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U.S. AGENCY FOR  
INTERNATIONAL  
DEVELOPMENT

SEP 30 1992

Mr. Stanette Kennebrew  
Vice President for Administration  
Charles R. Drew University of  
Medicine and Science  
1621 E. 120th Street  
Los Angeles, CA 90059

Subject: Grant No. PCE-5053-G-00-2029-00

Dear Mr. Kennebrew:

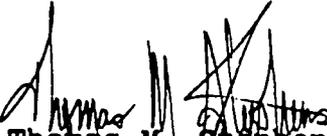
Pursuant to the authority contained in the Foreign Assistance Act of 1961 and the Federal Grant and Cooperative Agreement Act of 1982, as amended, the Agency for International Development (hereinafter referred to as "A.I.D.") hereby provides to Charles R. Drew University of Medicine and Science (hereinafter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

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

This Grant is made to the Grantee 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 Grant document and have been agreed to by your organization.

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

Sincerely yours,

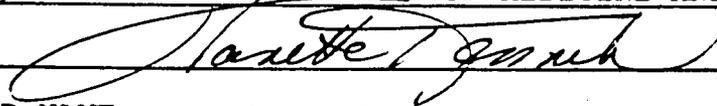
  
Thomas M. Stephens  
Grant Officer  
Chief, OP/B/PCE  
Office of Procurement

Attachments:

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

ACKNOWLEDGED:

CHARLES R. DREW UNIVERSITY OF MEDICINE AND SCIENCE

BY: 

TYPED NAME: Stanette Kennebrew, M.B.A., J.D.

TITLE: Vice President and Chief Financial Officer

DATE: October 14, 1992

FISCAL DATA

A. GENERAL

- A.1. Total Estimated A.I.D. Amount: \$99,988
- A.2. Total Obligated A.I.D. Amount: \$99,988
- A.3. Cost-Sharing Amount (Non-Federal): \$ - 0 -
- A.4. Other Contributions (Federal): \$15,802
- A.5. Project No.: 936-5053
- A.6. A.I.D. Project Office: R&D/UC, Brij Shrivastav
- A.7. Funding Source: A.I.D./W
- A.8. Tax I.D. No.: 95-6151-774
- A.9. CEC No.: 79-553-075H
- A.10. LOC No.: 72-00-1570

B. SPECIFIC

- B.1.(a) PIO/T No.: 936-5053-2691639
- B.1.(b) Appropriation: 72-1121021.8
- B.1.(c) Allotment: 248-36-099-00-20-21
- B.1.(d) BPC: DDHA-92-16900-KG11
- B.1.(e) Amount: \$100,000

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SCHEDULE

1A. PURPOSE OF GRANT

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

1B. PERIOD OF GRANT

The effective date of this Grant is the date of the Cover Letter and the estimated completion date is September 29, 1994. Funds obligated hereunder (see Section 1C.2. below) shall be used to reimburse the Grantee for allowable program expenditures incurred by the Grantee in pursuit of program objectives during such period. Funds obligated hereunder are anticipated to be sufficient for completion by the Grantee of the program described in Attachment 2 of this Grant by the estimated completion date.

1C. AMOUNT OF GRANT AND PAYMENT

1C.1. The total estimated amount of this Grant for its full period, as set forth in Section 1B. above, is \$99,988.

1C.2. A.I.D. hereby obligates the amount of \$99,988 for the purposes of this Grant 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 Grantee for any costs in excess of the obligated amount, except as specified in paragraph (f) of the Standard Provision of this Grant entitled "Revision of Grant Budget."

1C.3. Payment shall be made to the Grantee in accordance with procedures set forth in the Standard Provision of this Grant 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 Grant is \$118,790, of which A.I.D. may provide the amount specified in Section 1C.1. above, and the Grantee will provide \$ 9,000 in accordance with Section 1L. below.

