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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D C 20523

AUG 31 1990

Dr. Victoria Sheffield
Executive Director
International Eye Foundation
7801 Norfolk Avenue
Bethesda, Maryland 20814

Subject: Cooperative Agreement No. DAN-5116-A-00-0067-00

Dear Dr. Sheffield:

Pursuant to the authority contained in the Foreign Assistance Act of 1961 and the Federal Grant and Cooperative Agreement Act of 1982, as amended, the Agency for International Development (hereinafter referred to as "A.I.D.") hereby provides to the International Eye Foundation (hereinafter referred to as "IEF" or "Recipient") the sum set forth in Section 1C.2. of Attachment 1 of this Cooperative Agreement to provide partial financial support for the program described in Attachment 2 of this Cooperative Agreement entitled "Program Description."

This Cooperative Agreement is effective as of the date of this letter and funds obligated hereunder shall be used to reimburse the Recipient for allowable program expenditures for the period set forth in Section 1B. of Attachment 1 of this Cooperative Agreement.

This Cooperative Agreement is made to the Recipient on the condition that the funds will be administered in accordance with the terms and conditions as set forth in the attachments listed under my signature below, which together constitute the entire Cooperative Agreement document and have been agreed to by your organization.

Please acknowledge receipt and acceptance of this Cooperative Agreement by signing all copies of this Cover Letter, retaining one copy for your files, and returning the remaining copies to the undersigned.

Sincerely yours,



Jay M. Bergman
Agreement Officer
Chief, Food and Agriculture Branch
A.I.D./W Projects Division
Office of Procurement

Attachments:

1. Schedule
2. Program Description
3. Standard Provisions
4. Special Provision entitled "Restrictions on Lobbying"

ACKNOWLEDGED:

THE INTERNATIONAL EYE FOUNDATION
BY: Victoria M. Sheffield
TYPED NAME: Victoria M. Sheffield
TITLE: Executive Director
DATE: 09/07/90

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FISCAL DATA

A. GENERAL

A.1. Total Estimated A.I.D. Amount: \$100,000
A.2. Total Obligated A.I.D. Amount: \$100,000
A.3. Cost-Sharing Amount: \$33,335
A.4. Project No.: 936-5116
A.5. A.I.D. Project Office: S&T/N, F. Davidson
A.6. Funding Source: A.I.D./W
A.7. Tax I.D. No.: 52-0742301
A.8. DUNS No.: 08-234-3377
A.9. LOC No.: 72-00-1459

B. SPECIFIC

B.1.(a) PIO/T No.: 936-5116-0361481
B.1.(b) Appropriation: 72-1101021.3
B.1.(c) Allotment: 043-36-099-00-20-01
B.1.(d) BPC: DDNA-90-13600-IG11
B.1.(e) Amount: \$100,000

ATTACHMENT 1

SCHEDULE

1A. PURPOSE OF COOPERATIVE AGREEMENT

The purpose of this Cooperative Agreement is to provide partial financial support for the program described in Attachment 2 of this Cooperative Agreement entitled "Program Description."

1B. PERIOD OF COOPERATIVE AGREEMENT

The effective date of this Cooperative Agreement is the date of the Cover Letter and the estimated completion date is August 30, 1993. Funds obligated hereunder (see Section 1C.2. below) shall be used to reimburse the Recipient for allowable program expenditures incurred by the Recipient in pursuit of program objectives during such period. Funds obligated hereunder are anticipated to be sufficient for completion by the Recipient of the program described in Attachment 2 of this Cooperative Agreement by the estimated completion date.

1C. AMOUNT OF COOPERATIVE AGREEMENT AND PAYMENT

1C.1. The total estimated amount of this Cooperative Agreement for its full period, as set forth in Section 1B. above, is \$100,000.

1C.2. A.I.D. hereby obligates the amount of \$100,000 for the purposes of this Cooperative Agreement during the indicated period set forth in Section 1B. above, thereby fulfilling A.I.D.'s funding requirements. A.I.D. shall not be liable for reimbursing the Recipient for any costs in excess of the obligated amount, except as specified in paragraph (f) of the Standard Provision of this Cooperative Agreement entitled "Revision of Grant Budget."

1C.3. Payment shall be made to the Recipient in accordance with procedures set forth in the Standard Provision of this Cooperative Agreement entitled "Payment - Letter of Credit," as shown in Attachment 3.

1C.4. The total estimated amount of the program described in Attachment 2 of this Cooperative Agreement is \$133,336, of which A.I.D. may provide the amount specified in Section 1C.1. above, and the Recipient will provide \$33,336.

1D. COOPERATIVE AGREEMENT BUDGET

1D.1. The following is the Budget for the total estimated amount of this Cooperative Agreement (see Section 1C.1. above) for its full period (see Section 1B. above). The Recipient may not exceed the total estimated amount or the obligated amount of this Cooperative Agreement, whichever is less (see Sections 1C.1. and 1C.2., respectively, above). Except as specified in the Standard Provision of this Cooperative Agreement entitled "Revision of Grant Budget," as shown in Attachment 3, the Recipient may adjust line item amounts as may be reasonably necessary for the attainment of program objectives. Revisions to the budget shall be in accordance with Section 1C. above and the Standard Provisions entitled "Revision of Grant Budget" and "Cost Sharing (Matching)."

1D.2. Budget

<u>Cost Element</u>	<u>A.I.D.</u>	<u>Recipient/Others (Non-Federal)</u>	<u>Total</u>
Salaries	\$ 35,800	\$19,650	\$ 55,450
Travel			
International	5,700	1,800	7,500
Domestic	-0-	-0-	-0-
Subtotal	5,700	1,800	7,500
Per Diem			
International	4,000	1,500	5,500
Domestic	-0-	-0-	-0-
Subtotal	4,000	1,500	5,500
Equipment/Supplies	13,550	1,150	14,700
Eval./Consultants	16,450	1,100	17,550
Other Direct Costs	10,743	3,130	13,873
Subtotal	\$ 86,243	28,330	114,573
Overhead	13,757	5,006	18,763
TOTAL	\$100,000	\$33,336	\$133,336

1D.3. Inclusion of any cost in the budget of this Cooperative Agreement does not obviate the requirement for prior approval by the Agreement Officer of cost items designated as requiring prior approval by the applicable cost principles (see the Standard Provision of this Cooperative Agreement set forth in Attachment 3 entitled "Allowable Costs") and other terms and conditions of this Cooperative Agreement, unless specifically stated in Section 1I. below.

1E. REPORTING

1E.1. Financial Reporting

1E.1.(a) Financial reporting requirements shall be in accordance with the Standard Provision of this Cooperative Agreement entitled "Payment - Letter of Credit," as shown in Attachment 3.

1E.1.(b) All financial reports shall be submitted to A.I.D., Office of Financial Management, PFM/FM/CMPD/DCB, Room 700 SA-2, Washington, D.C. 20523-0209. In addition, three copies of all financial reports shall be submitted to the A.I.D. Project Office specified in the Cover Letter of this Cooperative Agreement, concurrently with submission of the Quarterly Technical Reports (See Section 1E.2. below).

1E.1.(c) The frequency of financial reporting and the due dates of reports shall be as specified in the Standard Provision of this Cooperative Agreement referred to in Section 1E.1.(a) above.

1E.1.(d) The Recipient's financial reports shall include expenditures of A.I.D. Cooperative Agreement funds provided hereunder, as well as non-federal matching funds in accordance with Section 1L. below.

