum* are expected to be diagnosed this year.

STEP 4. Isolation of parasites and extraction of DNA from infected and control blood specimens.

STEP 5. Amplification of the *P. falciparum* sequences using oligonucleotide primers and repeated cycles of DNA polymerization.

STEP 6. Quantitation of DNA synthesis using biotinylated nucleotides and avidin-linked enzyme action.

STEP 7. Characterization of the synthesized product using restriction enzymes.

STEP 8. Optimization of the methodology and determination of the specificity and sensitivity of the assay.

**Staffing:**

Ph.D. 1  
M.D. 1  
Technician 1

**Time for Completion:**

1 year.

**Time Before Field Use:**

5 years (approximately).

**Progress/Results:**

The oligonucleotide probes have been synthesized.

The oligonucleotide probes have been partially purified.

**Grant Title:**

Development of an ELISA to Monitor Quinine Levels in Malarious Patients.

**Grant #:**

860300049
Funding:

$19,948.00

Research Institution:

Institute of Zoology
London Zoological Society
London, England

Principal Investigator:

Alister Voller, Ph.D., D.Sc.

Specific Research Goals:

Development of a practical ELISA test (a simple photometric test with a total test time of 2 hours or less) for the measurement of plasma quinine levels.

Incidence of Disease:

See previous malaria projects.

Rationale:

Chloroquine-resistant strains of P. falciparum have emerged. Because of this, quinine will have to be used more often, as the resistant strains spread. A test for quinine plasma levels will be needed to be used alongside in vitro tests to determine the malaria parasites' drug resistance levels. It is anticipated that this approach will reduce the incidence of cerebral malaria, which has recently re-emerged as a commonplace infection, especially in Southeast Asia.

Techniques Used:

Enzyme assays/(ELISA)

Ultimate Goal:

Therapeutic drug monitoring.
Research Description:

**PHASE 1 (3 months):**

Quinine will be conjugated to ovalbumin, keyhole lympet hemocyanin (KLH), or thyroglobulin. These conjugates will be used to coat the wells in microstrips. Efforts will be made to stabilize the coated microstrips by various post-coating and drying procedures. An inhibition ELISA will then be performed on the quinine-coated microwells.

The format will be:

1. Dilute the quinine sample, and mix with antibody to quinine (rabbit anti-quinine serum).
2. Incubate the above mixture in quinine-coated microwells.
3. Assess the amount of antibody attached to quinine-coated wells by the addition of enzyme-labeled anti-rabbit Ig followed by the enzyme substrate. A color reaction will indicate if antibody was bound to the quinine coating in the wells. Quinine in the sample will reduce the intensity of the reaction in proportion to its concentration.

In this initial phase, reference aqueous preparations of quinine will be used.

**PHASE 2 (3-6 months):**

To determine if plasma or serum contain any factors which may interfere with the assay, prototype assays from Phase 1 will be evaluated as follows:

1. On plasma/serum samples with control amounts of quinine.
2. On plasma/serum samples from healthy volunteers given non-toxic doses of quinine.

This phase of testing will also establish the range of sensitivity of the assay in plasma and serum, and will enable the formulation of appropriate diluents and blocking agents.

A detailed progress will be provided at this point. If progress is judged to be acceptable, work will continue as follows.
PHASE 3 (6-10 months):

Plasma samples will be collected from patients at the Hospital for Tropical Diseases, London who have *falciparum* malaria infections and are being treated with quinine. The plasma samples will be tested by the quinine ELISA and by HPLC for quinine levels. The results will be analyzed along with parasitological and drug-resistance data. It is anticipated that 40-50 specimens will be assayed.

PHASE 4 (10-12 months):

On the basis of data generated in Phase 3, an attempt will be made to formulate the reagents into a relatively simple kit form. The kit will consist of:
1. Coated microstrips.
2. Specimen diluent.
3. Ready-to-use conjugate, if possible.
4. Substrate.
5. Reference standards of quinine.

Sets of reagents in the optimized form would be made available for approximately 5,000 tests. Subject to the responses of evaluators, a final kit form could be devised.

Staffing:

Technician  1

Time for Completion:

1 year.

Time Before Field Use:

1.5 years.

Collaborating Arrangements:

One clinical (Ph.D.) student from Columbia will participate. He is supported by his own funds.
Grant Title:

Colony-ELISA for the Detection of Bacteria Producing Shiga or Shiga-like Toxins.

Grant #:

860500063

Funding:

$111,242.00

Research Institution:

Henry M. Jackson Foundation for the Advancement of Military Medicine.

Principal Investigator:

Alison D. O'Brien, Ph.D.

Specific Research Goals:

Development of a colony-ELISA that will permit the detection in stool cultures of Escherichia coli that produce elevated levels of Shiga-like toxin I or its antigenic variant Shiga-like toxin II.

Incidence of Disease:

Not Available.

Rationale:

E. coli that produce elevated levels of Shiga-like toxins (I or II) have been strongly implicated by epidemiological studies as the cause of pediatric diarrhea, hemorrhagic colitis in children (and adults), and hemolytic-uremic syndrome in children. Currently there is no diagnostic assay available to distinguish such E. coli strains from normal flora E. coli in stool cultures. The colony-ELISA would be the first such process.

Techniques Used:

Hybridomas
Enzyme assays/probes (ELISA)
Western blotting
Ultimate Goal:
Diagnosis.

Research Description:

1. Purify Shiga-like toxin II from culture filtrates.
2. Determine the LD-50 of purified Shiga-like toxin II for BALB/c mice.
3. Inoculate BALB/c mice several times with sublethal doses of Shiga-like toxin II.
4. Harvest spleens when anti-shiga-like toxin II neutralizing antibodies are present in sera.
5. Fuse spleens with SP2/0-14 BALB/c mouse myeloma cells in the presence of polyethylene glycol.
6. Select hybrids by survival in a medium containing hypoxanthine, aminopterin, and thymidine.
7. Test culture supernatants of actively growing hybrids for anti-Shiga-like toxin II neutralizing antibodies.
8. Subclone cytotoxin-neutralizing hybrids by limiting dilution.
9. Characterize monoclonals for subunit binding specificity by Western blot.
10. Incorporate monoclonals to Shiga-like toxin II into existing colony-ELISA.
11. Test the sensitivity and specificity of the modified colony-ELISA assay with an available culture collection.

Staffing:

Ph.D. 1
Graduate student 1

Time for Completion:
2 years.

Time Before Field Use:
2-3 years.
Grant Title:

Visual Immunoassay (VIA) for Hepatitis B Surface Antigen and Cholera Enterotoxin: A Feasibility Study.

Grant #:

860300052

Funding:

$60,243.00

Research Institution:

Covalent Technology Corporation

Principal Investigator:

Michael G. Pappas, Ph.D.

Specific Research Goals:

To develop a rapid, simple, easy-to-perform immunoassay which is sensitive, specific, and reliable for the detection of hepatitis B surface antigen (HBsAg) and cholera exotoxin. The assay will be in the form of a dipstick immunoassay and is termed the Visual Immunoassay (VIA). The VIA dipstick employs purified (specific) antibody, covalently bound to the dipstick. Antigen in test samples will specifically bind to the antibody on the dipstick. Positive dipsticks will impart a blue color to the reactive area, while negative dipsticks appear colorless.

Incidence of Disease:

Cholera: Worldwide, 20-24 million are affected by cholera. In Africa, 1 thousand children under 5 years of age die annually, the total death incidence is about 20,000. In dia, 37,000 children under 5 years of age die annually, the total death incidence is about 102,400.

HBV: In Africa, 10%-20% of the population are carriers. Carriers get liver cancer at a rate 12-300X the rate of non-carriers. Twenty-five percent of carriers develop chronic cirrhosis. Ninety percent of children born to mothers with HBV infections become carriers.
Rationale:

Presently, the immunoassays most often used for the serodiagnosis of bacterial, viral, and parasitic diseases are ELISA and indirect immunofluorescence. These procedures are time-consuming, and have many steps. Additionally, both require skilled technicians and expensive, electrically operated photometers and fluorescent microscopes. The VIA maybe a significant advancement in rapid diagnostics for developing nations because it employs a simple-to-handle dipstick in a rapid, two-step procedure.

Techniques Used:

Hybridomas
Fluorescent assays/probes

Ultimate Goal:

Diagnosis.

Research Description:

Purified antigen and antibodies will be acquired (monoclonal and polyclonal). The VIA will be designed for the detection of HBsAg and cholera toxin (CT) using the following assay format:

1. The dipstick will be incubated in a small volume (less than 200 microliters) of sample (blood serum or plasma for HBsAg or stool for CT).

2. Wash dipstick.

3. Incubate the inoculated dipstick in a small volume of antibody-coated microparticles (of optimal size).

4. Wash the dipstick and allow to dry.

5. Read the dipstick for a positive reaction. Grade the reactions on a scale of 0 to +5 using standards provided with each kit. Steps 1 through 3 should not take any longer than 60 minutes to perform.

The amount of purified antibody to be covalently attached to the dipsticks, and the amount of antibody to be covalently attached to the microparticles will be optimized.

The minimum detectable limit of CT and HBsAg will be determined.
Two kits (25 tests per kit) will be produced for evaluation by PATH or its designee. A final report for submission with the test kits will be provided.

Staffing:

Ph.D. 2
Technician 1

Time for Completion:

1 year.

Time Before Field Use:

1.5 years.

Progress:

The project has not started yet.

Grant Title:

Serologic Detection of *Giardia lamblia* Antigen in Stool Samples

Grant #:

860700089

Funding:

$29,822.00

Research Institution:

Sero-Immuno Diagnostics, Inc.

Principal Investigator:

Irving G. Kagan, Ph.D.

Specific Research Goal:

Develop an antigen detection assay for *Giardia lamblia* in stool.
Incidence of Disease:

Worldwide.

Rationale:

Giardiasis is a serious parasitological disease in developed and developing countries. Currently, diagnosis (by detection of trophozoites or cysts in stool specimens) is labor intensive and requires highly trained personal. Additionally, diagnosis by strip test or biopsy is invasive, expensive, and labor intensive. The detection of antigen in stool specimens is a sign of an active infection. A simple serologic agglutination test would be very useful for diagnosis.

Techniques Used:

Hybridomas
Radiolabelled reagents

Ultimate Goal:

Diagnosis.

Research Description:

1. The optimal concentrations of antibody for antigen capture will be determined by various immunologic and radiolabeled methods.

2. Optimal methods for the attachment of polyclonal and monoclonal antibody to "micells" (nitrocellulose particles) will be determined. The following parameters will be studied:

   a. The concentration of micells used in the solid phase for maximal sensitivity in coupling with antibody.

   b. The types of substrates and surface to use (glass or plastic, plates or tubes).

   c. The best blocking agent to use after antibody attachment to obtain optimal sensitivity.

3. After the technical aspects of the coupling of micell to antibody have been accomplished, various polyclonal antibodies will be evaluated for their ability to bind antigen.
4. After the micell-bound antibody has captured antigens, a mouse monoclonal to the same antigen (but to a different epitope) will be introduced. This second antibody completes the sandwich. Several monoclonal antibody lines will be evaluated by immunologic methods (latex and red blood cells sensitized with purified mouse IgG) for binding capacity.

5. The last phase of the system is to evaluate the best detection system. Toward this end, latex particles, or red blood cells coated with purified anti-mouse IgG will be employed.

Staffing:

Ph.D. 2
Technician 1

Time for Completion:

6 months.

Time Before Field Use:

1.5 years.

Progress:

The project has not started yet.
Project Title:

Diarrheal Disease Research Program, ICDDR,B.

Project #:

936-5928

Agreement #:

DPE-5928-A-00-6002-00

Funding for Entire Project:

$9.0 million.

Project Goals:

To carry out research aimed at decreasing diarrheal disease morbidity and mortality.

Implementing Agency:

International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B).

Project Manager:

Jeffrey Harris, M.D.
S&T/Health

Grant Title:

Field Trial of Oral Cholera Vaccine

Funding:

1986 $900,000.00
1987 $1.1 million
1988 $1.1 million

Research Institution:

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).
Dhaka, Bangladesh.
Principal Investigator:
John Clemens, M.D.

Specific Research Goals:
1. To field test the Oral Killed Whole Cell/B-subunit vaccine for efficacy against cholera.
2. To study the determinants of immunity to cholera.
3. To determine factors related to childhood diarrheal morbidity and mortality.

Incidence of Disease:
Worldwide, 20-24 million people are affected by cholera. Case fatality ratios are 50-90%, and substantial morbidity occurs even with therapy. Current therapy involves either oral rehydration or intravenous infusion of fluids to replace the large volume of body fluid lost during the episodes of diarrhea.

Rationale:
The existing cholera vaccine is given by injection, and does not stimulate long-lasting immunity. Attention has been focussed on the use of oral cholera vaccines which will stimulate intestinal immunity more effectively. Oral vaccines consisting of killed cholera whole cells (WC) or whole cells with the B-subunit (BS) of the cholera toxin, have been successful in stimulating intestinal antibody formation in Bangladeshi volunteers. Additionally, neither the BS or WC components cause adverse reactions in humans, and both have shown protective activity in volunteers in America against challenge with virulent Vibrio cholerae organisms. This is the first field trial of an oral cholera vaccine in an area where cholera is endemic.

Techniques Used:
- Hybridomas
- Cell Culture
- Enzyme assays/probes
- Analytic separations
- Preparative separations

Ultimate Goal:
Prophylaxis.
Research Description:

The field trial was conducted in the Matlab area of the ICDDR,B. Cholera is endemic in this region. Participants were divided into groups of about 21,000 volunteers, consisting of males between the ages of 2 and 15 years, and females over the age of 2 years.

Group 1 received doses of 1 mg BS together with killed WC of 4 different serotypes of V. cholerae.
Group 2 received doses of WC without the BS component.
Group 3 received doses of a placebo of killed E. coli K-12.

The vaccination schedule called for an oral dose of BS-WC, WC, or placebo every 6 weeks, for a total of 3 doses.

The volunteers were then monitored for the incidence of naturally occurring cholera.

Time for Completion:

The initial phase was completed in 6 months. Monitoring of the groups will continue for several years.

Results:

In the first 6 months of the study, the BS-WC vaccine reduced the incidence of cholera by 85%. The WC vaccine reduced the incidence of cholera by 58%.

Publications:

Field Trial of Oral Cholera Vaccines in Bangladesh.
Clemens, John D., et al.

Immunogenicity and Reactogenicity of Oral Killed Cholera Vaccines.
Clemens, John D., et al.
Journal of Infectious Diseases
In press.
Grant Title:

Umbrella Shigellosis Grant

Funding:

1987 $520,000.00
1988 $600,000.00
1989 $840,000.00

Research Institution:

ICDDR,B

Principal Investigator:

David Sack, M.D.

Specific Research Goals:

Conduct clinical, laboratory, and epidemiological studies of shigellosis in preparation for a possible Shigella vaccine trial.

Disease Incidence:

Shigellosis is a major cause of morbidity and mortality in Bangladesh and other developing countries. The greatest incidence, and greatest mortality occurs in children under the age of 5 years. The disease is endemic in many countries, and epidemics do occur. A recent epidemic had a case fatality ratio of 41% for children under 1 year of age. In Matlab, dysentery associated deaths accounted for 29% of all deaths in children between the ages of 1 and 4 years.