1D. GRANT BUDGET

1D.1. The following is the Budget for the total estimated amount of this Grant (see Section 1C.1. above) for its full period (see Section 1B. above). The Grantee may not exceed the total estimated amount or the obligated amount of this Grant,

whichever is less (see Sections 1C.1. and 1C.2., respectively, above). Except as specified in the Standard Provision of this Grant entitled "Revision of Grant Budget," as shown in Attachment 3, the Grantee 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>Grantee/ Others (Non-Fed)</u>	<u>Grantee/ Others (Federal)*</u>	<u>Total</u>
Salaries	\$ 25,211	\$ 6,000	\$ 9,802	\$ 41,013
Fringe Benefits	\$ 6,655	\$ 0	\$ 0	\$ 6,655
Consultants	\$ 13,250	\$ 0	\$ 0	\$ 13,250
Travel	\$ 29,696	\$ 0	\$ 0	\$ 29,696
Equipment	\$ 2,000	\$ 0	\$ 0	\$ 2,000
Other Direct Costs	\$ 9,310	\$ 0	\$ 0	\$ 9,310
Site Rental	\$ 0	\$ 3,000	\$ 0	\$ 3,000
Indir. Costs (G&A)	\$ 13,866	\$ 0	\$ 0	\$ 13,866
Total	\$ 99,988	\$ 9,000	\$ 9,802	\$ 118,790

\*Note: Not subject to Cost Sharing Provision.

1D.3. Inclusion of any cost in the budget of this Grant does not obviate the requirement for prior approval by the Grant Officer of cost items designated as requiring prior approval by the applicable cost principles (see the Standard Provision of this Grant set forth in Attachment 3 entitled "Allowable Costs") and other terms and conditions of this Grant, 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 Grant 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, FA/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 Grant, 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 Grant referred to in Section 1E.1.(a) above.

1E.1.(d) The Grantee's financial reports shall include expenditures of A.I.D. Grant funds provided hereunder, as well as non-federal matching funds and any other contributions 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 Grant (see Section 1B. above), the Grantee shall prepare and submit to the A.I.D. Project Officer specified in the Cover Letter of this Grant five (5) copies of a project implementation plan, with critical path indicators (as described in Appendix A of A.I.D. Handbook 3), for the full term of this Grant.

1E.2.(b) Quarterly Reports

The Grantee 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 Grant. In addition, two copies shall be submitted to A.I.D., POL/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.(b)(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.(b)(2) Reasons why established goals were not met, if applicable.

1E.2.(b)(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.(c) Special Reports

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

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

1E.2.(d) Annual Activity Reports

Within thirty (30) days following the annual anniversary date of this Grant, the Grantee shall submit to the A.I.D. Project Office specified in the cover letter of this Grant 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 Grant. 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 Grantee'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.(e) Technical and Research Reports and Publications

The Grantee 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 Grant pertaining to publications.

1E.2.(f) Environmental Impact

If it appears that outputs of this project will result in an adverse environmental impact, the Grantee shall notify the A.I.D. Project Officer prior to implementation, in order to allow for orderly preparation of an environmental impact statement. The Grantee shall assure that appropriate U.S. Government and/or host country procedures are followed.

1E.2.(g) Final Report

Within 90 days following the estimated completion date of this Grant (see Section 1B. above), the Grantee shall submit five (5) copies of a final report to the A.I.D. Project Office specified in the cover letter of this Grant. In addition, two copies shall be submitted to A.I.D., POL/CDIE/DI, Washington, DC 20523-1802. It will cover the entire period of the Grant and include all information shown in Sections 1E.2.(b) through 1E.2.(f) above.

1E.2.(h) Protection of the Individual As A Research Subject

If the Standard Provision entitled "Protection of the Individual as a Research Subject" applies to this Grant (see section 1K. for applicability), the Grantee should be subject to review of all research involving human subjects as stated in Section (c) of said Standard Provision.

1F. TITLE TO PROPERTY

Title to property acquired hereunder shall vest in the Grantee, subject to the requirements of the Standard Provision of this Grant 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 1I. below.

1G. PROCUREMENT AND (SUB) CONTRACTING

1G.1. Applicability

This Section 1G. applies to the procurement of goods and services by the Grantee (i.e., contracts, purchase orders, etc.) from a supplier of goods and services (see the Standard Provisions of this Grant entitled "Procurement of Goods and

Services" and "AID Eligibility Rules for Goods and Services"), and not to assistance provided by the Grantee (i.e., a subgrant or [sub]agreement) to a subrecipient (see the Standard Provision of this Grant entitled "Subagreements").

#### 1G.2. Requirements

In addition to other applicable provisions of this Grant, the Grantee shall comply with paragraph (b)(1) of the Standard Provision of this Grant entitled "AID Eligibility Rules for Goods and Services," concerning total procurement value of less than \$250,000 under this Grant. If, under the order of preference set forth in paragraph (b)(1)(i) of said Standard Provision, the Grantee procures goods or services from cooperating country sources, the Standard Provision of this Grant 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 Grant 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 Grant 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 Grant Officer, nor any other terms and conditions of this Grant, unless specifically stated in Section 1I. below.