1E.2. Program Performance Planning and Reporting

1E.2.(a) Project Implementation Plan

Not later than sixty (60) days from the effective date of this Cooperative Agreement (see Section 1B. above), the Recipient shall prepare and submit to the A.I.D. Project Officer specified in the Cover Letter of this Cooperative Agreement five (5) copies of a project implementation plan, with critical path indicators (as described in A.I.D. Handbook 3), for the full term of this Cooperative Agreement.

1E.2.(b) Annual Workplans .

1E.2.(b)(1) The Recipient shall submit annual workplans for this Cooperative Agreement. Each annual workplan shall contain the following:

1E.2.(b)(1)(A) An action-oriented workplan describing planned activities during the next year, delineated by calendar quarter, and linked to the project goals and objectives. Planned activities shall be grouped by subject category, and then related to project objectives;

1E.2.(b)(1)(B) A projected budget, utilizing the same budget line items as are set forth in the budget of this Cooperative Agreement, for each calendar quarter, corresponding to the workplan, which describes the individuals to be involved, the activities to be conducted, and where and when they will be conducted; and

1E.2.'b)(1)(C) Publications, reports, workshops, seminars, and other information dissemination activities planned, by calendar quarter.

1E.2.(b)(2) The Recipient may develop the annual workplans in consultation with the A.I.D. Project Officer for this Cooperative Agreement.

1E.2.(b)(3) Three (3) copies of each annual workplan will be submitted to the designated A.I.D. Project Officer for this Cooperative Agreement and one copy submitted to the Agreement Officer. The first annual workplan covering the first year of this Cooperative Agreement shall be submitted by the Recipient not later than sixty (60) days from the effective date of this Cooperative Agreement (see Section 1B. above). Thereafter, the annual workplan for each successive year of this Cooperative Agreement shall be submitted by the Recipient not later than sixty (60) days prior to the beginning of each year.

1E.2.(c) Quarterly Reports

The Recipient shall submit five (5) copies of brief quarterly program performance reports, which coincide with the financial reporting periods described in Section 1E.1. above, to the A.I.D. Project Office specified in the Cover Letter of this Cooperative Agreement. In addition, two copies shall be submitted to A.I.D., PPC/CDIE/DI, Washington, DC 20523-1802. These reports shall be submitted within 30 days following the end of the reporting period, and shall briefly present the following information:

1E.2.(c)(1) A comparison of actual accomplishments with the goals established for the period, the findings of the investigator, or both. If the output of programs can be readily quantified, such quantitative data should be related to cost data for computation of unit costs.

1E.2.(c)(2) Reasons why established goals were not met, if applicable.

1E.2.(c)(3) Other pertinent information including the status of finances and expenditures and, when appropriate, analysis and explanation of cost overruns or high unit costs.

1E.2.(d) Special Reports

Between the required program performance reporting dates, events may occur that have significant impact upon the program. In such instances, the Recipient shall inform the A.I.D. Project Officer as soon as the following types of conditions become known:

1E.2.(d)(1) Problems, delays, or adverse conditions that will materially affect the ability to attain program objectives, prevent the meeting of time schedules and goals, or preclude the attainment of work units by established time periods. This disclosure shall be accompanied by a statement of the action taken, or contemplated, and any A.I.D. assistance needed to resolve the situation.

1E.2.(d)(2) Favorable developments or events that enable time schedules to be met sooner than anticipated or more work units to be produced than originally projected.

1E.2.(d)(3) If any performance review conducted by the Recipient discloses the need for change in the budget estimates in accordance with the criteria established in the Standard Provision of this Cooperative Agreement entitled "Revision of Grant Budget," the Recipient shall submit a request for budget revision to the Agreement Officer and the A.I.D. Project Officer specified in the Cover Letter of this Cooperative Agreement.

1E.2.(e) Annual Activity Reports

Within thirty (30) days following the annual anniversary date of this Cooperative Agreement, the Recipient shall submit to the A.I.D. Project Office specified in the cover letter of this Cooperative Agreement five (5) copies of an annual technical progress report which will be a description of the past year's activities, including technical, scientific, managerial, and fiscal information. The report shall include, both for each field site or subcontractor/subrecipient individually and for project activities as a whole, a review of program and problems to date, and a discussion of technical and managerial issues significant to the success or failure of this Cooperative Agreement. The report will also address regulatory issues related to the project. Although principally a technical document, it nevertheless must include pertinent statistics or quantitative information regarding the project and its

activities. An Impact Analysis Report will be appended to this report, which will be considered an instrument for Technology Transfer. The Impact Analysis Report will summarize and provide a feedback system for measurement and evaluation of the impact of the Recipient's activities in the public and private sector. The impact analysis will generally be qualitative in nature, and quantified only as appropriate. The Annual Activity Report shall also include an annual expenditure report corresponding to each annual workplan (see Section 1E.2.(b) above). These expenditure reports will cover A.I.D. and, if applicable, cost-sharing amounts by budget line item (see Section 1D.2. above) and by estimated distribution amongst project components, e.g., research, training, technical assistance, technology transfer, information dissemination, or networking.

1E.2.(f) Technical and Research Reports and Publications

The Recipient shall summarize technical and research activities of the project in reports, and distribute such reports to the appropriate USAID Missions, LDCs, and host country and international institutions in order to encourage use of the technology developed. Such reports will be completed within 60 days after completion of the activity. Journal articles and other publications are encouraged. See also Section 1I. of this Cooperative Agreement pertaining to publications.

1E.2.(e) Trip Reports

Within 30 days following the completion of each international trip, the Recipient shall submit 3 copies of a trip report summarizing the accomplishments of the trip to the A.I.D. Project Officer specified in the cover letter of this Cooperative Agreement. If several individuals are travelling together to one site, a single report representing the group will suffice. The report shall include the purpose of the trip, technical observations, suggestions and recommendations, overall impressions of the site situation (if appropriate), and a list of persons visited with their title and organization affiliation.

1E.2.(f) Final Report

Within 90 days following the estimated completion date of this Cooperative Agreement (see Section 1B. above), the Recipient shall submit three (3) copies of a final report to the A.I.D. Project Office specified in the cover letter of this Cooperative Agreement. In addition, two copies shall be submitted to A.I.D., PPC/CDIE/DI, Washington, DC 20523-1802. The final report shall include: (1) an Executive Summary of the Cooperative Agreement's accomplishments or failings; (2) a

description of the Cooperative Agreement's activities from its inception; (3) significance of these activities; (4) comments and recommendations; (5) significance of the Cooperative Agreement's activities to A.I.D. and S&T/N; and (6) a fiscal report that describes in detail how the Cooperative Agreement funds were used.

1F. SUBSTANTIAL INVOLVEMENT UNDERSTANDINGS

It is understood and agreed that A.I.D. will be substantially involved during performance of this Cooperative Agreement, as follows:

1F.1. Annual Workplan - The A.I.D. Project Officer will be consulted during the development of the annual workplans, and have the right of final approval of all areas of the workplan where A.I.D. funds are included.

1F.2. Workplan Revisions - The A.I.D. Project Officer will be consulted and have the right of approval for revisions of the annual workplan which involves the use of A.I.D. funds.

1F.3. Field Visits or Other Travel - Pursuant to the standard provision of this Cooperative Agreement entitled "Air Travel and Transportation," the A.I.D. Project Officer must provide advance approval of all international travel. The Recipient must make travel plans known sufficiently in advance to allow for USAID Mission travel concurrence.

1F.4. Consultants - The A.I.D. Project Officer must approve, in advance, the selection of consultants retained by the Recipient.