Rationale:

Even though much is known about the epidemiology of shigellosis, insufficient information is available about the occurrence of shigellosis in a large population. This absence of data makes it impossible to conduct a meaningful field trial of a Shigella vaccine.

Ultimate Goal:

Prophylaxis.
Research Description:

This is a large omni-project. Several individual projects will be pursued simultaneously in a coordinated fashion. Broadly, these projects include:

Clinical studies leading to improved case management.

Laboratory studies aimed at vaccine development.

Epidemiologic studies aimed at preparing the epidemiologic data base needed for a Shigella vaccine field trial.

The shigellosis research will include:

Stool examination for red and white blood cells as a diagnostic test.

Fecal bacteriology, media development, antibiotic sensitivity.

Development of a rapid co-agglutination test for diagnosis.

Clinical evaluation of antimicrobial therapy.

Therapy in addition to antibiotic treatment, ie. nutritional therapy.

A study of improved measures for the management and prevention of complications, such as seizures and toxic megacolon.

The role of Shiga toxin in clinical disease.

Determine the antibody response to shigellosis.

Identification of Shigella virulence antigens.

Analysis of Shigella plasmids with attention to their role in virulence and vaccine development.

Development of a surveillance system for cases of shigellosis.

Development of a rabbit model for shigellosis.
Staffing:

Ph.D.  3  
M.D.  4

Time for Completion:

The current grant is funded for 3 years.
Project Title:
Diarrheal Disease Research Program, WHO.

Project #:
936-5928

Agreement #:
DPE-5928-G-SS-4069-01

Funding for Entire Project:
$3.4 million.

Project Goals:
Development of new vaccines against cholera, Rotavirus diseases, shigellosis, typhoid fever, and enteropathogenic E. coli diseases.

Implementing Agency:
World Health Organization.
Contact: Dr. Michael Merson
1121 Geneva 27
Switzerland

Project Manager:
Jeffrey Harris, M.D.
S&T/Health.

Progress:
WHO has been contacted for information about research projects receiving AID funding.
Project Title:
Americares

Project #:
936-5957

Agreement #:
DPE-5957-G-SS-5039-00

Amount of Funding for Entire Project:
$800,000 in 1985-1989.

Project Goals:
Lower the burden of the disease caused by leprosy.
Cure the patient and prevent disabilities.
Interrupt the chain of transmission of leprosy.

Implementing Agency:
Americares Foundation
51 Locust Avenue
New Canaan, Connecticut 06840

Contact: Dr. Christina Kuhn
(203) 966-5195

Project Manager:
Clive Shiff, Ph.D.
S&T/Health

Grant Title:

Research Institution:
Institute of Biomedicine
Caracas, Venezuela.
Principal Investigator:
Dr. Convit

Specific Research Goals:
Evaluate the efficacy of Multi-drug therapy (MDT) on leprosy patients in a large-scale intervention in a less-developed country.

Survey leprosy contacts, and develop methods of early diagnosis.

Disease Incidence:
2-3/100,000 inhabitants.

Rationale:
Multi Drug Therapy (MDT) is the therapy of choice in leprosy. However, a model of treatment and surveillance is needed which can be applied to other LDC's.

Techniques Used:
Enzyme assays/probes (ELISA)
Electron Microscope

Ultimate Goal:
Diagnosis
Therapy
Prophylaxis
Eradication of Leprosy

Research Description:
Patients are skin-tested with lepromin, purified protein derivative (PPD), and soluble antigen to determine the status of their immunity and exposure to leprosy.

Antibodies to phenolic glycolipid Z are assayed for by ELISA to indicate exposure to leprosy by the formation of antibodies to this component of the bacterium.
Time for Completion:

5 years

Time Before Field Use:

5-10 years
Project Title:
Tropical Disease Research, WHO

Project #:
931-1126

Agreement #:
DPE-1126-G-1C-5048-00

Funding for Entire Project:
$32.3 million

Project Goals:
To support the World Health Organization (WHO) Special Program for Research and Training in Tropical Diseases Research (TDR). Objectives include finding better, more economical methods for the diagnosis, treatment, prevention, and control of six major tropical diseases:
onchocerciasis and other filariases,
schistosomiasis,
malaria,
trypanosomiasis,
leprosy,
leishmaniasis.

Training objectives include the creation of a global network of scientific institutions in both developed and less-developed countries involved in tropical disease research.

Project Manager:
Clive Shiff, Ph.D.
S&T/Health (703) 235-9788

Implementing Agency:
World Health Organization
1211 Geneva, Switzerland
Contact: Dr. Warren Furth
Grant Title:

Immunology of Leprosy (IMMLEP)

WHO has been contacted for information on the projects being supported by this ear-marked funding from AID.

A grant of $500,000.00 has been awarded for June 1986 to September 1987 in support of IMMLEP projects.

This information will be included when the Biomedical Research Portfolio is updated.
Project Title:
Malaria Immunity and Vaccine Research

Project #:
931-0453

Amount of Funding for Entire Project:
$75,112,000 for the period 1975-1989

Project Manager:
James Erickson, Ph.D.
S&T/Health

Rationale:
Malaria is the major cause of morbidity and mortality in the developing world. It is estimated that half of the world's population, some 2 billion people, are at risk of contracting malaria. There are currently 300-400 million persons infected with malaria. Annually, the disease kills about 2-3 million people, most of these are children under the age of 5 years, living primarily in Africa.

Progress:
Since its inception 22 years ago, the USAID malaria network has contributed substantially to our understanding of the pathogenesis of malaria. Over 90 major publications in the scientific literature have come from the malaria research funded by USAID in the past 3 years alone. More importantly, the research has resulted in the production of a synthetic sporozoite vaccine for falciparum malaria which is currently undergoing Phase I trials in human volunteers at the University of Maryland.
Title:
Development of Synthetic Anti-Malaria Vaccine Based on the Sporozoite Stage of the Parasite. Characterization of Protective Antigens and Their Corresponding Genes.

Subproject #: 931-0453.02

Funding: $4,739,000.00 for 1978-1985

Research Institution:
New York University
School of Medicine
Dept. Microbiology
550 First Avenue
New York, N.Y. 10016

Principal Investigator:
Ruth Nussenzweig, M.D., Ph.D.

Specific Research Goals:

1. Develop a synthetic vaccine using peptides exposed on the surface of the circumsporozoite (CS) proteins.

2. Develop a chemically defined synthetic anti-malarial vaccine.

3. Determine the immunogenicity of the synthetic peptides of the CS protein of P. falciparum, and detect a common blood stage antigen.

4. Use specific DNA probes to detect and quantify exoerythrocytic forms of malaria parasites.

5. Clone the CS gene, and get it expressed in yeast.

6. Determine the immunogenicity of the products of the CS protein gene of P. vivax and characterize protective blood stage antigens.

7. Clone and compare the CS protein gene of different strains of P. vivax.
8. Identify and characterize the CS protein of *P. brasilianum*, and compare this antigen with the CS protein of *P. malariae*.

**Techniques Used:**

- Hybridomas
- Cell culture
- Radiolabelled reagents
- Fluorescent assays/probes
- Recombinant DNA/splicing
- Enzyme assays/probes
- Analytic separations
- Electron microscope
- Northern blotting
- Southern blotting
- Western blotting

**Ultimate Goal:**

- Prophylaxis
- Epidemiology

**Staffing:**

- Ph.D. 4
- M.D. 3
- Post-Doc 4
- Graduate student 2
- Technician 7

**Time for Completion:**

- More than 5 years

**Time Before Field Use:**

- Approximately 5 years

**Publications:**

Nussenzweig, R.S., and Nussenzweig, V. Parasitology Today 1(60): 151-159, 1985


See Appendix II for additional references.

Results:

The first human synthetic peptide malaria vaccine trial began on November 3, 1986 at the University of Maryland.

Collaborating Arrangements:

Dr. Aikawa, Western Reserve

Dr. Hollingdale, Rockville Laboratories

University of Rome and Burkina Faso

Centers for Disease Control

Title:

The Effect of Interferons/Lymphokines Against Malaria in Rhesus Monkeys.

Subproject #:

931-0453.39

Funding:

$240,00

Research Institution:

Uniformed Services University of the Health Sciences Department of Pathology Bethesda, Maryland 20814-4799
Principal Investigator:

Dr. Radha K. Maheshwari

Research Goals:

To determine the possible role of interferons and other lymphokines in the control of malaria infections, especially in relapse cases.

Rationale:

Interferons may be useful in the control of relapsing malaria. Currently, only primaquine is effective against this stage of the infection. However, the toxicity of this drug limits its general use, and cannot be used in children, pregnant women, and people deficient in glucose-phosphate dehydrogenase.

Techniques Used:

Cell culture
Radiolabelled reagents
Fluorescent assays/probes
Electron microscope

Ultimate Goal:

Therapy
Prophylaxis

Research Description:

Rhesus monkeys will be infected with P. cynomolgi, and treated with gamma interferons and lymphokines. The mechanism of protection at both the cellular and humoral level will be investigated. This primate model closely relates to human infection with P. vivax, especially in terms of relapse.

Staffing:

Ph.D. 1
M.D. 1
Post-Doc 1
Technician 1

Time for Completion:

5 years
Time Before Clinical/Field Use:

5 years

Publications:

See Appendix II.

Results:

Prophylactic treatment with 0.1 mg of human gamma interferon per kilogram body weight per day completely suppressed experimental infection with P. cynomolgi b sporozoites in rhesus monkeys. Treatment with lower doses partially suppressed infection. Prophylactic treatment with human gamma interferon, however, had no protective effect against trophozoite-induced infection. This suggests that the interferon effect is limited to the exoerythrocytic stage of parasitic development.

Collaborating Arrangements:

Central Drug Research Institute,
Lucknow, India.

Hospital Salpetriere,
Paris, France.

Biomedical Research Institute,
Rockville, Maryland, USA.

Project Title:

Development of Asexual Stage Plasmodium falciparum Vaccine.

Subproject #:

931-0453.05

Funding:

$972,077.00 (1986-1987)

Research Institution:

University of Hawaii
Department of Tropical Medicine and Medical Microbiology
Honolulu, Hawaii 96816
Principal Investigator:
Dr. Wassim Siddiqui

Research Goals:
1. To identify protective antigens of *P. falciparum*.
2. To identify safe and efficacious adjuvants.
3. To demonstrate functional immunity in experimental animals.
4. To formulate a vaccine for clinical trials.

Techniques Used:
Hybridomas
Cell Culture
Radiolabeled reagents
Fluorescent assays/probes
Recombinant DNA/splicing
Enzyme assays/probes
Analytic separations
Electron microscope
Northern blotting
Southern blotting
Western blotting
Preparative separations

Ultimate Goal:
Prophylaxis.

Research Description:
Using hybridoma technology, specific monoclonal antibodies have been produced against the malaria parasite. These monoclonals are used as probes to identify and purify protective antigens. Once a native protein is shown to be protective, the specific gene for that protein will be cloned. Using recombinant DNA technology, these genes will be inserted into *E. coli* or yeast vectors to get expression of that protein. These proteins will be evaluated in experimental animals for their ability to induce functional immunity. The results of these studies will enable the formulation of a specific malaria vaccine for human use.
Staffing:

Ph.D. 3
Graduate student 3
Technician 5

Publications:

See Appendix II. At least 50 publications have come out of this grant since 1977.

Time for Completion:

4 years

Time Before Field/Clinical Use:

5 years

Results:

1. Using crude extracts of schizonts and merozoites as antigens, functional immunity has been induced in Aotus monkeys.

2. Specific monoclonal antibodies have been produced against P. falciparum.

3. Monoclonal antibodies have been used as probes to identify and purify specific protective proteins for vaccination studies.

4. A native protein (185,000 mw) has been shown to be effective in inducing sterile immunity in Aotus monkeys.

5. The gene for the 185,000 mw protein has been cloned.

6. The cloned protein is now being introduced into a yeast system for expression of the protein.

Collaborating Arrangements:

Currently being negotiated.
Title:

Molecular Cloning of the Dihydrofolate Reductase Gene of Plasmodium falciparum: A Model to Study Expression and Regulation of Malaria Genes.

Subproject #:

931-0453.35

Funding:


Research Institution:

Biomedical Research Institution
12111 Parklawn Drive
Rockville, Maryland 20852
(301) 881-3300

Principal Investigator:

Dr. J. Werner Zolg

Research Goals:

1. Use recombinant DNA technology to clone and express genes of the human malaria parasite, P. falciparum.

2. Determine molecular reasons for drug resistance.

3. Develop DNA probes for use as specific diagnostic tools to monitor the effect of malaria vaccines.

Techniques Used:

Cell culture
Radiolabelled reagents
Fluorescent assays/probes
Recombinant DNA/splicing
Enzyme assays/probes
Analytic separations
Southern blotting
Preparative separations

Ultimate Goal:

Diagnosis
Therapy
Staffing:

Ph.D. 3
Post-Doc 1
Technician 3

Time for Completion:

8 months

Time Before Field/Clinical Use:

Immediately. Tests are ongoing.

Publications:

Zolg, et al.
Molecular Biochemical Parasitology
1986 in press

Chen, et al
Molecular Pharmacology
1986 submitted for publication

Results:

1. Specific DNA probes have been isolated and sequenced. Techniques are now at hand to detect picogram quantities of P. falciparum DNA.

2. The kinetic and molecular properties of the target enzyme for antimalarial drug therapy (dihydrofolate reductase) based on antifolates has been completed.

Collaborating Arrangements:

Dr. M. Levine
Center for Vaccine Development
Baltimore, Maryland.

Dr. M. Hollingdale
Biomedical Research Institute
Rockville, Maryland.
Title:
The Development of Antigens for Use in Anti-blood Stage Vaccines (Merozoite Surface, Rhoptries, Erythrocyte Membrane) for *Plasmodium falciparum*.

Subproject #:
931-0453.17

Funding:
$844,069.00 for 1986-1987

Research Institution:
Scripps Clinic and Research Foundation
1066 North Torrey Pines Road
La Jolla, California 92037
(619) 333-2671

Principal Investigator:
Dr. Robert Reese

Specific Research Goals:
To identify a series of components in the blood stages of human malaria which can be used to stimulate a protective immune response in humans, or be used for immunodiagnostic purposes.

Techniques Used:
Hybridomas
Cell culture
Radiolabelled reagents
Fluorescent assays/probes
Recombinant DNA/splicing
Enzyme assays/probes
Analytic separations
Electron microscope
Northern blotting
Southern blotting
Western blotting
Preparative separations
Synthetic peptide chemistry
Circular dichroism spectroscopy
Nuclear magnetic resonance
Ultimate Goal:

Diagnosis
Prophylaxis

Research Description:

Mouse monoclonal antibodies (MAb) and monkey MAb's are being used to identify proteins on the parasite which it uses to attach to and enter red blood cells (RBC). (The technique for producing monkey MAb's was developed by this research group.) Monoclonal antibodies are also being used to identify proteins that the parasite inserts into the RBC membrane after it enters the RBC.