#### 1G.4. Title to Property

See Section 1F. above.

#### 1H. INDIRECT COST RATES

1H.1. Pursuant to the Standard Provisions of this Grant entitled "Negotiated Indirect Cost Rates - Predetermined" and "Negotiated Indirect Costs Rates - Provisional," a predetermined indirect cost rate or rates shall be established for each of the Grantee's accounting periods which apply to this Grant.

Pending establishment of predetermined indirect cost rates for the initial period (07/01/92 - 06/30/95), 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 (are) set forth below:

<u>Type</u>	<u>Rate</u>	<u>Base</u>
Provisional On-Campus	55.0%	1/

1/ Base of Application: Direct salaries and wages including vacation, holiday and sick pay but excluding other fringe benefits.

1H.2. Rates for subsequent periods shall be established in accordance with the Standard Provision of this Grant entitled "Negotiated Indirect Cost Rates - Predetermined."

## 1I. SPECIAL PROVISIONS

### 1I.1. Limitations on Reimbursement of Costs of Compensation for Personal Services and Professional Service Costs

#### 1I.1.(a) Employee Salaries

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

#### 1I.1.(b) Consultant Fees

Compensation for consultants retained by the Grantee hereunder shall not exceed, without specific approval of the rate by the Grant 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.

### 1I.3. Publications

1I.3.(a) The Grantee agrees to provide one copy 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.

1I.3.(b) In the case of publication of any of the reports described in Section 1E.2. of this Grant, 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 Grantee 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.

1I.3.(c) If A.I.D. does not choose to disclaim endorsement or dissociate itself from sponsorship or publication, the Grantee shall, in accordance with the Standard Provision of this Grant entitled "Publications," acknowledge A.I.D. support as follows:

"This publication was made possible through support provided by the University Center, Bureau for Research and Development, U.S. Agency for International Development, under Grant No. PCE-5053-G-00-2029-00."

1I.3.(d) In addition to providing one copy of all published works and lists of other written work produced under this Grant to the A.I.D. Project Officer, as required by paragraph (b) of the Standard Provision of this Grant entitled "Publications," the Grantee shall also provide two copies of such publications and lists to A.I.D., POL/CDIE/DI, Washington, D.C. 20523-1802.

#### 1I.4. Equipment and Other Capital Expenditures

##### 1I.4.(a) Requirement for Prior Approval

Pursuant to Sections 1D.3. and 1G.3. above and the Standard Provisions of this Grant entitled "Allowable Costs" and "Revision of Grant Budget," and by extension, Section J.13. of OMB Circular A-21, the Grantee must obtain A.I.D. Grant Officer approval for the following:

1I.4.(a)(1) Purchase of General Purpose Equipment, which is defined as an article of nonexpendable tangible personal property, the use of which is not limited only to research, medical, scientific, or other activities [e.g., office equipment and furnishings, air conditioning equipment, reproduction and other 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);

1I.4.(a)(2) Purchase of Special Purpose Equipment, which is defined as an article of nonexpendable tangible personal property, which is used only for research, medical, scientific, or other technical activities, and which has a useful life of more than two years and an acquisition cost of \$1,000 or more per unit); and

1I.4.(a)(3) Other Capital Expenditures, which is defined as the cost of the asset, including the cost to put it in place).

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1I.4.(b) Approvals

In furtherance of the foregoing, the Grant 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 Grant, whichever is less (see Section 1C. above):

one computer

1I.4.(c) Exception for Automation Equipment

Any approval for the purchase of automation equipment which may be provided in Section 1I.4.(b) above or subsequently provided by the Grant 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 Grantee must, under such circumstances, obtain the approval of the Grant Officer for the total planned system of any automation equipment, software, or related services.

1I.4.(d) Compliance with A.I.D. Eligibility Rules

Any approvals provided in Section 1I.4.(b) above or subsequently provided by the Grant Officer shall not serve to waive the A.I.D. eligibility rules described in Section 1G. of this Grant, unless specifically stated.

1I.5. Restricted Goods

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

1I.5.(a) Agricultural Commodities

Agricultural commodities may be purchased provided that they are of U.S. source (generally, the country from which the commodities are shipped) and origin (generally, the country in which the commodities are mined, grown, or produced) and

purchased from a U.S. supplier, except that wheat, rice, corn, soybeans, sorghums, flour, meal, beans, peas, tobacco, hides and skins, cotton, vegetable oils, and animal fats and oils cannot be purchased under any circumstances without the prior written approval of the Grant Officer.