1F.5. Participants - Where A.I.D. funds are used, the A.I.D. Project Officer must approve, in advance, the selection of technical trainees or scientists for participation in training activities.

1F.6. Staff - The A.I.D. Project Officer must approve, in advance, all changes in key staff. The Recipient shall advise A.I.D. of plans to convene, and shall invite A.I.D. to participate in advisory committees or groups with which the Recipient shall collaborate regarding the program supported by this Cooperative Agreement

1F.7. Changes - The Recipient agrees to work collaboratively with the A.I.D. Project Officer and agrees to keep the A.I.D. Project Officer informed, in advance, of proposed changes in the program through the reporting requirements set forth in Section 1E.2. of this Cooperative Agreement.

1F.8. Subcontracts and Subagreements - The A.I.D. Project Officer must approve, in advance, the terms of reference or scope of work of all subcontracts and subagreements awarded by the Recipient. If required by Paragraphs (b)(5) or (b)(6) of the Standard Provision entitled "Revision of Grant Budget," or the Standard Provision entitled "A.I.D. Eligibility Rules for Goods and Services," the Agreement Officer must approve subcontracts (see the Standard Provision entitled "Procurement of Goods and Services") and subagreements (see the Standard Provision entitled "Subagreements").

1G. PROCUREMENT AND (SUB)CONTRACTING

1G.1. Applicability

This Section 1G. applies to the procurement of goods and services by the Recipient (i.e., contracts, purchase orders, etc.) from a supplier of goods and services (see the Standard Provisions of this Cooperative Agreement entitled "Procurement of Goods and Services" and "AID Eligibility Rules for Goods and Services"), and not to assistance provided by the Recipient (i.e., a [sub]grant or subagreement) to a subrecipient (see the Standard Provision of this Cooperative Agreement entitled "Subagreements").

1G.2. Requirements

In addition to other applicable provisions of this Cooperative Agreement, the Recipient shall comply with paragraph (b)(1) of the Standard Provision of this Cooperative Agreement entitled "AID Eligibility Rules for Goods and Services," concerning total procurement value of less than \$250,000 under this Cooperative Agreement. If, under the order of preference set forth in paragraph (b)(1)(i) of said Standard Provision, the Recipient procures goods or services from cooperating country sources, the Standard Provision of this Cooperative Agreement entitled "Local Cost Financing" shall also apply. However, paragraph (b)(1) of the Standard Provision entitled "AID Eligibility Rules for Goods and Services" does not apply to: the restricted goods listed in paragraph (a)(3) of said Standard Provision and paragraph (e) of the Standard Provision entitled "Local Cost Financing," which must be specifically approved by the Agreement Officer in all cases, except to the extent that such approval may be provided in Section 1I. below; or to paragraph (d) of said Standard Provision pertaining to air and ocean transportation, to which the Standard Provisions entitled "Air Travel and Transportation" and "Ocean Shipment of Goods" apply, respectively. Paragraph (b)(2) of the Standard Provision entitled "AID Eligibility Rules for Goods and Services" does not apply.

1G.3. Approvals

Inclusion of costs in the budget of this Cooperative Agreement for the purchase of nonexpendable equipment obviates neither the requirement of Section J.13. of OMB Circular A-21 (for educational institutions) or Section 13 of Attachment B of OMB Circular A-122 (for nonprofit organizations other than educational institutions) for prior approval of such purchases by the Agreement Officer, nor any other terms and conditions of this Cooperative Agreement, unless specifically stated in Section II. below.

1G.4. Title to Property

Title to property acquired hereunder shall vest in the Recipient, subject to the requirements of the Standard Provision of this Cooperative Agreement entitled "Title To and Use of Property (Grantee Title)" regarding use, accountability, and disposition of such property, except to the extent that disposition of property may be specified in Section II. below.

1H. INDIRECT COST RATES

1H.1. Pursuant to the Standard Provisions of this Cooperative Agreement entitled "Negotiated Indirect Cost Rates - Provisional" an indirect cost rate or rates shall be established for each of the Recipient's accounting periods which apply to this Cooperative Agreement. Pending establishment of final or revised provisional indirect cost rates, provisional payments on account of allowable indirect costs shall be made on the basis of the following negotiated provisional rate applied to the base which is applied is set forth below:

<u>Type</u>	<u>Rate</u>	<u>Base</u>	<u>Period</u>
Provisional	18.15	*	Until Amended

1/ Base of Application: Total direct cost but excluding non-expendable project equipment.

11. SPECIAL PROVISIONS

11.1. Limitations on Reimbursement of Costs of Compensation for Personal Services and Professional Service Costs

11.1.(a) Employee Salaries

Except as the Agreement Officer may otherwise agree in writing, A.I.D. shall not be liable for reimbursing the Recipient for any costs allocable to the salary portion of direct compensation paid by the Recipient to its employees for personal services which exceed the highest salary level for a Foreign Service Officer, Class 1 (FS-1), as periodically amended.

11.1.(b) Consultant Fees

Compensation for consultants retained by the Recipient hereunder shall not exceed, without specific approval of the rate by the Agreement Officer: either the highest rate of annual compensation received by the consultant during any full year of the immediately preceding three years; or the maximum rate of a Foreign Service Officer, Class 1 (FS-1) (as periodically amended), whichever is less. A daily rate is derived by dividing the annual compensation by 2,087 and multiplying the result by 8.

11.3. Publications

11.3.(a) The Recipient agrees to provide two copies of the manuscript of any proposed publication to the A.I.D. Project Officer not later than submission to the publisher, and to give serious consideration to any comments received from the A.I.D. Project Officer.

11.3.(b) In the case of publication of any of the reports described in Section 1E.2. of this Cooperative Agreement, A.I.D. reserves the right to disclaim endorsement of the opinions expressed. For other publications, A.I.D. reserves the right to dissociate itself from sponsorship or publication. In both cases, the Recipient will consult with the A.I.D. Project Officer as to the nature and extent of any A.I.D. disclaimer of endorsement or dissociation from sponsorship or publication.

11.3.(c) If A.I.D. does not choose to disclaim endorsement or dissociate itself from sponsorship or publication, the Recipient shall, in accordance with the Standard Provision of this Cooperative Agreement entitled "Publications," acknowledge A.I.D. support as follows:

"This publication was made possible through support provided by the Office of Nutrition, Bureau for Science and Technology, U.S. Agency for International Development, under Cooperative Agreement No. DAN-5116-A-00-0067-00."

11.3.(d) In addition to providing one copy of all published works and lists of other written work produced under this Cooperative Agreement to the A.I.D. Project Officer, as required by paragraph (b) of the Standard Provision of this Cooperative Agreement entitled "Publications," the Recipient shall also provide two copies of such publications and lists to A.I.D., PPC/CDIE/DI, Washington, D.C. 20523-1802.

11.4. Equipment Purchases

11.4.(a) Requirement for Prior Approval

Pursuant to Sections 1D.3. and 1G.3. above and the Standard Provisions of this Cooperative Agreement entitled "Allowable Costs" and "Revision of Grant Budget," and by extension, Section 13 of Attachment B of OMB Circular A-122, the Recipient must obtain A.I.D. Agreement Officer approval for purchases of the following:

11.4.(a)(1) General Purpose Equipment, which is defined as an article of nonexpendable tangible personal property which is usable for other than research, medical, scientific or technical activities, whether or not special modifications are needed to make them suitable for a particular purpose (e.g., office equipment and furnishings, air conditioning equipment, reproduction and printing equipment, motor vehicles, and automatic data processing equipment), having a useful life of more than two years and an acquisition cost of \$500 or more per unit); and

11.4.(a)(2) Special Purpose Equipment, which is defined as an article of nonexpendable tangible personal property, which is used only for research, medical, scientific, or technical activities (e.g., microscopes, x-ray machines, surgical instruments, and spectrometers), and which has a useful life of more than two years and an acquisition cost of \$1,000 or more per unit).