The genes coding for these proteins are copied from the parasite, and cloned into bacteria. MAb's are then used to identify the bacterial clones which are producing the portions of these proteins which contain the antibody binding site.

The parasite DNA contained within the clones (cDNA, for copy DNA) is sequenced. From this information, the structure of the encoded protein is deduced using a series of computer programs. Computers are also used to make three-dimensional simulations of the conformation of the protein. Based on this information, peptides are synthesized which correspond to segments of the protein most important in stimulating an antibody response.

The synthetic peptides are characterized both immunologically and physically. Proof that the antigen (synthetic peptide) is truly on the surface of the parasite, or is involved in penetration of the RBC is done by immuno-electron microscopy. Peptides which show the most promise, based upon their biological and chemical properties, will be tested for their ability to stimulate immunity in Aotus monkeys. Additionally, some will be used for diagnosis.

Staffing:

Ph.D. 7
M.D. 1
Technician 5

Time for Completion:

6 years

Time Before Field/Clinical Use:

3-5 years
Publications:

See Appendix II. At least 27 support this research.

Results:

During the last 3-4 years, a series of MAb's have been produced which are capable of detecting most of the major proteins produced by the blood stage of \textit{P. falciparum}. Clones of cDNA encoding portions of 3 surface proteins have been isolated and sequenced. One of these has been subcloned by Smith, Kline, and Beckman so that large quantities of recombinant protein can be obtained. Large synthetic peptide models have been made which will also be tested for their capacity to induce strong protective immunity in monkeys.

Collaborating Arrangements:

Drs. S. Langreth  
W. Collins  
Gary Campbell  
M. Aikawa  
M. Hollingdale  
G. Losonsky  
M. Levine.

Title:

Development of Rapid, Field Applicable Diagnostic Tests for Malaria.

Subproject #:

931-0453.99

Funding:

$737,000.00 (1985-1988)

Research Institution:

KT and R Laboratories  
1491 Energy Park Drive  
St. Paul, Minnesota  55082
Principal Investigator:  
Dr. Gottfried Kellerman

Research Goals:
Development of a simple immunodiagnostic test for malaria.

Techniques Used:
Hybridomas  
Cell culture  
Western blotting

Ultimate Goal:  
Diagnosis

Research Description:
An ELSIA assay will be developed which will detect actual plasmodial infection during both clinical and subclinical (latent) forms of the disease. The performance of the research involves the use of a number of proprietary products and methods, especially in the specific methodology for the antigen binding immunoassays.

Staffing:
Ph.D. 3  
Technician 2  
Undergraduates 2

Time For Completion:
3 years

Time Before Field/Clinical Use:
3 years
Collaborating Arrangements:
Institutions in Venezuela and Columbia

Project Title:
Malaria Immunity and Vaccine Research - Centers for Disease Control.

Subproject #:
931-0453.24

Funding:
$207,480.00 for 1986-1987.

Research Institution:
Department of Health and Human Services
Centers for Disease Control
Division of Parasitic Diseases
Atlanta, Georgia 30333

Principal Investigator:
Carlos C. (Kent) Campbell, M.D., M.P.H.

Specific Research Goals:
1. To assess alternative non-human primate models for malaria.
2. To assess experimental malaria vaccines, developed by USAID funded research programs, in non-human primates.
3. To collect appropriate data on the primate colony.
4. To investigate vaccine parameters using primate malarias.
5. To provide malaria infected mosquitoes to support USAID network laboratories.
6. To develop immuno-assays for the detection of parasite antigens, DNA hybridization techniques for the detection of parasites, and serologic tests for the evaluation of the immune response.

7. To conduct research in Kenya to:

A) Develop and field test ELISA assays for the detection of sporozoites in mosquito vectors.

B) Develop and test ELISA using the synthetic CSP for different species of Plasmodium.

C) Measure the inoculation rates of species-specific Plasmodium sporozoites.

D) Assess the prevalence of antibodies to *P. falciparum* sporozoites.

E) Evaluate the effect of antibodies acquired at birth.

F) Determine the age-specific proportions of the population which have circulating infectious gametocytes.

G) Draw inferences on the effect of antisporezoite vaccines.

Techniques Used:

- Hybridomas
- Cell culture
- Radiolabelled reagents
- Fluorescent assays/probes
- Recombinant DNA/splicing
- Enzyme assays (ELISA)
- Analytical separations
- Northern blotting
- Southern blotting
- Western blotting
- Preparative separations

Ultimate Goal:

- Diagnosis
- Therapy
- Prophylaxis
Research Description:

All of the basic immunological techniques and methods are used for the development of monoclonal antibodies for the detection of antigens of the different species of Plasmodium. DNA probes are also generated using appropriate techniques. These procedures are then used to assess the immune responses of immunized non-human primates and naturally infected native populations.

Staffing:

- Ph.D. 3
- M.D. 4
- Post-Doc 3
- Technician 6

(Professional staff receives travel, supplies, and equipment, but not salary support.)

Time for Completion:

5 years

Time Before Field Use:

Current field applications in Kenya.

Publications:

See Appendix II.

Results:

1. Non-human primate models have been developed for the testing of sporozoite and blood stage vaccines using Aotus and Saimiri monkeys. Trials have been initiated to test the ring-stage-infected erythrocyte surface antigens (RESA), and sporozoite vaccines against *P. falciparum* and *P. vivax*.

2. Monoclonal antibodies have been developed against the blood stages and the circumsporozoite proteins (CS) of the four human malaria parasites. The monoclonals to the CS are being used to assess the infection rates of different species of mosquitoes in Kenya.

3. Synthetic peptides and DNA probes are being used to assess the presence of specific malaria antigens and antibodies in human populations in Kenya with different levels of malaria endemicity.
4. Simian models for malaria have been developed for the testing of the parameters for vaccine effectiveness in non-human primates.

5. Blood-stage parasites and mosquitoes infected with sporozoites have been provided to other USAID investigators for the development of DNA libraries and for reference antigens.

Collaborating Arrangements:

Biomedical Research Institute
University of Hawaii
University of Illinois
Scripps Clinic and Research Institute
New York University
Case Western Reserve University
National Institutes of Health

Title:
Operation of a Facility for the Study of Malaria Vaccines in Volunteers.

Subproject #:
931-0453.46

Funding:
$6,768,017.00 for 1985-1990.

Research Institution:
Center for Vaccine Development
Division of Geographic Medicine
Department of Medicine
University of Maryland School of Medicine

Principal Investigator:
Myron M. Levine, M.D., D.T.P.H.
Specific Research Goals:

To create a multidisciplinary professional team to conduct clinical and basic research on malaria and malaria vaccines using healthy volunteers. Outpatient safety and immunogenicity (Phase I) and inpatient efficacy (Phase II) studies will be conducted on candidate vaccines. An insect and Plasmodium culture laboratory will be maintained to support these activities. The humoral and cellular immune responses to vaccination and experimental malaria infections will be evaluated.

Techniques Used:

Hybrimomas
Cell culture
Radiolabelled reagents
Fluorescent assays/probes
Recombinant DNA/splicing
Enzyme assays/probes
Analytic separations
Northern blotting
Southern blotting
Western blotting
Preparative separations

Ultimate Goal:

Diagnosis
Prophylaxis
Therapy

Research Description:

The humoral and cellular response to vaccination will be evaluated as follows: Anti-sporozoite antibody will be measured three ways; by ELISA using a synthetic peptide antigen, by indirect immunofluorescence (IFA) using whole sporozoites, and by the circumsporozoite precipitation reaction. Humoral immune responses to blood stage antigens will utilize "classic" assays such as merozoite invasion inhibition, and IFA. Additionally, ELISA and Western blotting will be performed using purified, partially purified, or crude antigens to determine both the breadth and specificity of the antibody response to this complex group of antigens. The study of the cell-mediated arm of the immune response will involve sensitizing lymphocytes with various plasmodial antigens (or vaccine components) and measuring lymphocyte blast transformation, and the production of interleukins and interferons.
In conjunction with efficacy studies of malaria vaccines, applications of modern biotechnology will be applied to the rapid diagnosis malaria infections. The approach will utilize DNA probes to detect minute amounts of of Plasmodium DNA in the blood of infected volunteers. The probes will be prepared form cloned Plasmodium DNA or from synthetic DNA probes prepared from a DNA synthesizer. The probes will be either radiolabelled or biotin-labelled. Additionally, enzyme immunoassays using polyclonal and monoclonal antibodies will be used to detect Plasmodium antigens during the blood stages of infection.

Staffing:

Ph.D. 4
M.D. 4
Post-Doc 2
Technician 6

Time for Completion:
4.5 years

Time Before Field Use:
10 years

Results:

Low levels (less than 0.01%) of parasitemia occurring after sporozoite challenge can be detected and treated prior to the onset of symptoms. In 75% of cases this early treatment prevents any symptoms of clinical malaria. This model effectively eliminates significant hazards and minimizes the discomfort to volunteers who will participate in the efficacy trials of the sporozoite vaccines.

The safety and immunogenicity testing (Phase I) testing of a synthetic peptide P. falciparum vaccine commenced November 3, 1986, with 45 volunteers participating in the program.

Collaborating Arrangements:

New York University
Department of Parasitology

M. Hollingdale
Biomedical Research Institute

Scripps Institute
Title:

Studies on Malaria Antigens and Their Effects on Aotus Monkeys With Special Reference to the Nephrotoxic Syndrome.

Subproject #:

931-0453.36

Funding:


Research Institution:

Case Western Reserve University
Institute of Pathology
2085 Adelbert Road
Cleveland, Ohio 44106

Principal Investigator:

Masamichi Aikawa, M.D.

Specific Research Goals:

To determine the pathogenesis of renal disease with particular reference to the nephrotoxic syndrome occurring in uninfected, and P. falciparum infected Aotus monkeys.

To characterize the surface and intracellular antigens of P. falciparum and P. vivax by immuno-electron microscopy.

Rationale:

The nephrotoxic syndrome is one of the major complications of malaria. This research project will determine if malaria antigens used for malaria vaccines causes the nephrotoxic syndrome.

Techniques Used:

Hybridomas
Cell culture
Fluorescent assays/probes
Electron microscope
Western blotting
Ultimate Goal:

Therapy

Research Description:

Aotus monkeys will be experimentally infected with P. falciparum, and their renal tissues will be studied by light microscopy, electron microscopy, and immuno-electron microscopy. Sera from these monkeys will be analyzed for circulating immune complexes.

Staffing:

M.D. 2
Post-Doc 2

Time for Completion:

5 years

Time Before Field Use:

5 years

Publications:

See Appendix II.

Results:

During the past 2 years, the kidney tissues of 31 Aotus monkeys infected with P. falciparum have been studied. Both light and electron microscopy have demonstrated mesangial proliferative glomerulonephritis (inflamed kidneys). Immuno-histology of the tissues (using a peroxidase anti-peroxidase technique) showed that IgG, C3, and P. falciparum antigens were present in the majority of glomeruli.

A pathologic study was performed on 16 Aotus monkeys to evaluate the effect of malaria vaccine on kidney tissue. Five of nine Aotus monkeys showed deposits of P. falciparum antigens.

Collaborating Arrangements:

New York University
Scripps Research Institute
Project Title:


Subproject #: 931-0453.12

Agreement #: DPE 0453-C-00-3051

Funding: $2,863,000.00 for 1983-1986

Research Institution:

Biomedical Research Institute
1211 Parklawn Drive
Rockville, Maryland 20825

Principal Investigator:

Dr. Michael Hollingdale
(301) 881-3300

Specific Research Goals:

1. Produce mosquitoes infected with P. falciparum.

2. Develop the inhibition of sporozoite invasion (ISI) assay for use in clinical trials and areas endemic for P. falciparum and P. vivax.
3. Study the biochemistry of exoerythrocytic antigens.

4. Determine the ultrastructure of sporozoite invasion and exoerythrocytic development using electron microscopy.

Techniques Used:

- Hybridomas
- Cell culture
- Radiolabelled reagents
- Fluorescent assays/probes
- Enzyme assays/probes
- Analytic separations
- Electron microscope
- Western blotting
- Preparative separations

Ultimate Goal:

- Prophylaxis

Research Description:

Sera to candidate malaria antigens are prepared by hybridoma technology. The antibodies are labelled with fluoresceine or peroxidase, and tested against malarial parasites grown in cell culture. Parasite antigens are purified and analyzed biochemically, using, for example, polyacrylamide gel electrophoresis and western blotting. Immuno-gold labelling and electron microscopy are then used to study the process of parasite invasion and development. The interactions of the antibodies with purified antigens or synthetic peptides or recombinant proteins will determine the molecular basis for parasite invasion.

Staffing:

- Ph.D. 4
- Post-Doc 2
- Graduate student 2
- Technician 10

Time for Completion:

- 3 years (June, 1986)

Time Before Field Use:

- Immediate.
Publications:

See Appendix II.

Results:

Candidate malarial vaccines based on the P. falciparum circumsporozoite (CS) protein (the repeated epitope region) are currently in clinical trials. The ISI assay has been shown to be the only assay which correlates with protective antibodies, and is therefore being used during the clinical trials. The ISI assay is also being used (along with other serologic assays) to study the epidemiology of malaria immunity to different parameters of mosquito infections in Liberia, Tanzania, Kenya, Brazil, and Indonesia.

Other regions of the CS protein have been shown to directly mediate sporozoite infectivity, and are therefore candidates for second-generation vaccines. The synthesis of the CS protein by sporozoites has been shown by electron microscopy, and the fate of the CS protein during invasion has been described.

Mosquitoes infected with P. falciparum have been used to safely and effectively infect human volunteers. This is crucial for future Phase II clinical trials of the vaccine.

Collaborating Arrangements:

Case Western University: Dr. M. Aikawa

Centers for Disease Control: Drs. C. Campbell and W. Collins

Georgetown University: Dr. D. Taylor

Naval Medical Research Institute: Dr. S. Hoffman

New York University: Drs. R.S and V. Nussenzweig

University of Nijmegen (Medical Parasitology): Drs. J. Meuwissen and J. P. Verhave

Rockefeller University: Dr. J. Tam

Sao Paulo (SUCEM): Dr. Boulos

Tapachula: Dr. Mendez

University of Utrecht: Dr. H. J. Geuze
Walter Reed Army Institute of Research:
Dr. W.T. Hockmeyer

Title:
Anti-Blood Stage Vaccines: Soluble Plasmodium Antigens From Culture Fluids.

Subproject #:
931-0453.34

Agreement #:
DPE-0453-C-00-3059

Funding:
$2,387,000.00 for 1983-1986

Research Institution:
University of Illinois at Urbana-Champaign
College of Veterinary Medicine
2001 South Lincoln Avenue
Urbana, Illinois 61801

Principal Investigator:
Dr. Miodrag Ristic
(217) 333-2671

Specific Research Goals:
To develop a malaria vaccine for Plasmodium falciparum using in vitro culture-derived soluble exoantigens (or their purified components, synthetic analogues, or genetically engineered products). The Indochina I and Geneve strains of P. falciparum are being used.
Techniques Used:

Hybridomas
Cell culture
Radiolabeled reagents
Recombinant DNA/splicing
Fluorescent assays/probes
Enzyme assays/probes
Analytic separations
Electron microscope
Northern blotting
Southern Blotting
Western blotting
Preparative separations

Ultimate Goal:

Prophylaxis

Research Description:

When *P. falciparum* is cultivated in vitro, it freely releases proteinaceous molecules into the culture fluid. These molecules are antigenic, and are called exoantigens. The exoantigens are being used as a source of experimental vaccines. The exoantigens are being characterized both in vitro and in vivo for their immunological and biochemical properties. Squirrel monkeys (*Saimiri sciureus*) are being used as test animal model for efficacy studies.