#### 11.5.(b) Motor Vehicles

Motor vehicles, if approved for purchase under Section 11.4.(b) above or subsequently approved by the Grant Officer, must be of U.S. manufacture and must be of at least 51% U.S. componentry. The source 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.(c) 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 Grantee and the Standard Provision of this Grant entitled "Procurement of Goods and Services."

#### 11.5.(d) Pesticides

Pesticides may only be purchased if the purchase and/or use of such pesticides is for research or limited field evaluation by or under the supervision of project personnel. Pesticides are defined as substances or mixtures of substances: intended for preventing, destroying, repelling, or mitigating any unwanted insects, rodents, nematodes, fungi, weeds, and other forms of plant or animal life or viruses, bacteria, or other micro-organisms (except viruses, bacteria, or other micro-organisms on or living in man or other living animals); or intended for use as a plant regulator, defoliant, or dessicant.

1I.5.(e) Rubber Compounding Chemicals and Plasticizers

Rubber compounding chemicals and plasticizers may only be purchased with the prior written approval of the Grant Officer.

1I.5.(f) Used Equipment

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

1I.5.(g) Fertilizer

Fertilizer may be purchased if it is either purchased in the U.S. and used in the U.S., or if it is purchased in the cooperating country with local currency for use in the cooperating country. Any fertilizer purchases which do not comply with these limitations must be approved in advance by the Grant Officer.

1I.6. Limitation on Use of Funds

1I.6.(a) The Grantee shall not utilize funds provided by A.I.D. for any testing or breeding feasibility study, variety improvement or introduction, consultancy, publication, conference or training in connection with the growth or production in countries other than the United States of an agricultural commodity for export which would compete with a similar commodity grown or produced in the United States.

1I.6.(b) The reports described in Section 1E.2. shall contain a statement indicating the projects or activities to which United States funds have been attributed, together with a brief description of the activities adequate to show that United States funds have not been used for the purpose in Section 1I.6.(a) above.

1I.6.(c) The Grantee agrees to refund to A.I.D. upon request an amount equal to any United States funds used for the purposes prohibited by Section 1I.6.(a) above.

1I.6.(d) No funds provided by A.I.D. under this Grant shall be used to provide assistance, either directly or indirectly, to any country ineligible to receive assistance pursuant to the Foreign Assistance Act as amended, related appropriations acts, or other statutes and Executive Orders of the United States (also see the Standard Provision of this Grant entitled "Ineligible Countries").

1I.7. Disposition of Property

With reference to Sections 1G.4. and 1I.4.(b) above, disposition of nonexpendable property acquired hereunder shall be as follows:

To be donated to host country institution.

**1J. RESOLUTION OF CONFLICTS**

Conflicts between any of the Attachments of this Grant 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

**1K. STANDARD PROVISIONS**

The Standard Provisions set forth as Attachment 3 of this Grant consist of the following Standard Provisions denoted by an "X" which are attached hereto and made a part of this Grant:

**1K.1. Mandatory Standard Provisions For U.S.,  
Nongovernmental Grantees**

- ( X ) Allowable Costs (November 1985)
- ( X ) Accounting, Audit, and Records (September 1990)
- ( X ) Refunds (September 1990)
- ( X ) Revision of Grant Budget (November 1985)
- ( X ) Termination and Suspension (May 1986)
- ( X ) Disputes (November 1989)
- ( X ) Ineligible Countries (May 1986)
- ( X ) Debarment, Suspension, and Other Responsibility Matters (March 1989)
- ( X ) Nondiscrimination (May 1986)
- ( X ) U.S. Officials Not to Benefit (November 1985)
- ( X ) Nonliability (November 1985)
- ( X ) Amendment (November 1985)
- ( X ) Notices (November 1985)

**1K.2. Additional Standard Provisions For U.S.,  
Nongovernmental Grantees**

- ( X ) Payment - Letter of Credit (November 1985)
- ( ) Payment - Periodic Advance (January 1988)
- ( ) Payment - Cost Reimbursement (November 1985)
- ( X ) Air Travel and Transportation (November 1985)
- ( X ) Ocean Shipment of Goods (May 1986)
- ( X ) Procurement of Goods and Services (November 1985)
- ( X ) AID Eligibility Rules for Goods and Services (November 1985)
- ( X ) Subagreements (November 1985)
- ( X ) Local Cost Financing (November 1988)
- ( X ) Patent Rights (November 1985)
- ( X ) Publications (November 1985)
- ( X ) Negotiated Indirect Cost Rates - Predetermined (May 1986)
- ( X ) Negotiated Indirect Cost Rates - Provisional (May 1986)