11.4.(b) Approvals

In furtherance of the foregoing, the Agreement Officer does hereby provide approval for the following purchases, which shall not be construed as authorization to exceed the total estimated amount or the obligated amount of this Cooperative Agreement, whichever is less (see Section 1C. above):

N/A

11.4.(c) Exception for Automation Equipment

Any approval for the purchase of automation equipment which may be provided in Section 11.4.(b) above or subsequently provided by the Agreement Officer is not valid if the total cost of purchases of automation equipment (e.g., computers, word processors, etc.), software, or related services made hereunder will exceed \$100,000. The Recipient must, under such circumstances, obtain the approval of the Agreement Officer for the total planned system of any automation equipment, software, or related services.

11.4.(d) Compliance with A.I.D. Eligibility Rules

Any approvals provided in Section 11.4.(b) above or subsequently provided by the Agreement Officer shall not serve to waive the A.I.D. eligibility rules described in Section 1G. of this Cooperative Agreement, unless specifically stated.

11.5. Restricted Goods

Pursuant to Section 1G. above, paragraph (a)(3) of the Standard Provisions of this Cooperative Agreement entitled "AID Eligibility Rules for Goods and Services," and, if applicable (see Section 1K. below for applicability), paragraph (e) of the Standard Provision of this Cooperative Agreement entitled "Local Cost Financing," the Agreement Officer's approval is required for purchase of the restricted goods described therein. In furtherance thereof, the Agreement Officer does hereby provide such approval to the extent set forth below. The Agreement Officer's approval is required for purchases of such restricted goods if all of the conditions set forth below are not met by the Recipient. Any approval provided below or subsequently provided by the Agreement Officer shall not serve to waive any terms and conditions of this Cooperative Agreement unless specifically stated.

11.5.(a) Motor Vehicles

Motor vehicles, if approved for purchase under Section 11.4.(b) above or subsequently approved by the Agreement Officer, must be of U.S. manufacture and must be of at least 51% U.S. componentry. The origin of the motor vehicles, and the nationality of the supplier of the vehicles, must be in accordance with Section 1G.2. above. Motor vehicles are defined as self-propelled vehicles with passenger carriage capacity, such as highway trucks, passenger cars and busses, motorcycles, scooters, motorized bicycles, and utility vehicles. Excluded from this definition are industrial vehicles for materials handling and earthmoving, such as lift trucks, tractors, graders, scrapers, and off-the-highway trucks.

11.5.(b) Pharmaceuticals

Pharmaceuticals may be purchased provided that all of the following conditions are met: (1) the pharmaceuticals must be safe and efficacious; (2) the pharmaceuticals must be of U.S. source and origin (see Section 1G. above); (3) the pharmaceuticals must be of at least 51% U.S. componentry (see Section 1G. above); (4) the pharmaceuticals must be purchased from a supplier whose nationality is in the U.S. (see Section 1G. above); (5) the pharmaceuticals must be in compliance with U.S. Food and Drug Administration (FDA) (or other controlling U.S. authority) regulations governing United States interstate shipment of pharmaceuticals; (6) the manufacturer of the pharmaceuticals must not infringe on U.S. patents; and (7) the pharmaceuticals must be competitively procured in accordance with the procurement policies and procedures of the Recipient and the Standard Provision of this Cooperative Agreement entitled "Procurement of Goods and Services."

11.5.(c) Used Equipment

Used equipment may only be purchased with the prior written approval of the Agreement Officer.

1J. RESOLUTION OF CONFLICTS

Conflicts between any of the Attachments of this Cooperative Agreement shall be resolved by applying the following descending order of precedence:

- Attachment 1 - Schedule
- Attachment 3 - Standard Provisions
- Attachment 4 - Special Provision entitled "Restrictions on Lobbying"
- Attachment 2 - Program Description

ATTACHMENT 2

PROGRAM DESCRIPTION

The Recipient's proposal entitled "Increasing the Consumption of Carotene-Containing Foods as a Long-term and Sustainable Strategy to Reduce Hypovitaminosis A in Guatemala" dated March, 1990 is attached hereto as the Program Description (Attachment 2) and is made a part of this Cooperative Agreement.

19/11

TITLE;

**INCREASING THE CONSUMPTION OF CAROTENE-CONTAINING FOODS AS A
LONG-TERM AND SUSTAINABLE STRATEGY TO REDUCE HYPOVITAMINOSIS A
IN GUATEMALA**

A collaborative project of the

International Eye Foundation of Bethesda, Maryland

**The Medical Division of the National Committee for the Blind and
Deaf of Guatemala, Guatemala City, Guatemala**

**The Center for Studies of Sensory Impairment, Aging and
Metabolism, the research branch for the National Committee for
the Blind and Deaf of Guatemala, Guatemala City, Guatemala**