Staffing:

Ph.D. 6
Post-Doc 1
Graduate student 3
Technician 3

Estimated Time For Completion:

3 years (June, 1989)

Time Before Field/Clinical Use:

3 years (Spring, 1989)

Results:

1. Partially-purified exoantigens have demonstrated protection from clinical disease in the Bolivian squirrel monkey model.
2. A synthetic polypeptide, derived from the amino acid sequence of the amino terminal of an 83,000 mw protein, has been constructed and shown to be immunogenic.

Publications:

See Appendix II.

Collaborating Arrangements:

University of Illinois
Department of Pathobiology, and the Department of Biochemistry

Institute Mérieux
Lyon, France

Project Title:

Develop Monkey Models Using Wild Caught Monkeys

Subproject #: 931-0453.38

Agreement #: BST-0453-P-HZ-4068

Funding: $941,065.00 (length of project)

Research Institution:

National Institutes of Health
Building 102
Room 102
Bethesda, Maryland 20205

Principal Investigator:

Dr. David Renquist
(301) 496-9416
Research Goals:

This is an agreement with the National Institutes of Health and the Pan American Health Organization to coordinate and supply AID with monkeys from Peru.

Project Title:

Pathogenesis and Ultrastructure of Plasmodia and RBC

Subproject #:

931-0453.14

Agreement #:

BST-0453-P-02-4022

Funding:

$947,652.00 for 1984-1987

Research Institution:

Dept Microbiology
Uniformed Services University of the Health Sciences
Room B 4126
4301 Jones Bridge Road
Bethesda, Maryland 20852

Principal Investigator:

Dr. Susan Langreth
(301) 295-3310

Did not respond.
Project Title: Vaccine Development and Health Research (PASA)

Project #: 936-5947
Agreement #: BST-5947-P-HI-4265-02

Funding: $6,000,000 for 1984-1989

Project Goals:
To develop new and improved vaccines to reduce the incidence of major preventable diseases in developing countries. Current investigation includes an improved, less reactive pertussis vaccine, a measles vaccine effective in children younger than 5 months of age, a rotavirus vaccine, and a typhoid vaccine.

Contact:
George Curlin, M.D.
Fogarty International Center
Building 38A
Room B2N-13
National Institute of Health
Bethesda, Maryland 20892
(301) 496-2516
Agreement Title:  
Evaluation of Vaccine Injection Technologies-Ezeject and Conventional

Contract #:  
282-86-0010

Funding:  
$132,662.23

Research Institution:  
Pan American Health Organization  
Johns Hopkins School of Hygiene and Public Health

Principal Investigators:  
Neal A. Halsey, M.D.  
Ciro C. A. deQuadros, M.D.

Specific Research Goals:  
To compare two vaccine injection technologies, a unit-dose system (EZEJECT) and a conventional needle-and-syringe system. The comparisons involved:

1. Necessary training of health workers.
2. Seroconversion rates of the recipients.
3. Local reactions to the injection.
4. Efficiency of the vaccination program.

Rationale:  
Transmission of disease (including hepatitis and AIDS) from the re-use of needles is a serious health problem. A single-dose, non-reusable injection unit would eliminate the spread of infections by this route.

Additionally, vaccines are currently provided in multi-dose vials. There is a tendency to waste vaccine when only a few doses are needed.

The EZEJECT system, which is a single-dose non-reusable vaccine injection unit would remedy both of these problems.
Incidence of Disease:

The distribution of needle-transmitted infections is ubiquitous in LDC's. The incidence of these infections varies, but often occur in large outbreaks.

Techniques Used:

Enzyme assays/probes (ELISA)

Ultimate Goal:

Prophylaxis

Research Description:

Children in Guatemala, aged 9-23 months, were inoculated with measles vaccine using either EZEJECT (Merck, Moraten measles strain) or conventional needle-and syringe methods (Schwarz measles strain, Merieux Institute). The rates of seroconversion were measured by ELISA 4-6 weeks after vaccination using a commercial ELISA assay (Measlestat MA, Bioproducts). The speed and technique of the people performing the injections were also monitored.

Staffing:

M.D. 4
Graduate student 1
Technician 1
Trainer 1

Time for Completion:

9 months. Project was completed in August, 1986.

Time Before Field/Clinical Use:

12 months

Results:

The seroconversion rates were equal for the EZEJECT method and the conventional method. The antibody titer was slightly higher in the EZEJECT group. Inoculation with EZEJECT was faster than conventional methods. The newly-trained vaccinators had difficulty remembering to aspirate for blood before injection during vaccination.
Publications:

Field Evaluation of Ezeject: A Simplified Unit Dose Syringe for Administration of Measles Vaccine.
Neal A. Halsey, M.D. et al.
Department of International Health
School of Hygiene and Public Health
Johns Hopkins University
Baltimore Maryland

Results of Ezeject Studies in Guatemala
Juan Jose Arroyo Hernandez, M.D., M.O.H., Guatemala

Collaborating Arrangements:

Contracts with PAHO (Pan American Health Organization)

Agreement Title:

Phase I Studies of the Rhesus Rotavirus Vaccine-University of Maryland

Agreement #:

OIH-007-285

Funding:

$71,160

Research Institution:

University of Maryland
Center for Vaccine Development

Principal Investigator:

Margaret Rennels, M.D.

Specific Research Goals:

To evaluate the immunogenicity, infectivity, and side effects from a low-dose of Rhesus rotavirus vaccine strain MMU 18006
Rationale:

Diarrheal disease is one of the major killers of young children, especially those under 1 year of age. The number of deaths in the less developed countries of the world caused by diarrhea has been estimated at 5-10 million per year. Rotavirus has been identified in the stool of 30-48% of children under 2 years of age with diarrhea. A vaccine preventing diarrhea caused by rotavirus would be an important strategy against infant and childhood mortality.

Incidence of Disease:

Rotaviral diseases are ubiquitous in their geographic distribution.

Techniques Used:

Cell culture
Enzyme assays/probes

Ultimate Goal:

Prophylaxis

Research Description:

The experimental groups were vaccinated with doses of 1,000 pfu (plaque-forming-units) or 10,000 pfu of the rotavirus vaccine. The immunogenicity of vaccine was measured by ELISA, and the volunteers were monitored for side-reactions.

Staffing:

M.D. 7
Technician 1

Time for Completion:

3 months

Time Before Field Use:

2 years
Results:

The dose of 10,000 pfu produced 100% seroconversion in the test groups (all of the children responded to the vaccine). The incidence of fever was the same in both groups, and was related to the age of the subject.

Collaborating Arrangements:

PASA subagreements with National Institute of Allergy and Infectious Disease

NIAID contract with University of Maryland.

Agreement Title:

Rhesus Monkey Rotavirus Vaccine (RRV)-Field Trials, Sweden

Agreement #:

OIH-005-258

Funding:

$84,955

Research Institution:

University of Umea
Sweden

Principal Investigator:

Lief Gothefors, M.D.

Specific Research Goals:

To evaluate the Rhesus rotavirus vaccine (RRV) with regard to:

1. Side effects
2. Sero-conversion
3. Efficacy
Techniques Used:

- Cell culture
- Enzyme assays/probes (ELISA)
- Electron microscope

Research Description:

Two experimental groups were inoculated with either a high dose of RRV at 30,000 pfu, or a low dose at 3,000 pfu (a pfu is a plaque-forming-unit, or the number of viable viruses). The groups were monitored for seroconversion rates, and diarrheal disease rates.

Staffing:

- M.D. 7
- Technician 1

Time for Completion:

- 1.5 years

Time Before Field Use:

- 2 years

Results:

1. The high dose of RRV produced unacceptable levels of fever and loose stools in the subjects.

2. The overall seroconversion rate was 93%.

3. The vaccinated groups experienced fewer bouts of diarrhea, and the diarrhea was less severe than the unvaccinated group.

Collaborating Arrangements:

- PASA subagreements with the National Institutes of Allergy and Infectious Diseases (NIAID)
- NIAID contract with the University of Umea, Sweden
Agreement Title:
   Rhesus Monkey Rotavirus Vaccine-Field Trials

Agreement #:
   OIH-002-258

Funding:
   $115,000

Research Institutions:
   National Institute of Dermatology
   Caracas, Venezuela

   University of Tampere
   Finland

Principal Investigators:
   Jorge Flores, M.D.
   Irene Perez-Schael, M.Sc.
   Timu Vesikari, M.D.

Specific Research Goals:
   To evaluate the Rhesus Rotavirus Vaccine with regard to:
       1. Side effects
       2. Sero-conversion
       3. Efficacy

Techniques Used:
   Cell culture
   Enzyme assays/probes
   Electron microscope

Ultimate Goal:
   Prophylaxis
Research Description:

Experimental groups were vaccinated with either a high dose or a low dose of RRV, control groups received a placebo. The rates of seroconversion and diarrhea were determined for all groups.

Staffing:
M.D. 6
Technician 2

Time for Completion:
1.5 years

Time Before Field Use:
2 years

Results:

Seroconversion (a measure of antibody formation) was induced in 59% of the children who received the 3,000 pfu dose, and 82% of children who received the 30,000 pfu dose. Side effects were not significant. In Finland, diarrhea rates were similar, signifying a lack of protection in the face of a type 2 rotavirus epidemic. In Venezuela, the vaccine protected type 3 rotavirus diarrhea. Thus RRV appears to protect against infection with rotavirus of the same serotype as the vaccine, but not against other serotypes.

Grant Title:
PENDING

Grant #:
Not available.

Funding:
Not available.

Research Institution:
Institute for Nutritional Investigation
Lima, Perú
Principal Investigator:
Claudio Lanata, M.D.

Specific Research Goals:
To test the resultant serotype strains derived from RRV type 1 and type 2 human rotaviruses.

Techniques Used:
Cell culture
Enzyme assays/probes

Ultimate Goal:
Prophylaxis

Research Description:
The three vaccine strains (RRV, RRVXD, RRVXD1) will be given to 3 experimental groups, at three months of age; a fourth group will be given placebo.

The occurrence of side effects, and the rates of sero-conversion, and virus shedding will be measured in the short term. Long-term surveillance will assess the efficacy and duration of protection.

Staffing:
Ph.D. 3
M.D. 6
Technician 3

Time for Completion:
2.5 years

Time before Field Use:
3 years

Collaborating Arrangements:
OIH contract to PAHO

Results:
Not available.
Agreement Title:

Human Diploid Cell (HDC) Measles Vaccine Studies-Phase I & II.

Agreement #:

OIH 001-1258 and amendment No. 2

Funding:

$568,040

Research Institution:

Ministry of Health, Mexico

Centers for Disease Control, U.S.A.

Principal Investigators:

Drs. Jaime Sepulvida, Jose Valdespino, Lauri Markowitz, Roger Bernier

Specific Research Goals:

To compare the Edmunston-Zagreb or Schwartz measles vaccines at three widely varying doses for the ability to immunize children at 6 months of age, and to compare its immunogenicity with that observed in children who receive the vaccine at the recommended age of 9 months.

Rationale:

The current recommended age for vaccinating infants with the available measles vaccine is 9 months. Up to 30% of the measles cases (and an even larger portion of measles-related mortality) occur before this age. A vaccine is needed which will be effective in inducing immunity in this age group.

Incidence of Disease:

Measles contributes to the deaths of 2 million children per year.

Techniques Used:

Cell culture
Enzyme assays/probes
Ultimate Goal:

Prophylaxis

Research Description:

Seroconversion will be measured by ELISA and plaque neutralization.

Staffing:

Ph.D.  1
M.D.  1
Graduate student  4
Technician  3

Time for Completion:

1 year

Time Before Field Use:

2 years

Agreement Title:

Typhoid Vaccine Field Trial-Pilot Study

Agreement #:

OIH 009-258 and amendment No.1

Funding:

$14,110

Research Institution:

Nepal Typhoid Project and Tegu Hospital
Kathmandu, Nepal

National Institute of Child Health and Human Development
NIH

Principal Investigator:

Dr. I. L. Acharya
Dr. John B. Robbins
Specific Research Goals:

To establish the attack rates for typhoid fever in an urban and a rural setting in Nepal. This is in preparation for a field trial of typhoid Vi polysaccharide vaccine.

To perform a pilot study of the safety and immunogenicity of the Vi vaccine.

Incidence of Disease:

In Africa, there are more than 2.5 million cases of typhoid fever resulting in 130,000 deaths annually. In Asia, there are more than 13 million cases of the disease, with 430,000 fatalities.

Rationale:

Typhoid fever is caused by the bacterium Salmonella typhi. The organism causes erosive ulcers in the intestine, resulting in the passage of blood and mucous in the stool. The organism has several groups of antigens, one of which, the Vi polysaccharide, has vaccinogenic potential.

Techniques Used:

Radiolabelled reagents

Ultimate Goal:

Diagnosis
Prophylaxis

Research Description:

An epidemiologic survey of the proposed study area will be performed. Small numbers of children and adults will be inoculated with the Vi polysaccharide and observed for side effects from the vaccination. The levels of antibody formed in response to vaccination will also be studied.

Staffing:

M.D. 6
Technician 2

Time for Completion:

7 months
Time Before Field Use:
Immediate

Results:
1. Vaccination produced an antibody response in 98% of the subjects.
2. A vaccine dose of 25 micrograms was as effective as a dose of 50 micrograms.
3. No serious side effects were observed.

Agreement Title:
Typhoid Vaccine Field Trial-Phase II

Agreement #:
OIH 010-258 and amendment No.1

Funding:
$202,311

Principal Investigators:
Dr. I. L. Acharya
Dr. John B. Robbins

Specific Research Goals:
To study the efficacy of the typhoid Vi polysaccharide vaccine in preventing typhoid fever.

Techniques Used:
Radiolabeled reagents

Ultimate Goal:
Prophylaxis

Research Description:
Approximately 7,000 children and young adults were inoculated with either vaccine or placebo. The efficacy of the typhoid vaccine, determined by surveillance for typhoid fever during the first year of observation, was over 70%.
Staffing:

M.D. 6
Technician 2

Time for Completion:

2 years

Time Before Field Use:

2 years

Agreement Title:

Acellular Pertussis Vaccine Research-Phase I

Agreement #:

OIH 003-258

Funding:

$233,205

Research Institution:

Centers for Disease Control
Atlanta, Georgia

Swedish National Bacteriological Laboratory
Sweden

Principal Investigators:

Lars Olaf Kallings, M.D.
Patrick Olin, M.D.

Specific Research Goals:

To test a new vaccine for pertussis (whooping cough) which should produce fewer side-effects than the currently used vaccine.