prepared by

**Noel W. Solomons, M.D.
CeSSIAM**

**Jesus Bulux, M.D.
CeSSIAM**

**Gustavo Hernandez-Polanco, M.D.
Medical Director, NCBD**

Contacts

**Jack Blanks, M.A.
IEF**

**John Barrows, M.P.H.
IEF**

March, 1990

EXECUTIVE SUMMARY

GUATEMALA IS A COUNTRY OF 9,000,000 INHABITANTS WITH A LONG HISTORY OF ENDEMIC HYPOVITAMINOSIS A. AFTER HAVING UTILIZED MASSIVE DISTRIBUTION OF HIGH-DOSE VITAMIN A CAPSULES TO PRESCHOOL CHILDREN, AND NATIONWIDE FORTIFICATION OF TABLE SUGAR WITH RETINYL PALMITATE, THERE IS A CONSENSUS OF INFORMED OPINION THAT THE ONLY SUSTAINABLE, LONG-TERM STRATEGY FOR ERADIATING VITAMIN A DEFICIENCY IS INCREASING THE INTAKE OF THE VITAMIN FROM FOODS AND BEVERAGES. OVER SEVENTY-PERCENT OF THE VITAMIN A CURRENTLY CONSUMED IN GUATEMALA COMES FROM PLANT SOURCES IN THE FORM OF CAROTENOIDS. THIS CHEMICAL FORM IS INTRINSICALLY SAFER THAN RETINOL AS IT CANNOT INDUCE VITAMIN A TOXICITY. THE PRESENT PROPOSAL COMBINES THE RESPECTIVE TALENTS OF THE PRIVATE VOLUNTARY ORGANIZATION THE, INTERNATIONAL EYE FOUNDATION, THE PRIVATE, NON-PROFIT ENTITY, THE NATIONAL COMMITTEE FOR THE BLIND AND DEAF OF GUATEMALA AND ITS RESEARCH UNIT, CENTER FOR STUDIES OF SENSORY IMPAIRMENT, AGING AND METABOLISM, THREE INSTITUTIONS THAT HAVE COMBINED EFFORTS IN THE PAST IN VITAMIN A INTERVENTIONS AND ACCOMPANYING OPERATIONAL RESEARCH. THE PRESENT PROPOSAL SEEKS TO FURTHER THE INFORMATION NECESSARY TO SUSTAIN HIGHER INTAKES OF VITAMIN A IN THE GUATEMALAN POPULATION THROUGH RELIANCE ON INCREASED CONSUMPTION OF CAROTENE-RICH FOODS. IT INVOLVES THE CHEMICAL ANALYSIS OF THE EDIBLE WILD GREEN PLANTS AND DOMESTICATED VEGETABLES CURRENTLY CONSUMED IN THE RURAL DIET, AND A PILOT INTERVENTION INVOLVING THE DISTRIBUTION OF VARIOUS FORMS OF CAROTENE-RICH FOODS (INCLUDING THE GENETICALLY-IMPROVED BETA-III CARROT), TO DETERMINE THE ACCEPTABILITY AND BIOLOGICAL EFFECT. THREE HAMLETS IN AN AREA OF THE SANTA ROSA PROVINCE, IN WHICH THE LOWEST INDIVIDUAL VITAMIN A INTAKES IN PRESCHOOL CHILDREN HAVE BEEN RECORDED, WILL BE ENROLLED. THE SPECIFIC OBJECTIVES OF THE STUDY ARE: 1) (INTERVENTION) WEEKLY DISTRIBUTION OF a) AN EDIBLE GREEN PLANT (E.G. QUILETE), b) OF A CONVENTIONAL VARIETY OF CARROT, AND c) OF THE BETA-III CARROT; 2) (BASIC RESEARCH) DETERMINE THE CAROTENOID PATTERN OF THE EDIBLE WILD GREEN PLANTS AND DOMESTICATED VEGETABLES CURRENTLY CONSUMED IN THE RURAL DIET; 3) (OPERATIONAL RESEARCH) DETERMINE THE ACCEPTABILITY AND LEVELS OF CONSUMPTION OF THE PLANT FOODS; 4) DETERMINE THE EFFECT OF THE AVAILABILITY OF PLANT FOODS ON THE BASIC PATTERN OF DIET; 5) DETERMINE THE BIOLOGICAL IMPACT OF THE DEGREE OF CONSUMPTION OF THE CAROTENE-RICH FOODS IN RELATION TO HOUSEHOLD PARTICIPATION AND CAROTENE-CONTENT OF THE FOOD ITEM; AND 6) DETERMINE THE INFLUENCE OF INTESTINAL PARASITE INFESTATION ON THE UTILIZATION OF DIETARY CAROTENES. A TOTAL OF 1500 PERSONS WILL BE DIRECTLY BENEFITTED BY THE INTERVENTION OF VITAMIN A-RICH PLANT DISTRIBUTION AND EVENTUAL DE-PARSITIZATION. NEW RESOURCES AND ENHANCEMENT OF RESEARCH CAPACITY WILL RESULT FOR THE NCBD AND THE CESSIAM. NEW UNDERSTANDING OF THE POSSIBILITIES, LIMITATIONS AND NUTRITIONAL CONSEQUENCES IN INTERVENTIONS TO INCREASE VITAMIN A CONSUMPTION FROM LOCALLY-AVAILABLE PLANT FOODS WILL BE DERIVED.

INTRODUCTION AND BACKGROUND

Guatemala a country of 9,000,000 inhabitants, is the northernmost republic in the Central American chain. It is still largely agrarian, and more than 50% of the population live in settings that are considered to be rural. Since the time of the Central American Nutrition Survey in 1965-67, conducted by the InterDepartmental Committee on Nutrition in National Defense and the Institute of Nutrition of Central America and Panama (1), it has been known that hypovitaminosis A was a public health problem. It was manifest by rates of up to . . .% of the preschool children in rural areas with circulating levels of retinol of <20 ug/dl. The percentage of children in this age-group consuming less than the dietary recommendation for that vitamin in that era, 25 years ago,

The National Committee for the Blind and Deaf of Guatemala has a vested interest in the sustained improvement of the vitamin A status of the Guatemalan population as the xerophthalmic ocular lesions are a form of preventable nutritional blindness (2). The revelations from Indonesia in the early 1980s (3,4) of an effect of vitamin A deficiency on producing excess mortality and morbidity from childhood illnesses, and, that, conversely, child survival and health could be improve by supplementation with high doses of vitamin A. These findings have been confirmed by recent work presented at the meeting of the International Vitamin A Consultative Group in Kathmandu, Nepal. The NCBD, with its background in vitamin A issues, has seen it within its mission to join the battle to reduce hypovitaminosis A in service to improved child survival as well.

Elements of the Guatemalan strategy for the conquest of hypovitaminosis A have been agreed upon by common accord among the Ministry of Health and the various institutions concerned. One stage was the nationwide distribution of the massive-dose 200,000 IU capsule to the under-6-year-olds. This occurred on October 15 and 16, 1988. This was to replete the hepatic reserves of the preschool population, a group that might be at specific risk of infection. The second stage is the fortification of a universal, common vehicle -- table sugar was chosen -- with retinyl palmitate. As specified, this would deliver 14 RE per gram of sugar (5). This was reiniciated with the 1987-88 sugar harvest, with the first lots of fortified sugar coming onto the national market in May of 1988, the fortification has been continued apace in the subsequent two harvests. If the populace consumes the estimated 40 g of table sugar daily, some 540 RE would be derived from this approach on average. However, the onus of the financing of the program is on the sugar refining industry. In the past there was resistance to requirement, and the program is always vulnerable to the vicisitudes of political will.

The final phase in the strategy is sustained improvement of dietary intake of vitamin A from foods and vegetables in the diet. This is the subject addressed by this proposal. This is the manner in which the industrialized countries of the world have put the problem of hypovitaminosis A into their history. The issue of the artificial and dependent nature of the supplementation campaigns and the fortification schemes with retinyl palmitate are not the only consideration, however. The safety of preformed retinol in high doses being put into the environment of Third World countries has caused some legitimate concern. Retinol can be toxic (6,7). The elderly are more susceptible to tissue damage from high intakes of retinol than younger adults. Pregnant women in the first trimester are vulnerable to teratogenic effects of high intakes of vitamin A or its analogues on the embryo (8). Beta-carotene and the other carotenoids with provitamin A activity, on the other hand, cannot produce vitamin A intoxication. The conversion of the provitamin A compounds to vitamin A is governed and regulated by the gut (9). The human body, in its wisdom, will not convert active vitamin A than it needs.

Economic and ethnic diversity of the Guatemalan people has been the subject of studies by social scientist for decades. In fact, on the macro-dietetic level, there is some consensus as to the basic elements of the diet. The triad of black beans, corn tortillas (or tamalitos) and squash as been considered the core of the diet (10). Detailed studies, such as that by Lic. Eugenia Saenz de Tejada, the anthropologist on the IEF project (see below), show that the rural diet is much more varied and complex, a consequence that would impact on the micronutrient intake (11). Sixty-five percent of the population of Guatemala is of indigenous, Mayan descent while 35% are classified as ladino. The first language of the latter group is Spanish, whereas the former population constitutes a total of 23 distinct language groups. Having 90% of calories from beans, corn and other cereals does not leave much room for variety in the diet. However, how the tail of the diet is constructed with the vegetable, fruits and foods of animal origin, is a major determinant of vitamin A intake. It could also be postulated that how that same tail of the diet is constructed would be a determinant of the resistance to, or acceptance of, intervention initiatives to increase the consumption of plant sources of vitamin A. The hot:cold paradigm is a strong influence on food selection and cuisine in Guatemala (12). It remains to be seen how much of an increment in vitamin A intake from plant sources can be produced.

Genetic improvement of beta-carotene-containing plants is another and final dimension to the argument. If much more vitamin A can be contained in a plant, then lesser amounts need be consumed to provide a similar vitamin A value. The work by Phil Simon at the University of Wisconsin in horticultural

genetics led to the development of the Beta-III "supercarrot." (13). Genetically improved varieties of sweet potatoes is another direction. It is of interest to determine how acceptable are these new varieties to various populations.

Home and family gardens have been a mechanism for increasing dietary diversity employed with varying success in developing nations (14).

PRELIMINARY EXPERIENCE AND FINDINGS

The last five years have seen a resurgence of interest in vitamin A nutrition in Guatemala. This coincides with the international events cited above and the linking of vitamin A status to child survival. A local component was the prolonged lapsing in the legally-mandated fortification of table sugar with retinyl palmitate. At the Center for Studies of Sensory Impairment, Aging and Metabolism, the research branch for the National Committee for the Blind and Deaf, fundamental issues of vitamin A biology have represented one of the pillars of interest since 1987. In the same year, the International Eye Foundation, in collaboration with the NCDB, began effort to develop cooperative programs in community-level intervention. The first co-operative effort was sponsored under the Office of Nutrition's Competitive Vitamin A Intervention program and it involved the distribution of the vitamin A-enriched, convalescent refeeding food, NutriAtol, in three counties in Alta Verapaz. This activity was headed by Dr. Gustavo Hernandez-Polanco who is also the Medical Director of the Medical Division of the NCBD. This project had AID financing for two years of activities, and still continues with internal funding from the IEF. NutriAtol is also being distributed to the 3000 preschool children in the county of San Pedro Yepocapa, in Chimaltenango under a grant from the Hoffmann - La Roche, Sight and Life.

Operational research projects were added to the original Vitamin A intervention grant. One of these was a project to define the baseline, culturally-defined patterns of distribution within the household (Intrahousehold Distribution) and to determine any impact on this pattern by 1) childhood illness; and by 2) the introduction of NutriAtol to the populace. This project is taking advantage of the situation of the novel introduction of the post-illness weaning food to San Pedro Yepocapa.

CeSSIAM has collaborated with the aforementioned activities in service to the NCBD and IEF. This assistance includes technical assistance in the writing of the proposals and the progress reports for the intervention projects and operational research. Specific technical assistance was provided in testing 1) the acceptability of NutriAtol; 2) its short-term biological

impact on healthy children and diarrheal children; and 3) the biological impact in the field of NutriAtol distribution in a population of schoolchildren in Alta Verapaz. Finally, CeSSIAM professionals have provided specific technical training to the field personnel for the intervention projects.

Broader aspects of CeSSIAM research mandate in vitamin A have included: 1) innovation and evaluation in vitamin A assessment techniques such as dietary questionnaire instruments, conjunctival impression cytology, and relative dose response; 2) studies on beta-carotene bioavailability and bioconversion by the human intestine; and 3) the effects of parasites on vitamin A status and carotene metabolism. The former series of studies were conducted with the collaboration of the Johns Hopkins University and the USDA Human Nutrition Research Center on Aging. The bioavailability research was a cooperative effort with the University of Arizona. A series of publications and abstracts have been derived from these efforts (see Appendix).

Research both in CeSSIAM and from the IEF applied-inquiry efforts has centered around the description of the use of edible green plants in rural areas of the Republic. The University of Arizona graduate student, Claire Zizza, undertook a Masters thesis to describe the food-chain events in the acquisition, storage and preparation of vitamin A-containing foods. From this study we learned that most foods not grown or gathered are obtained on market-days twice per week (15). With CeSSIAM nutritionist, Julieta Quan de Serrano, the intake of vitamin A was assessed using a 7-day food-frequency instrument. A median intake of 100 RE was found in the preschoolers. This is the lowest estimated median intake of any preschool population so far studies in the Republic (16). McGill University doctoral candidate, Ms Sarah Booth, at the School of Dietetics and Human Nutrition, in conjunction with CeSSIAM professional, Dr. Yolanda Lopez, has been studying the pattern of edible green plant use among the Kekchi-speaking indigenous households of the Alta Verapaz Province (17). They first defined the range of plants consumed. The work is currently extending to an examination of a rural-to-urban and seasonal-variation context. With a more practical end of determining what would be the range of plants, both wild, indigenous edible greens as well as domesticated green, yellow and orange vegetables, Mr. William Scott of the I.E.F. staff in Guatemala, with the participation of University of Pennsylvania medical student, Jeremiah Goldstein. This was the other element of applied inquiry financed by the Office of Nutrition under the Vitamin A Intervention study. It led to the report: **Consumption Practices in the Use of Domesticated and Indigenous Carotene Sources in the Guatemalan Diet.** (18) and basically defined the types of plants used, the degree of participation by different populations in township, hamlet, and plantation

settings, and the form (bought, bartered, gathered, donated, or cultivated) that the specific items were obtained by the households.

JUSTIFICATION, RATIONALE AND SIGNIFICANCE:

That vitamin A deficiency is a major public health problem has been confirmed in several contexts. The original was its role in nutritional blindness. Its contemporary context now includes the reduction in infantile and juvenile infectious morbidity and mortality. Less needs to be studied about the problem of human hypovitaminosis A than the search for rational, safe, effective and sustainable solutions.

In the proposed package, we combine populations of a country with both a history of chronic, endemic hypovitaminosis A in which reliable information on patterns of beta-carotene intake are available, with an international team experienced in developing community-level delivery of food-interventions, with researchers with a track record in the evaluation of nutritional status, dietary intake, nutritional anthropology, biological availability and food analysis related to vitamin A, as well as a background in intestinal parasite research. This is a key constellation of force.

There are many levels of significance to the proposed project. From the point of view of the nutrition, it is likely that direct benefits will nutritional and health benefits will accrue to the participants in the participating communities. From the point of useful scientific knowledge, we shall evaluate the biological impact of dietary vitamin A sources and the modification of biological availability/ retention by parasitic infestations. From institutional and disciplinary perspectives, the proposal will further strengthen the ties between the US PVO and the Guatemalan service and research institutions, as well as contributing new resources for each of the triad to do their specific tasks. A larger disciplinary implication includes 1) application of behavioral studies to the solution of vitamin A problems in developing countries and 2) the stimulation of increased efforts at the development of high-beta-carotene varieties. Under the best outcome scenario, far-ranging policy implications. It could lead to decreased the reliance on dependency-creating -- and even potentially hazardous -- intervention programs that involve chemical forms of the preformed vitamin A such as the retinyl palmitate that is applied to sugar as a fortificant and is packaged in 200,000 IU capsules. A viable alternative course, using intrinsically safe provitamin A, would allow community-level focus and community-level control of vitamin A supplementation activities.

OBJECTIVES

ANALYZE THE BETA-CAROTENE AND OTHER CAROTENE CONTENT OF INDIGENOUS AND DOMESTIC PLANTS AVAILABLE TO RURAL FAMILIES.

DETERMINE THE DIFFERENTIAL EFFICIENCY OF A THE BETA-CAROTENE IN A GREEN LEAFY INDIGENOUS PLANT VERSUS CONVENTIONAL CARROTS TO CORRECT LOW RETINOL LEVELS AND INCREASE CIRCULATING BETA-CAROTENE LEVELS.

ASSESS THE DIFFERENTIAL EFFICIENCY ON A GRAM-OF-CARROT-CONSUMED BASIS OF CONVENTIONAL CARROTS AND HIGH-CAROTENE VARIETIES TO CORRECT LOW RETINOL LEVELS AND INCREASE CIRCULATING BETA-CAROTENE LEVELS.