Incidence of Disease:

In unvaccinated Swedish children the incidence rate is 2.4% to 5% per year.
Rationale:

Pertussis is a severe disease resulting from a respiratory tract infection with the bacterium *Bordetella pertussis*. (The classic symptoms of the disease are a severe, paroxysmal cough, with the patient gasping for air in between coughs-giving the characteristic "whoop"). Some 30,000 cases of pertussis occur annually. The current vaccine is based on a preparation of killed whole bacteria. It often produces a lowgrade fever and tenderness at the site of injection. More serious side-effects occur, but less often.

Many mothers in less developed countries avoid having their children vaccinated with the currently available preparation because it causes noticeable side-effects and discomfort. A new vaccine would decrease the amount of side effects, and should thereby increase the levels of compliance for existing vaccine programs. Enhanced safety should also encourage the establishment of new programs.

Techniques Used:

Not Available

Ultimate Goal:

Prophylaxis

Staffing:

Ph.D.  2
M.D.  6

Time for Completion:

8 months

Time Before Field Use:

2 years

Results:

A final protocol for a definitive field trial of two acellular pertussis vaccines (Biken FHA + PT and Biken PT) has been developed with the cooperation of WHO and Swedish investigators.
Agreement Title:

Efficacy Trial of an Acellular Pertussis Vaccine in Sweden, Phase II.

Agreement #:

OIH 006-258

Funding:

$750,000

Research Institution:

Swedish National Bacteriological Laboratories

Principal Investigators:

Lars Olof Kallings, M.D.
Patrick Olin, M.D.

Specific Research Goals:

To study the efficacy of two acellular pertussis vaccines, and to compare the two vaccines directly.

Rationale:

See previous pertussis

Incidence:

See previous

Techniques Used:

Cell culture
Radiolabelled reagents
Fluorescent assays/probes
Enzyme assays/probes

Ultimate Goal:

Prophylaxis
Research Description:

Two experimental groups will receive the Biken PT (pertussis toxin, or lymphocytosis promoting factor) or the Biken PT with FHA (filamentous hemagglutinin). The placebo group will receive neither. The rates of seroconversion and the incidence of pertussis will be measured in all three groups.

Staffing:

Ph.D. 2
M.D. 6
Technician 4

Time for Completion:

2 years

Time Before Field Use:

3 years.
Project Title:
Pediatric Chronic Diarrheal Disease Research Training

Project #:
936-5940

Agreement #:
DPE-5940-A-00-4019-00

Funding for Entire Project:
$2.8 million for 1984-1987

Project Goals:
To define the changes which occur during malnutrition in pregnant mothers and newborn children with respect to digestion and absorption.

To identify the factors in the gut which affect the course of acute diarrhea and the development of chronic diarrhea.

Implementing Agency:
International Institute for Infant Nutrition and Gastrointestinal Diseases, Children's Hospital of Buffalo 219 Bryant Street Buffalo, New York.

Contact:
Emmanuel Lebenthal, M.D.
P.C. Lee, Ph.D.

Incidence of Disease:
The estimated yearly morbidity and mortality from diarrheal disease for children under 5 years of age in Africa, Asia, and Latin America were 744-1,000 million episodes of diarrhea and 4.6 million deaths. Most of these episodes were accompanied by malnutrition.
Rationale:

Poor digestion caused by malnutrition occurs even when nutritional rehabilitation is provided for the patient. This may be due to impaired development of pancreatic enzymes during the period of malnutrition. A possible reason for the impaired development of the pancreatic enzymes may be mediated through a change in the receptors for hormones of the glucocorticoid group. A better knowledge of the interaction between malnutrition and the development of pancreatic enzymes is needed to provide the basis for intervention strategies.

The role of specific micronutrients in the development of the gastrointestinal tract and its associated organs has not been explored in detail. Deficiencies in vitamin D and calcium almost always occur together, and may affect the development of gastrointestinal enzymes. Micronutrient deficiency in a pregnant or lactating mother may have potential harmful effects on her fetus or infant. Deficiencies in dietary vitamin A have been associated with increased susceptibility to infection, and higher levels of mortality when compared with adequate dietary intake. Vitamin A deficiency is most often associated with xerophthalmia, and xerophthalmia is often accompanied by an increased incidence of diarrheal diseases. Vitamin A may be involved in maintaining the integrity of the mucosal surface barrier of the gastrointestinal tract, or in the adherence of bacterial pathogens (eg. Salmonella) to the intestinal mucosa.

Acute gastroenteritis and its interaction with environmental factors is the main cause of chronic diarrhea and consequent chronic malnutrition. Some food components (particularly lectins, carbohydrate-binding proteins) from beans and other vegetables may exaggerate carbohydrate malabsorption by causing damage to the intestinal mucosa during acute gastroenteritis. Localized malnutrition of the cells of the colon (colonocytes) can impair the growth and maturation of these cells, and may therefore interfere with attempts at nutritional rehabilitation.

Breast milk provides an effective immunoprotective factor for infants during their first months of life. Malnourished mothers have been shown to secrete decreased amounts of immunoglobulins in their milk, but the selective effects of different parameters of malnutrition (calories vs. protein) have not been investigated. Evaluation of the immunological components in the serum during the suckling period would provide further insight to immunodefense potentials following malnourishment.
Title of Study:
Ontogeny of Glucocorticoid Receptors in Rat Pancreas.

Principal Investigator:
Rong-Bao Lu, M.D. (Peoples Republic of China)

Specific Research Goals:
1. To study the changes in development of receptors for the hormone glucocorticoid in the nucleus and cytoplasm of normal rat pancreas.
2. To relate these changes in receptors to the development of exocrine pancreatic enzymes.
3. To examine the effect of malnutrition on the development of these hormone (glucocorticoid) receptors, and determine its possible influence on the maturation of the pancreatic enzymes and ultimately the digestive capacity of animals.

Techniques Used:
Radiolabelled reagents
Fluorescent assays/probes
Enzyme assays/probes
Northern blotting
Preparative separations

Ultimate goal:
Prophylaxis

Research Description:
Experimentally induced malnutrition during pregnancy and lactation in rats will be used as a model to see if malnutrition could affect pancreatic development via changes in the glucocorticoid hormone-receptor system. The overall study will contribute to a better understanding of the interaction among pancreatic development, corticosteroid action, and nutrition.

Time for Completion:
3 years
Time Before Clinical Use:

5 years

Results:

The amount of binding of dexamethasone (a glucocorticoid hormone) in the pancreas of newborn and adult rats was determined. It was relatively low in newborn rats, by day 15 a significant rise was seen. Binding capacity was greatest at weaning (3rd to 5th weeks). When a thyroid hormone (L-thyroxine, T4) was administered to pups, an increase in glucocorticoid hormone binding was observed, as well as an increase in the serum concentration of corticosterone.

Publications:


Title of Study:

The Effect of Malnutrition on the Development of Pancreatic Exocrine Enzymes.

Principal Investigator:

Kazuo Shimizu, M.D. (Japan)

Specific Research Goals:

To examine the effect of malnutrition on the development of the exocrine (excreted) enzymes of the pancreas by following the changes in their rates of synthesis.
Techniques Used:

Radiolabelled reagents
Enzyme assays/probes
Preparative separations
Preparation of antiserum

Ultimate Goal:

Investigation

Research Description:

The rates of synthesis of pancreatic enzymes in normal and malnourished rats will be measured by determining the amount of radio-labelled amino acids incorporated into specific pancreatic enzymes. Rats will be injected with radiolabelled amino acids and their pancreas removed; the amount of radiolabelled enzymes will be measured by the immunoprecipitation technique. The activities of the enzymes will be determined and correlated with the synthesis rates obtained. Similar measurements will also be performed on rats following nutritional rehabilitation.

Time for Completion:

2 years

Results:

The enzymes amylase and trypsinogen have been purified from rats. Anti-amylase and anti-tyrpsinogen serum prepared in rabbits are being characterized at present.

Title of Study:


Principal Investigator:

Subilanto Sudarmo, M.D. (Indonesia)

Specific Research Goals:

T. examine the effect of intrauterine and postnatal malnutrition on the development of intestinal and pancreatic enzymes.
To follow the recovery of intestinal and pancreatic enzymes of malnourished rats during a period of nutritional rehabilitation.

**Techniques Used:**

Enzyme assays/probes

**Ultimate Goals:**

Therapy
Prophylaxis

**Research Description:**

Pregnant rats will be divided into groups such that some have unrestricted intake of food and water, and some receive half of the unrestricted amount. After the pups are born, they will be divided among the mother rats to create 4 groups of nutritional and nursing status. At 21 days after birth, the pups will be weaned to laboratory chow. The intestinal and pancreatic enzyme profile will be determined each week, beginning 1 week after weaning until adulthood is reached.

**Time for Completion:**

2 years

**Title of Study:**

Effect of Vitamin D and Calcium Repletion on Pancreatic Exocrine Function in Vitamin D and Calcium Restricted Rats.

**Principal Investigator:**

Pipop Jirapinyo, M.D. (Thailand)

**Specific Research Goals:**

1. To determine the effect of vitamin D deficiency on the development of the gastrointestinal tract, particularly with reference to the enzymes of the pancreas and small intestine.

2. To determine the relative importance of vitamin D and calcium on the development of enzymes in the exocrine pancreas and the small intestine.
3. To determine the functional changes resulting from vitamin D and calcium deficiency.

**Ultimate Goal:**

Prophylaxis

**Research Description:**

The effects of vitamin D and/or calcium on the development of gastrointestinal enzymes will be evaluated during the critical phases of both suckling and weaning. The nutritional deficiency of the pregnant or lactating mother may be of particular relevance to harmful effects on the fetus and infant.

**Time for Completion:**

2 years

**Time Before Clinical Use:**

3-4 years

**Results:**

Deficiency of vitamin D and calcium caused a marked decrease in food intake and a reduction in the growth of weanling rats. Vitamin D and calcium deficiency particularly affected both the growth of the pancreas and the functional development of pancreatic secretion. Supplementation with vitamin D and calcium promptly corrected the effect.

Vitamin D deficiency during pregnancy and lactation leads to a slower growth rate of the pups. This slow growth rate was similar to that observed following diet restriction. Thus, vitamin D deficiency in mothers caused growth retardation in their offspring.

**Publications:**

Effects of vitamin D and calcium repletion on pancreatic exocrine functions in vitamin D and calcium restricted rats. Gastroenterology, 1986; Submitted for publication.

Title of Study:
Effects of Vitamin D and Calcium Restriction on Pepsinogen Secretion in Rats.

Principal Investigator:
Achiuri Bakri, M.D. (Indonesia)

Specific Research Goals:
To evaluate the effect of calcium and vitamin D depletion on the secretion of pepsinogen.

Techniques Used:
Enzyme assays/probes

Ultimate goal:
Therapy

Research Description:
Weanling rats will be fed a diet lacking vitamin D and calcium for 8 weeks. Control rats will be fed a regular diet with full complements of vitamin D and calcium. The gastric mucosa from these rats will be removed, and the secretion of pepsinogen by gastric glands will be evaluated in vitro. The responsiveness of the isolated gland preparations will be compared following stimulation by known secretion-inducers. Vitamin D and calcium repletion of the weanling rats will also be carried out to determine if the pepsinogen secretory responses can be normalized after depletion of vitamin D and calcium.

Time for Completion:
2 years

Time Before Clinical Use:
3-5 years

Results:
Currently being collated.
Title of Study:

A) Pepsinogen Release from Isolated Gastric Glands: The Relation With Metabolism of Membrane Phospholipids.

B) The Effects of Vitamin D and Calcium Repletion on Pepsinogen Release From Isolated Gastric Glands.

Principal Investigator:

Hernando Lyons, M.D. (Colombia)

Specific Research Goals:

1. a) To determine the role of the breakdown of phosphoinositide in mediating the release of the digestive enzyme pepsinogen caused by cholecystokinin (CCK, an intestinal hormone which probably stimulates the contraction of the gallbladder and enzyme secretion by the pancreas).

   b) To examine the interaction of calcium mobilization within the cytoplasm and the activation of protein kinase C in triggering and sustaining pepsinogen secretion.

   c) To examine the effect of inhibitors of calcium and its metabolism on the inhibition of pepsinogen secretion caused by petides related to CCK.

   d) To examine the effect of protein kinase C inhibitors on pepsinogen release stimulated by TPA (a phorbol diester).

2. To compare the amounts of pepsinogen released from isolated gastric glands in rats deficient in vitamin D and calcium, with rats which receive normal dietary levels of calcium and vitamin D.

Time For Completion:

1 year.

Time Before Field/Clinical Use:

4 years
Title of Study:

Adherence and Invasiveness of Salmonella typhimurium to Small Intestinal Enterocytes of the Rat.

Principal Investigator:

Bo Lindquist, M.D., Visiting Scientist (Sweden)

Specific Research Goals:

1. Characterize and study the mechanisms for the interaction between the bacterium S. typhimurium and enterocytes (intestinal cells) of the small intestine.

2. Study the effect of various factors which may promote or inhibit the binding of S. typhimurium to the small intestine.

3. Determine the molecular basis for the pathogenesis of bacterial infections of the small intestine.

Techniques Used:

Radiolabelled reagents
Electron microscope
Bacterial culture

Ultimate Goal:

Prophylaxis

Research Description:

Enterocytes will be isolated from the small intestine, and the binding of radiolabelled S. typhimurium will be quantitated. Light microscopy and electron microscopy will be used to study the interaction of the bacterium with the intestinal cells. The component of the microbial and intestinal surfaces responsible for the binding interaction will be isolated and characterized.

Time for Completion:

2 years

Time Before Clinical Use:

5 years
Publications:

1. Adherence and invasiveness of S. typhimurium to small intestinal enterocytes of the rat. (Submitted for publication).

Results:

1. The basic methodology for the study has been established.

2. The binding of the bacteria to enterocytes is specific for fimbriae producing strains of S. typhimurium.

3. The binding pattern of the bacteria is biphasic, showing an initial period of adherence, followed by invasion.

4. The sugar mannose inhibits the binding in a dose-dependent fashion.

Collaborating Arrangements:

Dr. J.M. Merrick
Department of Microbiology
SUNY at Buffalo.

Title of Study:

Adherence of Salmonella typhimurium to Isolated Rat Enterocytes During Postnatal Development.

Principal Investigator:

Ali Moustafa Abd Monem, M.D. (Egypt)

Specific Research Goals:

1. To determine the pattern of the adherence of S. typhimurium to enterocytes during postnatal development.

2. To determine the effect of milk factors on adherence.

3. To examine the effect of early weaning on adherence of S. typhimurium to enterocytes.

Techniques Used:

Radiolabelled reagents
Bacterial culture
Research Description:

Epithelial cells from the small intestine (enterocytes) are isolated from different age groups of rats. The isolated enterocytes are then incubated with radiolabelled S. typhimurium for 30 minutes, and bacteria which are bound to the enterocytes are collected onto a filter. The enterocytes to which bacteria are bound are then placed in a beta-emission detector to determine the amount of radiolabel bound to them. From this, the number of bound bacteria/enterocytes is calculated.

Time for Completion:

6 months

Time Before Clinical/Field Use:

2-3 years

Results:

The adherence of S. typhimurium to rat enterocytes is age-related. Binding of the bacteria becomes higher as the rat gets older, until it reaches the adult level. Early weaning does not seem to have a significant effect on the adherence of S. typhimurium to isolated rat enterocytes.