ASSESS THE ACCEPTABILITY OF AN INCREASED HOUSEHOLD AVAILABILITY OF CAROTENE-CONTAINING PLANTS, THEIR DEGREE OF ACTUAL CONSUMPTION, AND ANY IMPACT ON THE PATTERN OF USE OF OTHER FOODS.

HYPOTHESES

THAT THE APPARENT ACCEPTANCE OF CARROTS WILL BE EQUIVALENT TO THAT OF THE EDIBLE GREEN PLANTS.

THAT, ON A BASIS OF GRAMS PLANT CONSUMED, THE RISE IN BETA-CAROTENE AND REDUCTION IN LOW RETINOL LEVELS WILL BE GREATER FROM THE CONVENTIONAL CARROT THAN FROM THE GREEN LEAFY HERB.

THAT THERE WILL BE NO DIFFERENTIAL ACCEPTANCE OF CARROTS BASED ON THEIR PIGMENT CONTENT, THAT IS THE ORDINARY CARROT AND THE BETA-III CARROT WILL HAVE EQUAL RATES OF ACCEPTANCE AND CONSUMPTION.

THAT, ON A BASIS OF GRAMS CARROT CONSUMED, THE RISE IN BETA-CAROTENE AND REDUCTION IN LOW RETINOL LEVELS WILL BE GREATER FROM THE BETA-III CARROT THAN FROM THE ORDINARY VARIETY.

THAT THE REGULAR AND GRATIS PROVISION OF CARROTS OR EDIBLE GREENS WILL ALTER THE PATTERN OF CONSUMPTION OF FOODS BY THE MEMBERS OF THE HOUSEHOLDS AT A COMPARABLE PERIOD OF THE YEAR.

PROCEDURES

INTERVENTION DESIGN

The principal intervention is the distribution at the community level to individual enrolled households of the assigned edible plant being in one village an edible green, in another village, ordinary carrots, and in a final village beta-III carrots. The primary agents of this intervention will be the promoters.

In each of the selected communities, the households will be interviewed by both the promoters and a professional from the investigative side, and the nature, purpose, obligations of participation and the benefits, inconveniences, discomforts and risks of the intervention and the monitoring of biological effects will be explained.

The households to participate in each community will be numbered, the age-gender composition of each household member will be documented. A per capita assignment of estimated vitamin A activity (in terms of beta-carotene only) will be given, in which 1000 IU (6000 ug) of beta-carotene-derived vitamin A from the edible green plant; 1000 IU of beta-carotene-derived vitamin A from ordinary carrots (58 g), and approximately 3500 IU from beta-III carrot (58 g) will be given for each person over two years of age. This is equivalent to 100 RE or 40% of the young preschool recommended daily intake of the WHO (19) for each of the first two treatments, and to 350 RE or 140% of the daily intake for the beta-III carrot, using current estimates from our own assays or from that published in food tables or scientific articles. The amount of the edible green quilete to be consumed daily would be 116 grams, or 812 grams per person per week. The amount of the the ordinary carrot to be consumed daily would be 58 grams, or 406 grams per person per week. The amount of the beta-III carrot to be consumed daily would be the same 406 grams.

Note: Direct analysis of the carotenoid pattern of the edible green plant, of the conventional carrot, and of the Beta-III supercarrot as grown in Guatemala by the prospective sources, will be undertaken in the Laboratory of Prof. Louise Canfield at the Department of Biochemistry of the University of Arizona in Tucson.

A principal wholesale vendor (and a back-up vendor) will be identified for the edible plant and for the ordinary carrot. The supercarrot will be cultivated in an farm, either at the National Committee's Training Farm for the Rural Blind (ideally) or from a private contractor. The total weekly volume of the edible plants needed will be established based on the number of enrolled participants in each of the hamlets. Based on 500 persons (max) per hamlet, this would be 450 lbs of carrot or super-carrot, and 900 lbs of quilete per hamlet each week.

The promoters will receive the purchases of vendors/producers once per week. The family allowance will be weighed out by the promoters intake once weekly on the first or second day after the hamlet's market day into numbered (by household) and color-coded (by hamlet) bags. The household-allotments will be delivered to a distribution point within each village where the enrolled families can receive their allocation. A record of the receipt will be kept. Unclaimed packages will be returned to the promoters on the following day.

Deparasitization: At the conclusion of the study, stools will be examined microscopically diagnosis of pathogenic protozoa and helminths. Based on the results, the persons who have stools positive for *Giardia lamblia* or *Entamoeba histolytica* will be given a course of metronidazole. Persons positive for a one of the roundworm species (*Ascaris lumbricoides*, *Trichuris trichiura*, *Enterobius* and hookworm) will be provided with a three-day course of mebendazole.

MONITORING OF DIETARY INTAKE

For reasons both of resource constraints and essential public health significance, the monitoring of dietary intake (and of biological impact, below) will be focused on the children from 1 to 6 years of age.

Food-Pattern Estimates: The pattern of intake of specific members of the family -- with focus on the preschool child -- will be studied using a pictorial instrument similar to the that devised by Licda. Carolina Galindo for use in San Pedro Yepocapa. After a preliminary survey of 24 h intakes, a series of pictures will be developed which represent commonly used foods in the community, a final instrument will be devised. At the beginning and the end of the study, each participating household will have the instrument passed to determine whether some food has been displaced by the passage of time/introduction of the intervention food. This will be administered by research physicians and nutritionists from CeSSIAM.

Food-Frequency Estimates: For a more quantitative estimate of intake, specifically related to vitamin A-containing foods (and table sugar or sweetened foods), in order to assign a "usual" vitamin A intake estimate, the intake of preschool children and of the female head of household, will be estimated using a 7-day time-frame. An adaptation of the food-frequency instrument developed by Licda. Julieta Quande Serrano will be used (16). The seven-day, vitamin A-focused food-frequency instrument will be administered for the appropriate members of the enrolled household at the beginning (baseline) and in the final month (follow-up) of the study, in the same climatic season of the year. This will be administered by research physicians and nutritionists from CeSSIAM.

Monitoring of acceptance of the intervention foods: This will be among the routine processes, and will be conducted by the field promoter personnel. It will register the frequency of times (weeks/year) in which the enrolled family picked up, or otherwise received their weekly allotment of the community intervention.

INVESTIGATION OF BIOLOGICAL VARIABLES

Plasma sampling for retinol and beta-carotene: During the month prior to the initiation of the intervention, all of the subjects aged 1 year to 5 years of age in the participating households will undergo extraction of 5 ml of whole blood. This will be anticoagulated with heparin, and centrifuged. This is estimated to be about 80 to 100 preschoolers per hamlet, and will generate a total of 300 tubes of plasma. The plasma will be separated, protected from light, and stored frozen in screw-top vials (Cryotubes) first at -20°C in Guatemala, then at -70°C in Arizona, for about 12 months time. During the twelve month of the intervention, and in the same calendar month of the original sampling, the same cohort of preschool children, now aged 2 to 6 years, will again undergo extraction and processing of blood for plasma assay to generate another 300 samples.

The plasma samples will be transported to the laboratory of Dr. Louise Canfield in the Department of Biochemistry of the University of Arizona in Tucson. The plasma will be assayed for its concentration of retinol and beta-carotene using a modification of the method of Peng and Beaudry (20) which is a high precision liquid chromatography method which involves protection of the sample from oxidation with EDTA/ascorbic acid mixture the extraction with ethylacetate:THF (1:1 v/v) and injection of a 50 uL volume into the HPLC column. Beta-apo-8 carotenol is used as the internal standard.

Note: Hematocrit determinations will be made using heparinized capillary tubes and a microhematocrit centrifuge. The families will be given immediate feedback with regard to the existence or not of anemia, as defined by a hematocrit of <37%.