Collaborating Arrangements:

Department of Microbiology
SUNY at Buffalo

Title of Study:

Vitamin A Deficiency and Bacterial Adherence to Rat Small Intestinal Mucosal Cells.

Principal Investigator:

Elizabeth Gabriel, M.D. (Philippines)

Specific Research Goals:

1. To determine the effect of vitamin A deficiency on the adherence of bacteria to cells lining the small intestine.

Techniques Used:

Radiolabeled reagents
Bacterial culture
Ultimate Goal:

Investigation

Research Description:

Rats are fed diets with normal or deficient levels of vitamin A. Enterocytes are isolated from the small intestine using a mechanical shaker incubator, and are incubated with radiolabelled bacteria. After a 30 minute incubation, the enterocytes and the radiolabelled bacteria bound to them are collected by filtration. Quantitation of the bound bacteria is performed by liquid scintillation counting.

Time for Completion:

1 year

Time Before Field/Clinical Use:

2-3 years.

Results:

1. Vitamin A deficiency resulted in a decline in body growth rate.

2. Enterocytes isolated from the proximal (upper) small intestine exhibited increased adherence to the bacterium *S. typhimurium*.

Collaborating Arrangements:

Department of Microbiology
SUNY at Buffalo

Title of Study:

Effects of Malnutrition on the Adherence of *Salmonella typhimurium* to Enterocytes of Rats.

Principal Investigator:

Irekopo U. Omoike, M.D. (Nigeria)
Specific Research Goals:

1. To determine the pattern of adherence of fimbriae-producing (thread-like projections) and non-fimbriae producing strains of *S. typhimurium* to epithelial cells from different anatomic segments of the small intestine.

2. To compare the adherence pattern in well-fed and malnourished rats.

Techniques Used:

- Radiolabelled reagents
- Electron microscope
- Bacterial culture

Ultimate Goals:

- Therapy
- Prophylaxis

Research Description:

An animal model of malnutrition will be produced by restricted feeding. Enterocytes will be isolated from malnourished and control rats, and then incubated with radiolabelled *S. typhimurium* which either possess or lack fimbriae. Unbound bacteria are separated from bound bacteria by membrane filtration, and the adherence of the bacteria to the enterocytes is measured by liquid scintillation counting.

Time for Completion:

18 months

Results:

1. The strains of *S. typhimurium* studied adhered to enterocytes from both the proximal and distal (upper and lower) sections of the small intestine.

2. Although both the fimbriated and non-fimbriated strains adhered to the enterocytes, the fimbriated strain showed significantly higher binding.

3. There was no significant difference between the nutritionally deprived rats and the well-fed rats with regard to bacterial adherence when isolated enterocytes were used.
4. The bacterial adherence to enterocytes in vitro was variable, and independent of the extent of malnutrition.

5. An in vivo model of bacterial adherence was developed and preliminary data showed a significant effect of malnutrition on \textit{S. typhimurium} adhesion in such testing.

\textbf{Publications:}

Effect of malnutrition on the adherence of \textit{S. typhimurium} to enterocytes from rats. (Manuscript in preparation).

\textbf{Collaborating Arrangements:}

Dr. Bo Lindquist  
Children's Hospital of Buffalo

Dr. J.M. Merrick  
Department of Microbiology  
SUNY at Buffalo

\textbf{Title of Study:}

Effect of Phytohemagglutinin (PHA) on Rat Small Intestine.

\textbf{Principal Investigator:}

C.H. Lin, M.D. (Taiwan)

\textbf{Specific Research Goals:}

1. To investigate the changes in enzymes and cell structure in the small intestine after chronic feeding on a diet containing purified PHA from red kidney beans.

2. To determine if the changes observed are the result of an increased cellular proliferation.

3. To determine if the observed cell proliferation is caused by an increase in polyamine synthesis.

\textbf{Techniques Used:}

Radiolabelled reagents  
Enzyme assays/probes  
Preparative separations  
Affinity chromatography

\textbf{Ultimate Goal:}

Investigation
Research Description:

The lectin PHA (phytohemagglutinin) is isolated from red kidney beans by affinity chromatography. The isolated PHA preparation is mixed with chow and fed to weanling rats for a fixed period of time. Structural and biochemical changes in the small intestine are followed by enzymatic and morphologic assays. The degree of cellular proliferation is determined by the rate of incorporation of radiolabelled thymidine into DNA. The level of the enzyme ornithine decarboxylase is measured, as well as the concentration of polyamines.

Time for Completion:

2 years

Time Before Field/Clinical Use:

4 years

Results:

When rats as fed with partially purified PHA, there is an increase in the wet weight of the small intestine. The biosynthesis of DNA and polyamines also increases.

Publications:


Title of Study:

Effect of Food Lectins (PHA) on Intestinal Absorption of Carbohydrates.

Principal Investigator:

Mohamed A.A. Saad, M.D. (Egypt)

Specific Research Goals:

1. To establish a basal absorption pattern for various carbohydrates using an infusion technique in adult rats.

2. To study the effect of specific lectins (PHA) from red kidney beans on the absorption of various carbohydrates.
Techniques Used:
Radiolabelled Reagents

Ultimate Goal:
Prophylaxis

Research Description:
Rats will be anesthetized, and the PHA and carbohydrates will be directly instilled into the duodenum of the small intestine. Blood samples will be drawn from the portal vein every 30 minutes after the dose of PHA and carbohydrates.

Time for Completion:
2 years

Time Before Field/Clinical Use:
3 years

Results:
The rat model for infusion has been established.

Title of Study:
Effect of Phytohemagglutinin on Isolated Small Intestinal Enterocytes of Rats

Principal Investigator:
Rodney Abud, M.D. (Brazil)

Specific Research Goals:
1. To study the carbohydrate absorption in rats fed with PHA using the perfusion technique.

2. To study bacterial adherence to the small intestine in the presence of PHA.

Techniques Used:
Radiolabelled reagents
Analytic separations
**Ultimate Goal:**

Investigation

**Research Description:**

Phytohemagglutinin will be isolated from red kidney beans by affinity chromatography. PHA will be fed to adult rats for a period of seven days before infusion with carbohydrate. The rate of carbohydrate absorption will be measured and correlated with the effects of PHA injection. The activity of acute PHA ingestion on absorption of carbohydrates will be evaluated by the direct instillation of PHA into the duodenum.

**Time for Completion:**

2 years

**Results:**

The basic model for perfusion has been established. The normal levels of carbohydrate absorption, using glucose, have been established.

**Title of Study:**

Substrate Utilization by the Epithelial Cells of the Rat Colon and Small Intestine.

**Principal Investigator:**

Duna Penn Schmidt-Sommerfield, M.D. (West Germany)

**Specific Research Goals:**

1. To determine the relative contributions of representative substrates (glucose, glutamine ketone bodies, and fatty acids) to energy production in the epithelial cells of the small intestine and colon throughout development.

2. To examine the effects of pre-natal and post-natal malnutrition on the above.

3. To investigate the possible role of the plant alkaloid carnitine in modulating the changes in energy-producing mechanisms in the mucosal cells of the colon and small intestine.
Techniques Used:

Cell isolation
Radiolabelled reagents
Fluorescent assays/probes
Enzyme assays/probes
Analytic separations
Preparative separations

Ultimate Goal:

Therapy

Time for Completion:

3 years

Time Before Field/Clinical Use:

3-5 years

Results:

The basic methods for cell isolation and metabolic assays in the animal model for malnutrition have been established.

Title of Study:

The Effect of Malnutrition on the Metabolic Activities of the Colonocytes.

Principal Investigator:

Agus Firmansyah, M.D. (Indonesia)

Specific Research Goals:

1. To investigate the influence of postnatal malnutrition on the following parameters of colonic growth in the weanling rat: a) colonic weight and length, b) colonic mucosal weight, c) DNA content, d) protein content.

2. To investigate the influences of postnatal malnutrition on the metabolic activities of colonocytes (cells of the colon) in weanling rats.

3. To determine if the possible colonic changes caused by post-natal malnutrition are reversible by nutritional rehabilitation.
Techniques Used:

Fluorescent assays/probes
Enzyme assays/probes

Ultimate Goal:

Prophylaxis

Research Description:

Using the rat as a model for malnutrition, isolated colonocytes will be analyzed for their metabolic activities in terms of utilization of energy sources. The parameters of DNA and protein content, colonic weight, and colonic length will be used to measure colon growth in normal and malnourished animals.

Time for Completion:

2 years

Time Before Field/Clinical Use:

4 years

Results:

Colonocytes have been isolated from various age groups of rats. Preliminary experiments on the production of lactate from glucose have just been completed. Experiments are currently being performed to examine the utilization of beta-hydroxy butyrate in vitro using isolated colonocytes.

Title of Study:

IgG and IgA Levels in the Milk of Protein or Calorie Malnourished Rat Dams.

Principal Investigator:

Yoram Elitzur, M.D. (Israel)

Specific Research Goals:

To investigate the specific role of calorie vs. protein malnutrition on immunoglobulin secretion in the milk of lactating dams.
Techniques Used:

Rocket immunoelectrophoresis

Ultimate Goal:

Prophylaxis

Research Description:

Pregnant female rats who are malnourished will have their breast milk analyzed for its content of immunoglobulins (Ig). The levels of Ig in the milk will be referred back to known variants in their diets. The data should lead to a rational nutritional support program for malnourished mothers during pregnancy.

Time for Completion:

10 months

Results:

In comparison with well nourished rats, malnourished rats had higher levels of IgA and IgG in their milk. No major difference was found between calorie and protein malnourished rats with regard to their secretion of IgA and IgG in milk.

Title of Study:

Ontogenic Changes in Serum Immunoglobulin and Complement in Normal, Vitamin D Deficient, and Malnourished Rat Pups.

Principal Investigator:

Nualanong Srimaruta, M.D. (Thailand)

Specific Research Goals:

1. To study the changes in development in serum immunoglobulin and the third component of complement (C3) in normal rat pups.

2. To study these changes in rats which are nutritionally deficient, either protein-energy-malnourished (PEM) or vitamin D deficient.
Techniques Used:

Rocket immunoelectrophoresis

Ultimate Goal:

Investigation

Research Description:

Sera are collected from rat pups at various ages from groups which are well-nourished, PEM malnourished, or vitamin D deficient. The concentrations of immunoglobulin and C3 are then quantitated by rocket immunoelectrophoresis using specific antibody to IgG, IgA, and C3.

Time for Completion:

2 years

Time Before Field/Clinical Use:

5 years

Results:

In rats the Ig and C3 levels are very low at birth, but increase rapidly after weaning to the adult level at 35 days of age. PEM malnutrition does not significantly affect the IgG or C3 levels. Vitamin D deficiency lowers the serum IgG and C3 levels only in newborn pups born from vitamin D deficient dams. IgA and IgM do not differ among the nutritional groups studied.

Title of Study:

Mucosal Injury and Carbohydrate Absorption

Principal Investigator:

Hug. Madariago, M.D. (Peru)

Specific Research Goals:

1. To evaluate carbohydrate digestion and absorption in prolonged intestinal mucosal injury.

2. To evaluate the effect of an an inhibitor of the enzyme alpha-glucosidase (Acarbose) on the absorption of simple and complex carbohydrates.
Techniques Used:

Enzyme assays/probes

Ultimate Goals:

Diagnosis
Therapy

Research Description:

Adult rats are used in an animal model for mucosal injury. Carbohydrates are directly instilled into the duodenum, and the content of glucose in the portal venous blood is assayed to monitor the uptake of carbohydrate hydrolysis. After this perfusion study, the protease and disaccharidase activity of the mucosa of the small intestine will be determined. The degree of injury to the mucosa will be quantitated by both biochemical and microscopic methods.

Time for Completion:

2 years

Time Before Field/Clinical Use:

4 years

Results:

1. When the intestinal mucosa is damaged by loading with highly concentrated mannitol solutions (20%), the digestion and absorption of carbohydrates is suppressed, and the activity of the disaccharidase enzymes decreases.

2. Acarbose had no effects on the absorption of glucose and lactose. Acarbose did affect the absorption of maltose and sucrose. The inhibition of sucrose absorption was dose-dependent. A good correlation existed between the residual sucrase activity and rise in blood glucose.
Title of Study:

Small Intestinal Mucosal Injury: A New Model as Induced by Difluoromethyl Ornithine

Principal Investigator:

Pedro Alarcon, M.D. (Peru)

Specific Research Goals:

1. To develop a new model of small intestinal mucosal injury using the cell proliferation-inhibiting agent difluoromethyl ornithine (DFMO).

2. To examine the relative sensitivity of weanling and adult animals to DFMO.

3. To characterize the changes in the mucosa both biochemically and morphometrically.

4. To examine the reversibility of these changes upon withdrawal of DFMO.

5. To evaluate the effect of malnutrition on the sensitivity of the mucosa to DFMO and the subsequent recovery of the small intestinal mucosa.

Ultimate Goal:

Prophylaxis

Research Description:

DFMO is an anti-proliferative agent. It inhibits the normal proliferative process of the intestinal mucosa which is required to maintain the integrity of the intestinal lining. Treatment with DFMO essentially leads to a situation very similar to mucosal atrophy as seen in many clinical situations following chronic diarrhea and/or malnutrition. DFMO is non-toxic, and its biological half-life is short enough to allow recovery from the damages. As such, DFMO is very useful in controlled studies of mucosal injury and its subsequent recovery. Nutritional manipulation, in addition to using DFMO, enables the individual components that constitute mucosal injury and recovery to be investigated.
Time for Completion:

2 years

Time Before Field/Clinical Use:

5-6 years

Results:

Feeding with DFMO led to mucosal injury which was similar to that found in chronic diarrhea and malnutrition in terms of both the biochemical and structural changes. The mucosal injury was accompanied by diarrhea. DFMO was found to be effective in weanling rats, but not in adult rats. This suggests a difference in the small intestinal characteristics between weanling and adult rats. The DFMO induced mucosal injury was reversible, but required prolonged periods of recuperation. Malnutrition in the immediate postnatal period did not seem to predispose the small intestine to more severe damage as caused by DFMO. Malnutrition itself caused intestinal damage in this rat model.

Publications:


Title of Study:

Studies of Secretory Diarrhea in Animal Model

Principal Investigator:

Golam H. Rabbani, M.D. (Bangladesh)
Specific Research Goals:

1. To gain understanding of the underlying mechanisms of secretory diarrheal diseases.
2. To evaluate antisecretory drugs for better treatment of secretory diarrheal diseases.

Techniques Used:

- Radiolabelled reagents
- Analytic separations
- Enzyme assays/probes
- Electron Microscope

Ultimate Goal:

Therapy

Research Description:

Cholera toxin will be directly infused into the small intestine of adult rats. The kinetics of secretion, including dose response, and the effect of malnutrition on the host response will be evaluated. Various antisecretory drugs (in their developmental stages) will be tested in this model for efficacy.