DATA ANALYSIS:

Data on household participation in the supplementation will be reduced to expressions of estimated per capita intake of the foods. Conversion to the per capita intake of supplemental beta-carotene will be done, based on food analyses of the distributed foods. Descriptive statistics by population, and disaggregated by age-group, will be computed in terms of mean, median, range, for circulating ROH, circulating BC, parasite intensity. Changes in dietary intake of vitamin A from both total and from non-intervention foods from baseline to follow-up will be calculated as will changes in the pattern of food use through the same interval.

Interactions between the change/stability of food pattern for the same season of the year with 1) type of food (green, carrot, supercarrot) and 2) with within-community, level of household participation will be assessed.

Change in plasma ROH and BC as a function of 1) type of food (green, carrot, supercarrot) and 2) within-community, level of household participation with change in pattern will be assessed alone, and adjusted for parasite infection level and other variables such as age and sex.

We shall look at parasites status and participation in the intervention as a possible index of parasitosis-induced anorexia. Initial ROH and BC status will be examined as a function of initial parasite status.

For within-subject interactions, bivariate and multivariate regression analyses will be performed with appropriate transformation of the data when distributions of variables are not normal within populations. For contrasts across the three communities with their respective food intervention, appropriate parametric (ANOVA) and non-parametric techniques (Chi-squared, Fisher exact test of proportions) will be used as appropriate to the nature of the distribution (normal, skewed) and of the variable (discrete, continuous).

LIMITATIONS OF THE PROJECT

The experience that the collaborating entities in this proposal have garnered in field work over the past five years allows us to create the design and strategies. The same familiarity with the realities of the communities and the biochemical technology allows us to recognize the limitations of the project.

The feasibility of mounting the elements in the communities is a consideration. Political unrest or community rejection could impede the access to or the continuance of the project. The Santa Rosa hamlets around Santa Cruz Naranjo have already expressed interest in receiving some of the benefits of field activities. The area is a relatively short drive (60 min) from the capital city. The proposed project does not include an indigenous culture for comparative purposes. We do know that intakes are almost as low in A.V., but the budget for logistics to two zones did not permit.

The model of gratis distribution of the food item is not a model for a reasonable public health intervention. In the real world, subsidies or home-garden promotion would be the models. However, dependency of the biological revelations on a relative efficiency of the intervention component requires that we see that the households get the carotene-containing foods into their larder.

Even thus, we cannot sample all of the population, but have concentrated on the under-six-year-old preschoolers for the primary biological-impact data. Large number of subjects are needed to generate a large volume of data, but costs preclude more than 600 plasma retinol and beta-carotene analyses.

The ethical considerations have dictated the timing of the analysis of both the plasma samples and the parasite status. The sample of plasma from both sampling-periods will be analyzed in the latter phases to obviate the imperative to respond therapeutically to the knowledge of the vitamin A status. At the conclusion of the study, individuals with "low" or "deficient" ROH levels will be given a therapeutic treatment with retinyl palmitate in supplement form. Similarly, since we plan to treat individuals found to have parasites, this assessment will be reserved until the end of the study.

Even with the successful execution of the intervention and the collection of the data, there are limitations in the inferential conclusions that one can gather. The communities may have intrinsic differences of non-comparability that make strick comparisons invalid. The within-individual variability of a number of the variables is relatively large. This is known for vitamin A intake (21), and studies in CeSSIAM (22) show that day-to-day excretion of parasite eggs is variable. The power of correlation analysis is dependent on the intraindividual stability (precision) of the variable (23). Also assumptions about the steady-state nature of parasites within an individual for correlation of what one finds at the conclusion of a year with what was present 12-months earlier. Also, the consequences of intraindividual variability of egg counts (22), correlation analysis using helminth intensity as a variable suffers a relative lack of within-individual precision.

BASIC CALENDAR OF EVENTS

FIRST PHASE (SIX MONTHS)

SURVEY OF PLANT CONTENT

CULTIVATION OF SUPERCARROTS

PUT MANAGEMENT INFORMATION SYSTEM IN PLACE

APPROACH COMMUNITIES TO ENROLL THEIR PARTICIPATION

BASELINE FOOD-FREQUENCY PATTERN

BASELINE PLASMA SAMPLING

FIRST EXTERNAL EVALUATION OF PROJECT

SECOND PHASE (THIRTEEN MONTHS)

**ESTABLISH DISTRIBUTION THREE HAMLETS IN SANTA ROSA RANDOMLY
ASSIGNED TO RECEIVE EDIBLE GREENS, CONVENTIONAL CARROTS AND THE
BETA-III "SUPERCARROT" AS THE FOOD INTERVENTION**

**MONITORING OF ACCEPTANCE AND CONSUMPTION OF DISTRIBUTED ITEM
WITHIN HOUSEHOLDS**

12-MONTH REPEAT PLASMA SAMPLING

12-MONTH REPEAT FOOD-FREQUENCY PATTERN

**SURVEY OF THE INTESTINAL PARASITE STATUS OF THE PARTICIPATING
HOUSEHOLDS**

NOTE: NUMBER OF INDIVIDUALS BENEFITTED 1500 IN 300 HOUSEHOLDS

THIRD PHASE (FIVE MONTHS)

ANALYSIS OF BLOOD SAMPLES

CONTINUE PARASITE SURVEY

PROVIDE ANTI-HELMINTHIC TREATMENT TO THE INFECTED POPULATION

ANALYSIS OF DATA

FINAL EXTERNAL EVALUATION OF PROJECT

PREPARATION OF FINAL REPORT

CONCEPT OF DISTRIBUTION OF RESPONSIBILITY

THE IEF/NCDB STRUCTURE IN GUATEMALA ORGANIZES THE INTERVENTION.

- A. SELECTION AND ENROLLMENT OF COMMUNITIES
- B. PROCUREMENT OF PLANTS
- C. DISTRIBUTION OF PLANTS

THE CeSSIAM WOULD BE RESPONSIBLE FOR THE VARIABLES

- A. BETA-CAROTENE CONTENT OF PLANTS
- B. BETA-CAROTENE (AND RETINOL) STATUS OF THE POPULATIONS
- C. BEHAVIORAL RESPONSE OF INTRAHOUSEHOLD CONSUMPTION
- D. PARASITE SURVEY OF THE POPULATION

THE IEF/NCDB/CeSSIAM WOULD ALL BE INVOLVED IN THE DATA ANALYSIS AND REPORTING

AN EXTERNAL ADVISOR, FAMILIAR WITH THE BIOLOGY OF VITAMIN A AND WITH EXPERIMENTAL DESIGN AND DATA ANALYSIS FROM FIELD STUDIES WOULD BE CONTRACTED TO PERFORM AN EXTERNAL EVALUATION IN THE FIRST AND THE THIRD PHASES OF THE STUDY.

STAFF CONSIDERATIONS

INTERNATIONAL EYE FOUNDATION, BETHESDA

Jack Blanks

John Barrows, MPH

NATIONAL COMMITTEE/Guatemalan INTERNATIONAL EYE FOUNDATION

Guastavo Hernandez-Polanco, M.D.

William Scott

Eugenia Saenz de Tejada

CENTER FOR STUDIES OF SENSORY IMPAIRMENT, AGING AND METABOLISM

Noel W. Solomons, M.D.

Jesus Bulux, M.D.

Carmen Yolanda Lopez, M.D.

Lic Guisela Leche

UNIVERSITY OF ARIZONA

Louise Canfield, Ph.D.

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