Time for Completion:

2 years

Time Before Field/Clinical Use:

1-2 years

Collaborating Arrangements:

ICDDR, B
Dhaka, Bangladesh
Project Title:
Applied Diarrheal Disease Research Project

Agreement #:
DPE 5952-A-00-5073-00

Funding:
$9,998,630.00 for 1985-1990.

Project Manager:
Jeffrey Harris, M.D.

Institution.
Harvard Institute for International Development
One Elliot Street
Cambridge, Massachusetts 02138

Contact:
Richard Cash, M.D. (617) 495-9791

Project Goals:
To foster applied diarrheal disease research in selected developing countries, and to develop indigenous research capacity.

Grant Title:
Epidemiology of Prolonged Diarrhea in Lima, Peru.

Funding for Entire Grant:
$31,000.00

Research Institution:
Instituto de Investigacion Nutricional

Principal Investigator:
Dr. Claudio Lanata
Specific Research Goals:

A. To define prolonged diarrhea based on outcomes.

B. To describe and quantify the problem of prolonged diarrhea in one population.

C. To identify important risk factors.

Rationale:

Identification of risk factors may enable the design of appropriate, specific interventions that would reduce the incidence or duration of prolonged diarrheal diseases in infants and children.

Ultimate Goal:

- Diagnosis
- Therapy
- Prophylaxis

Research Description:

This project is a continuation of a Nestle and WHO funded project. Children under 3 years of age from a sample of 400 families are under twice-weekly home surveillance. Clinical data is recorded from all diarrheal disease episodes detected. Monthly anthropometry and feeding pattern data are collected. A survey of feeding during diarrhea, and a skin test for immunocompetance are also done.

Staffing:

- Ph.D. 1
- M.D. 6
- Technicians 5
- Research staff 1
Grant Title:
Development and Field Testing of a Soup-Based Oral Rehydration Solution in Lima, Peru.

Funding for Entire Grant:
$182,896.00

Research Institution:
Universidad Peruana Cayetano Heredia and Instituto de Investigacion Nutricional.

Principal Investigator:
Dr. Eduardo Salazaar-Lindo

Research Goals:
A. To develop and test a starch-based oral rehydration solution
B. To examine the effects of early home use of an oral rehydration solution.

Rationale:
Identification of a soup that can be prepared in the home and used as an ORS may lead to wider use of the ORS in this form. Thus reducing morbidity and mortality due to dehydration.

Ultimate Goal:
Therapy

Research Description:
Phase 1: Identification of a soup that can be prepared in the home which can be used as an ORS.
Phase 2: Clinical Trial.
Phase 3: Field Test.
Staffing:

M.D.  4
Technician  1
Research staff  12

Estimated Time for Completion:

3 years

Time Before Clinical Use:

Immediately upon completion.
APPENDIX I

USAID MALARIA RESEARCH SUPPORT
Institution:  
American Institute for Biological Sciences  

Subproject #:  
931-0453.26  

Funding:  
$1,398,003.00 for 1986-1987.  

Goals:  
To coordinate technical activities of the USAID malaria network, and to plan and coordinate malaria vaccine field trials.  

Contact:  
Dorothy Jordan, Project Manager  
Phillip Winter, M.D. Director of Clinical Medicine  

Progress:  
Two international conferences on malaria in the developing world have been sponsored. The meetings on malaria in Latin America, and Africa brought together malaria researchers and public health officials from the respective areas to discuss the prospects and requirements for field trials of potential malaria vaccines.
Institution:
Batelle Northwest Laboratories

Subproject #:
93i-0453.42

Funding:
$750,000.00 for 1984-1989.

Contact:
Charles R. Watson, Ph.D.
Richard E. Weller, Ph.D.

Goals:
To establish a data management system for the USAID malaria network. Batelle Northwest's central data base will be available to all malaria network researchers to provide detailed information on the demography and clinical status of the more than 500 non-human primates owned by the Agency. Data derived from this project will be used to support the use of both Aotus and Saimiri monkeys as the definitive animal models for candidate malaria vaccines to the Food and Drug Administration.

Time for Completion:
September, 1989

Results:
Demographic data an all non-human primates in USAID facilities has been entered in the database. The first annual report on the status of the primate colony, including data on hematology, reproduction, and clinicopathology will be prepared and published in January, 1987.

Publications:
See appendix II.
APPENDIX II

USAID MALARIA NETWORK PUBLICATIONS
University of Hawaii - W. Siddiqui


"Plasmodium Falciparum: Protein Antigens Identified by Analysis
of Serum Samples from Vaccinated Aotus Monkeys".

University of Illinois - M. Ristic

"Purification and Characterization of Culture-Derived Exoantigens of
Plasmodium Falciparum".
Molecular and Biochemical Parasitology, 17, pp. 299-310.

2. Kakoma, I.; James, M.; Jackson, W.; Bennett, G., and Ristic, M.
"Hematologic Values of Normal Bolivian Squirrel Monkeys (Samiri
Sciureus): A Comparison Between Wild-Caught and Laboratory-Bred
Male Animals".

"Malaria and Babesiosis: Similarities and Differences".
In: Malaria and Babesiosis: Research Findings and Control Measures. 
(M. Ristic, J.P. Kreier and P. Ambroise-Thomas, Eds.) Martinus

"Immunology of Malaria". In: Malaria and Babesiosis: Research
Findings and Control Measures. (M. Ristic, J.P. Kreier and P. 

"Induction of Protective Immunity to Plasmodium Falciparum in
Samiri Sciureus Monkeys with Partially Purified Exoantigens".

"In Vitro and In Vivo Adaptation of the Geneve/SGE-1 Strain of
Plasmodium Falciparum to Growth in a Squirrel Monkey (Samiri
Sciureus) Model".
In Press: Americal Journal of Tropical Medicine and Hygiene

Uniformed Services University of the Health Services - R. Maheshwari

1. Maheshwari, R.; Czarniecki, C.; Dutta, G.; Puri, S.; Dhawan, B.,
and Friedman, R. (1986).
"Recombinant Human Gamma Interferon Inhibits Simian Malaria".


7. Howard, R.; Ardehiri, F., and Reese, R. "DNA Sequence Analysis of cDNA Clones for the M 185,000 Glycoprotein of Plasmodium Falciparum". Research Institute of Scripps Clinic, Publ. No. 4274-IMM


8. Nussenzweig, V. and Nussenzweig, R. "Recent Advances in the Development of a Sporozoite Vaccine for Malaria". Publication of the Dept. of Medical and Molecular Parasitology, NYU School of Medicine.


Biomedical Research Institute - W. Zolg


"Characterization of Plasmodium Falciparum Antigens Using Monoclone Antibodies".

2. Cochrane, F.; Collins, W., and Nussenzweig, R.
"Monoclonal Antibodies Identify the Circumsporozoite Protein of Plasmodium Malariae and Detect a Common epitope of P. Brasilianum Sporozoites."

3. Collins, W.; Schwartz, I.; Skinner, J., and Broderson, J.
"Studies on the Uganda I/CDC Strain of Plasmodium Malariae in Bolivian Aotus Monkeys and Different Anophelines".

"Infection of Aotus Azarae Boliviensis Monkeys with Different Strains of Plasmodium Vivax".

Mehaffey, P., and Sutton, B.
"Studies on the North Korean Strain of Plasmodium Vivax in Aotus Monkeys and Different Anophelines".
Journal of Parasitology 71

6. Guo, S-C.; Collins, W.; Campbell, C., and Chin, W.
"Plasmodium Fragile: The Inhibition of Culture-Grown Plasmodium Fragile by Serum from Rhesus Monkeys Immunized with Homologous Parasites."

7. Hollingdale, M.; Collins, W.; Campbell, C., and Schwartz, A.
"In Vitro Culture of Two Populations (Mitotically Active and Non-Active) of Exoerythrocytic Parasites of Plasmodium Vivax".

8. Howard, R.; Stanley, H.; Campbell, G., and Reese, R.
"Proteins Responsible for a Punctate Fluorescence Pattern in Plasmodium Falciparum Merozoites".


17. Campbell, C. C. Practical problems of chemotherapy of malaria in Southeast Asia and the South Pacific. Infection (Submitted manuscript).


60. Lobel, H. O., Campbell, C. C., Pappalayanou, M., and Huong, A. Y. Use of malaria prophylaxis by American Travelers to Africa and Haiti. Journal of the American Medical Association (Submitted Manuscript).


Publications


Abstracts

APPENDIX III

HISTORICALLY BLACK COLLEGES AND UNIVERSITIES

HEALTH PROPOSALS FUNDED, 1986
1) Atlanta University - Samy Sidky, "Problems and Solutions in Developing a Medical Knowledge Database Diagnostic System for Childhood Diseases in Rural Egypt" $99,950

2) Benedict College - James T. Kinard, "Toxic Metal Accumulation in Plantain--A Major Food Source to the Caribbean and West African Nations" $88,942


4) Charles R. Drew Postgraduate Medical School - R. King, "Public Health Implications of Average Peak Serum Concentration of Rifampin in Indonesian Subjects" $82,386

5) Charles R. Drew Postgraduate Medical School - R. King, "The Use of Traditional and Western Medicine for the Same Illness in Selected Populations in Zaire" $98,421

6) Florida A&M University - S.S. Lamba, "Phytochemical Screening of Solanum Species Indigenous to Jamica (West Indies)" $53,835

7) Florida A&M University - A. Asker, "Influence of Storage under Tropical Conditions on the In-Vitro vailability of Certain Drugs from Tablets" $36,386

8) Howard University - O.L. Oke, "Cyanide-Malnutrition Synergism-Effect on Thyroid Function" $99,944

9) Howard University - Sally Gravely, "Plasmodial Induction of Epstein-Barr Virus" $34,485

10) Howard University - Kunle Kassim, "Immunosuppression to Bacterial Vaccines in Schistosomiasis" $58,535

11) Howard University - Margaret E. Grigsby, "A Latex Slide Agglutination Test for the Detection of Malarial Antigen in the Blood of Mice Infected with Plasmodium yoelli 17XNL" $93,589

13) **Howard University** - Carl A. Reindorf,  
"Model for the In-Vitro Generation of Human Epidemal Autographs for Potential use in the Treatment of Sickle Cell Leg Ulcers"  
$98,388

14) **Meharry Medical College** - Manuel S. Valenzuela,  
"Replication of Trypanosome Kinetoplast DNA"  
$88,206

15) **Meharry Medical College** - Fernando Villalta,  
"Trypanosoma cruzi Membrane Components Involved in the First Steps of Parasite-Host Cell Association"  
$98,291

16) **Meharry Medical College** - F. M. Hatcher,  
"Trypanosome RNA Transcription"  
$100,000

17) **Morehouse School of Medicine** - Paul Urso,  
"Changes in T lymphocytes of Progeny and Mothers after Exposure to Benzo(a)pyrene during Pregnancy (Midgestation)"  
$71,714

18) **Morehouse School of Medicine** - Dr. Gordon B. Bailey,  
"Identification of Mammalian Target Cell Molecules Involved in Attachment and Stimulation of Attack by Entamoeba histolytica"  
$81,045

19) **Morehouse School of Medicine** - Gordon Bailey,  
"Preparation of Monoclonal Antibodies to Detect and Isolate Entamoeba histolytica Membrane Antigens Essential for Attack Upon Human Target Cells"  
$89,489

20) **Morehouse School of Medicine** - Gordon Leitch,  
"Role of Host Nutrition in the Pathogenesis of Intestinal Amebiasis"  
$96,604

21) **Morehouse School of Medicine** - Gordon J. Leitch,  
"Role of the Intestinal Mucus Blanket in Secretory Diarrhea, Intestinal Amebiasis, and the Absorption of Oral Replacement Fluids"  
$85,382

22) **Morgan State University** - Ruhul Amin,  
"From Demonstration to General Program: The Companiganj Health Care Delivery Experiment in Rural Bangladesh"  
$99,475

23) **Prairie View A&M University** - E. Brans  
"Toxic Trace Metals in Acid Soils of the Humid Tropics: Adulteration of the Indigenous Food Chain"  
$100,000

24) **Texas Southern University**, A.L. Jadhav,  
"Development of Antischistosomal Compounds - Purine Derivatives: Structure Activity Relationships in the Inhibition of Hyposantnine-Guanine Phosphoribosyltransferase"  
$46,390
25) **Texas Southern University** - A.L. Jadhav, "Kinetic Characteristics of the Enzymes of the Purine Salvage Pathways of S. Mansoni" $76,930

26) **Xavier University of Louisiana** - Richard F. Ochillo, "Ethnopharmacologic Research: Discovering New Prototype Drugs that Will be Useful in the Clinic" $35,000
APPENDIX IV

USAID SCIENCE ADVISOR'S OFFICE

PROPOSALS FUNDED IN

BIOTECHNOLOGY/IMMUNOLOGY AND VECTOR CONTROL, 1981-1985
AID/SCI PROPOSALS FUNDED IN FY-81 THROUGH 85 LIMITED TO: 'BIOT/I' *MODULE and ('VECT' *MODULE)

KEY TO ABBREVIATIONS

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<th>MODULES</th>
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<th>SECTOR COUNCIL (Closest)</th>
<th>SECTOR (Other Special Groups)</th>
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A = Agriculture
B = Biomass Conversion
C = Computers & Commun.
D = Disaster Assist.
F = Field Sciences
G = Engineering
H = Health
MAR = Marine/Aquacult.
N = Nutrition
GEN = Genetic Resources
P = Population
M = Marine Sciences
S = Science Policy
V = Vector Control
R = Human Resources
STR = Strengthening
OTHER = Other

CURRENT STATUS

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<th>ACC = Accept</th>
<th>REJ = Reject</th>
<th>IR = Initial Review by SCI</th>
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<td>ASS = Assist</td>
<td>RES = Resubmit</td>
<td>SC = Sector Council Review</td>
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<td>FUN = Funded</td>
<td>TRA = Transfer</td>
<td>PR = Ext.Panel Peer Review</td>
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<td>HOL = Hold</td>
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NUMBER TITLE **SHORT FORM**
STATUS INVESTIGATOR P.I. INSTITUTION REGION/COUNTRY

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<td>CARSON, C.A.</td>
<td>Development of Modified Live Vaccine Against Bovine Babesiosis</td>
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### AID/SCI Proposals Funded in FY-81 Through 85

**Limited To: 'VECT' Module**

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<td>PIMENTEL, D.</td>
<td>you/PR</td>
<td>Cornell U.</td>
<td>USA /USA/NY</td>
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<td>Biological Control of the Cowpea Aphid in Indonesia</td>
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<td>U. Maryland</td>
<td>USA /USA/Md.</td>
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<td>Experimental and Field Studies on Poss. Biological Control Agents of The Human Disease Bilharziasis (Schistosomiasis)</td>
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<td>6.202</td>
<td>A Small-Scale Field Testing of Echinostoma liaii As a Biocontrol Agent against Schistosoma mansoni in Egypt</td>
<td>YOUSIF, F.</td>
<td>TBRI</td>
<td>NE /Egypt</td>
<td>$133,000</td>
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<td>6.375</td>
<td>Ecology of a Virus and Mollicutes of Maize and of Their Dalbulus Vectors in Costa Rica and Mexico</td>
<td>GORDON, D.</td>
<td>Ohio State U.</td>
<td>USA /USA/CA</td>
<td>$142,503</td>
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<td>6.390</td>
<td>Identification and Cloning of Surface Antigens and Genes of Schistosoma japonicum (Chinese &amp; Philippines)</td>
<td>SOBHON, P.</td>
<td>Mahidol U.</td>
<td>ASIA/Thailand</td>
<td>$150,000</td>
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APPENDIX V

INDO-U.S. COOPERATION IN
SCIENCE AND TECHNOLOGY
from
A Report by the Embassy of the
United States of America, New Delhi
# Health & Biological Sciences

<table>
<thead>
<tr>
<th>Project</th>
<th>Participating Indian Institutions</th>
<th>U.S. Institutions</th>
<th>U.S. Funding (Rs. 12.00 = $1.00)</th>
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<td><strong>INFECTIOUS &amp; PARASITIC DISEASES</strong></td>
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<tr>
<td>Synthesis and Screening of New Anti-Malarial Drugs</td>
<td>Central Drug Research Institute, Lucknow, Uttar Pradesh</td>
<td>Walter Reed Army Institute of Research, Washington, D.C.</td>
<td>Rs. 10,025,000</td>
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<td>Studies on Transmission Blocking Immunity in P. Cynomolgi and P. Knowlesi</td>
<td>Central Drug Research Institute, Lucknow, Uttar Pradesh</td>
<td>National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland</td>
<td>Annual budget for eight STI projects is Rs. 2,686,404</td>
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<td>Studies of Antigenic Variations During Relapses of P. Cynomolgi Infections</td>
<td>Central Drug Research Institute, Lucknow, Uttar Pradesh</td>
<td>New York University School of Medicine, New York</td>
<td>See above</td>
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<td>Immunization of Primates With Purified Malarial Antigens</td>
<td>Central Drug Research Institute, Lucknow, Uttar Pradesh</td>
<td>National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland</td>
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<td>Detection of Infected Mosquitoes Using the Two-Site Immunoradiometric Assay</td>
<td>Malaria Research Centre, New Delhi</td>
<td>New York University School of Medicine, New York</td>
<td>See above</td>
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<tr>
<td>Immunological and Clinical Aspects of Human Filariasis</td>
<td>Tuberculosis Research Center, Madras</td>
<td>National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland</td>
<td>See above</td>
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<tr>
<td>Immune Response Dynamics in Filariasis Characterization of Immunosuppression and Evaluation of Antibody—Mediated Killing of Wuchereria Bancrofti Infected Larvae</td>
<td>National Institute of Communicable Diseases, New Delhi</td>
<td>University of Michigan, Ann Arbor</td>
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<td>Detection of Circulating Parasite Antigens as a Clinical and Epidemiological Tool for Filariasis</td>
<td>National Institute of Communicable Diseases, New Delhi</td>
<td>Washington University of Medicine, St. Louis, Missouri</td>
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<td>Antigen, Antibody and Immune-Complex Profiles as Applied to the Immunodiagnosis of Human Filariasis</td>
<td>Mahatma Gandhi Institute of Medical Sciences, Wardha, Maharashtra</td>
<td>University Medical School, University of Texas, Houston</td>
<td>See above</td>
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<tr>
<td>Immunodiagnosis and Immunopathogenesis in Bancroftian Filariasis Inclusive of the Tropical Pulmonary Eosinophilia Syndrome</td>
<td>Tuberculosis Research Center, Madras</td>
<td>National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland</td>
<td>Rs. 2,500,900</td>
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<td>Epidemiological Studies on Viral Hepatitis and related problems</td>
<td>National Institute of Virology, Pune, Maharashtra</td>
<td>National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland</td>
<td>Rs. 2,068,000</td>
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<td>Biomedical Research</td>
<td>National Institute of Communicable Diseases, New Delhi</td>
<td>Centers for Disease Control, Atlanta, Georgia</td>
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<td>a) Field Epidemiology</td>
<td>Director General of Health Services, New Delhi</td>
<td>Rockefeller Foundation, New York</td>
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<td>b) Laboratory Support</td>
<td>Central Bureau of Health Intelligence, All India Institute of Medical Sciences, New Delhi</td>
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<td>c) Clinical Epidemiology</td>
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<td>d) Malaria Information Systems</td>
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<td>LEPROSY</td>
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<td>Development, Validation and Application of Immuno-Diagnostic Techniques for the Diagnosis of Clinical Tuberculosis</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>State University of New York, Brooklyn</td>
<td>Annual budget for eight STI projects is Rs. 1,995,200</td>
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<td>Comparison of the FLA-ABS Test Using Whole M. Leprae, and ELISA Test for Antibody to Phenolic Glycolipid, for the Detection of Subclinical Infection in Leprosy</td>
<td>Central JALMA Institute for Leprosy, Agra, Uttar Pradesh</td>
<td>University of Washington, Seattle</td>
<td>See above</td>
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<tr>
<td>Demonstration and Characterization of Specific Glycolipid in Infected Human Tissues and Human Derived M. Leprae</td>
<td>Foundation for Medical Research, Bombay</td>
<td>Colorado State University, Fort Collins</td>
<td>See above</td>
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<td>Studies on Macrophage Metabolism and Functions After M. Leprae Infections as an Indication of Host-Parasite Interaction</td>
<td>Foundation for Medical Research, Bombay</td>
<td>Rockefeller University, New York City</td>
<td>See above</td>
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<td>Production, Characterization and Supply of Monoclonal Antibodies to M. Leprae</td>
<td>All India Institute of Medical Sciences, New Delhi Foundation for Medical Research, Bombay Central JALMA Institute for Leprosy, Agra, Uttar Pradesh</td>
<td>University of Washington, Seattle</td>
<td>See above</td>
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<td>Development of M. Leprae-Specific Human T. Cell Clones</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland</td>
<td>See above</td>
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<td>The Role of Dendritic Cell in Antigen Lymphoproliferative Responses in Leprosy</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>Rockefeller University, New York City</td>
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<td>Evaluation of Biological Significance of Antigen Derived from M. Leprae</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>Colorado State University, Fort Collins</td>
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<td>Genetic Studies of Leprosy Based on HLA and B Cell Typing</td>
<td>Central JALMA Institute for Leprosy, Agra, Uttar Pradesh</td>
<td>Centers for Disease Control, Atlanta, Georgia</td>
<td>Rs. 1,740,230</td>
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<td>Supply of Biopsy Specimens of Leprosy</td>
<td>Acworth Leprosy Hospital, Bombay</td>
<td>Centers for Disease Control, Atlanta, Georgia</td>
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<td>Detection of Drug Resistance and Screening of Drugs for Leprosy</td>
<td>Foundation for Medical Research, Bombay</td>
<td>Wayne University, Detroit, Michigan</td>
<td>Rs. 1,702,355</td>
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<td>Role of Transfer Factor in Management of Leprosy</td>
<td>Schieffelin Leprosy Research and Training Center, Karigiri, Tamil Nadu</td>
<td>Gillis W. Long Hansen's Disease Center, Carville, Louisiana</td>
<td>Rs. 826,476</td>
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**CANCER**

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<td>Oral Cancer and Precancerous Lesions in Rural Indian Population</td>
<td>Tata Institute of Fundamental Research, Bombay</td>
<td>Center for Health Sciences, University of Tennessee, Nashville</td>
<td>Rs. 8,164,020</td>
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<td>Liposomes in Delivery of Partially Thiolated Polynucleotides as Potential Antitumor Agents</td>
<td>Bose Institute, Calcutta</td>
<td>State University of New York, Buffalo</td>
<td>Rs. 1,441,100</td>
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<td>Study on Pelvic vs Extended Radiotherapy in Cancer of Cervix Stage III</td>
<td>Postgraduate Institute of Medical Education and Research, Chandigarh</td>
<td>Allegheny General Hospital, Pittsburgh, Pennsylvania</td>
<td>Rs. 5,269,611</td>
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<td>New Therapeutic Agents Against Cancer</td>
<td>Central Drug Research Institute, Lucknow, Uttar Pradesh</td>
<td>National Cancer Institute, National Institutes of Health, Bethesda, Maryland</td>
<td>Rs. 1,731,300</td>
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<td>Coordinated Research Project on Mycotoxins in Foods</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>Food and Drug Administration, Rockville, Maryland</td>
<td>Rs. 8,577,460</td>
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<td>Investigations on Prevention, Elimination and Inactivation of Aflatoxin and Other Mycotoxins From Cereals and Oilseeds With Special Reference to Maize and Mustard</td>
<td>Bhagalpur University, Bihar</td>
<td>U.S. Department of Agriculture, Agricultural Research Service, New Orleans, Louisiana</td>
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<td>Control and Prevention of Iron Deficiency Anemia in Rural Indian Populations</td>
<td>National Institute of Nutrition, Hyderabad, Andhra Pradesh</td>
<td>National Center for Health Statistics, Hyattsville, Maryland</td>
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<td>Clinical Research Center for Nutritional Blindness</td>
<td>National Institute of Nutrition, Hyderabad, Andhra Pradesh</td>
<td>National Eye Institute, National Institutes of Health, Bethesda, Maryland</td>
<td>Annual budget for six STI projects is Rs. 5,557,909</td>
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<td>Anterior Segment Collagenase Activity in Relation to Keratomalacia</td>
<td>National Institute of Nutrition, Hyderabad, Andhra Pradesh</td>
<td>National Eye Institute, National Institutes of Health, Bethesda, Maryland</td>
<td>See above</td>
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<td>Absorption of Vitamin A in Diarrhea Treatment With and Without Oral Rehydration Solution</td>
<td>National Institute of Nutrition, Hyderabad, Andhra Pradesh</td>
<td>National Eye Institute, National Institutes of Health, Bethesda, Maryland</td>
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<td>Use of Monoclonal Antibodies for the Detection of Early Vitamin A Deficiency</td>
<td>National Institute of Nutrition, Hyderabad, Andhra Pradesh</td>
<td>National Eye Institute, National Institutes of Health, Bethesda, Maryland</td>
<td>See above</td>
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<td>Laboratory and Clinical Studies of Eale's Disease</td>
<td>Madurai Kamaraj University, Tamil Nadu</td>
<td>National Eye Institute, National Institutes of Health, Bethesda, Maryland</td>
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<td>Case Control Study of Senile Cataract</td>
<td>Rajendra Prasad Center for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi</td>
<td>National Eye Institute, National Institutes of Health, Bethesda, Maryland</td>
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<td><strong>CHILD HEALTH/FAMILY PLANNING</strong></td>
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<td>Integrated Child Development Services</td>
<td>National Institute of Public Cooperation and Child Development, New Delhi</td>
<td>Montefiore Hospital and Medical Center, Albert Einstein College of Medicine, Bronx, New York</td>
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<td></td>
<td>Director of Health and Medical Services and Medical Education, Government of Gujarat, Ahmedabad</td>
<td>John Snow Public Health Group, Boston, Massachusetts</td>
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<td>Secretary, Rural Development Department, Mantralya, Government of Maharashtra, Bombay</td>
<td>American Public Health Association, Washington, D.C.</td>
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<td>Indian Council of Medical Research, New Delhi</td>
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<td>Family Planning Communications and Marketing</td>
<td>Ministry of Health and Family Welfare, New Delhi</td>
<td>American Public Health Association, Washington, D.C.</td>
<td>Rs. 31,200,000</td>
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<td>Contraceptive Development: Reproductive Immunology</td>
<td>National Institute of Immunology, New Delhi National Biotechnology Board, Department of Science and Technology, New Delhi</td>
<td>Program for Applied Research on Fertility Regulation, Chicago, Illinois</td>
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<td>Effects of Immunization with FSH in Primates</td>
<td>Indian Institute of Science, Bangalore, Karnataka</td>
<td>School of Medicine, New Orleans, Louisiana</td>
<td>Annual budget for six STI projects is Rs. 8,320,580</td>
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<td>Studies on Biochemically and Immunologically Fractioned Agents</td>
<td>Kothari Center of Gastroenterology, Calcutta</td>
<td>National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland</td>
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<td>Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Protein</td>
<td>Postgraduate Institute of Medical Education and Research, Chandigarh</td>
<td>Baylor College of Medicine, Houston, Texas</td>
<td>See above</td>
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<td>Reproductive Biology and Population Studies</td>
<td>Indian Council of Medical Research, New Delhi</td>
<td>National Institute for Child Health and Human Development, National Institutes of Health, Bethesda, Maryland</td>
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<td>Establishment of Reagent Bank</td>
<td>Indian Council of Medical Research, New Delhi</td>
<td>National Institute for Child Health and Human Development, National Institutes of Health, Bethesda, Maryland</td>
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<tr>
<td>Development of an Anti-Fertility Vaccine Based on Immunization Against LHRH in the Male</td>
<td>National Institute of Immunology, New Delhi</td>
<td>Population Council, New York</td>
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<td>A Longitudinal Study of Outcome and Survival of Birth Cohort</td>
<td>Safdarjung Hospital, New Delhi</td>
<td>International Institute for Vital Registration and Statistics, Rockville, Maryland</td>
<td>Rs. 1,771,095</td>
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<td>Collaborative Study on Urolithiasis</td>
<td>Indian Council of Medical Research, New Delhi</td>
<td>National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland</td>
<td>Rs. 1,356,000</td>
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<td>Studies on Biology of Fungi Causing Superficial and Systematic Infections in Man</td>
<td>Maulana Azad Medical College, New Delhi</td>
<td>U.S. Department of Agriculture Agricultural Research Service, Ames, Iowa</td>
<td>Rs. 737,650</td>
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<td>Conformational Structure of Polynucleotides, Nucleotides and Nucleosides</td>
<td>Tata Institute of Fundamental Research, Bombay</td>
<td>National Institute of Arthritis, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland</td>
<td>Rs. 8,297,440</td>
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<td>MENTAL HEALTH/NEUROSCIENCES</td>
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<td>Research Collaboration in Mental Health and Substance Abuse</td>
<td>National Institute of Mental Health and Neuro-Sciences, Bangalore, Karnataka</td>
<td>Alcohol, Drug Abuse and Mental Health Administration, Rockville, Maryland</td>
<td>Rs. 2,781,159</td>
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<td>Non-Volitional Biofeedback Study of Higher Nervous Functions and its Applications to Therapy of Nervous Disorders</td>
<td>Voluntary Health Services, Madras</td>
<td>California College of Medicine, Orange</td>
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<td>Collaborative Study on Head Injuries</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>University of Virginia School of Medicine, Charlottesville</td>
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<td>Rehabilitation of Rural Cancer Patients and Their Families</td>
<td>Indian Cancer Society, Bombay</td>
<td>National Institute of Handicapped Research, Washington, D.C.</td>
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<td>Rehabilitation of the Blind in Southern Parts of Tamil Nadu State</td>
<td>Madurai Medical College, Tamil Nadu</td>
<td>National Institute of Handicapped Research, Washington, D.C.</td>
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<td>A Comprehensive Model Rehabilitation Services System for India—The Establishment and Support of Six Model District Rehabilitation Centers</td>
<td>Ministry of Social and Women’s Welfare, New Delhi</td>
<td>National Institute of Handicapped Research, Washington, D.C.</td>
<td>Rs. 13,000,000</td>
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