- ( X ) Regulations Governing Employees (November 1985)
- ( ) Participant Training (May 1986)
- ( ) Voluntary Population Planning (August 1986)
- ( X ) Protection of the Individual as a Research Subject (November 1985)
- ( ) Care of Laboratory Animals (November 1985)
- ( X ) Government Furnished Excess Personal Property (November 1985)
- ( X ) Title To and Use of Property (Grantee Title) (November 1985)
- ( ) Title To and Care of Property (U.S. Government Title) (November 1985)
- ( ) Title To and Care of Property (Cooperating Country Title) (November 1985)
- ( X ) Cost Sharing (Matching) (November 1985)
- ( ) Use of Pouch Facilities (November 1985)
- ( ) Conversion of United States Dollars to Local Currency (November 1985)

**1L. COST SHARING AND OTHER CONTRIBUTIONS**

1L.1. The Grantee agrees to expend an amount not less than (a) the amount shown in the budget of this Grant for financing by the Recipient and/or others from non-federal funds (see Sections 1D. and/or 1H.), and (b) the amount shown in the budget of this Grant for financing by the Recipient and/or others from other federal funds.

1L.2. The Standard Provision of this Grant entitled "Cost Sharing (Matching)" makes reference to project costs. "Project Costs" are defined in Attachment E of OMB Circular A-110 as all allowable costs (as set forth in the applicable cost principles [see the Standard Provision of this Grant entitled "Allowable Costs"]) incurred by a Grantee and the value of in-kind contributions made by the Grantee or third parties in accomplishing the objectives of this Grant during the program period.

1L.3. The restrictions on the use of A.I.D. funds provided hereunder, as set forth in this Grant, do not apply to cost-sharing (matching) or other contributions unless such restrictions are stated in the applicable federal cost principles and/or imposed by the source of such cost-sharing (matching) funds or other contributions.

PROGRAM DESCRIPTION

The Grantee's proposal entitled "Hypertension in Dominica: Training Detection and Understanding " and dated October 1, 1991 (Principal Investigator: Clarence E. Grim, MD) is attached hereto as the Program Description (Attachment 2) and is made a part of this Grant.

**"HYPERTENSION IN DOMINICA: TRAINING, DETECTION, AND UNDERSTANDING"****A1. SUMMARY****ABSTRACT**

The long term goal of this research team is to eliminate hypertension, the leading cause of death and disability in adults and the aging population in all nations of sub-Saharan African descent. The immediate goal of this project is to understand the present day epidemiology of hypertension in Dominica so that, in the future, preventative and/or therapeutic strategies can be developed, tested and applied to Dominica. This goal will be met by carrying out the following specific aims:

- 1) Estimate the prevalence of hypertension through a population-based random sample.
- 2) Evaluate the association between salt intake and blood pressure.
- 3) Utilize the power of the study of twins to understand the role of heredity *(and environment)* on the variation in blood pressure, *the first study to examine genes in a representative sample of blacks living in a less developed country.*

In a 1988 issue of WHO Statistical Quarterly it was predicted that high blood pressure, or hypertension, will soon be the major cause of morbidity and mortality in less developed countries (LDCs) (1,2). WHO strongly suggests that the best way to stem the costly epidemic of hypertension in LDCs is to prevent the disease from developing in the first place. The best way to prevent is to understand risk factors associated with hypertension.

Today, hypertension is already the major underlying cause of morbidity and mortality in blacks in the Western hemisphere, far surpassing the morbidity and mortality from hypertension in other ethnic groups. Unfortunately we are not certain why hypertension disproportionately affects black populations in the Western hemisphere, but, there is some evidence that salt intake and genetics play major roles. This project will use the power of the study of twins to understand the heritability of blood pressure variability in Dominica. Dominica is an island-country in the West Indies with a predominantly black population of about 80,000. In a hospital admission survey in 1981, 55% of Dominicans over the age of 35 had hypertension! And in 1987 it is estimated that underlying hypertension was responsible for up to 61% of the total deaths in the country.

To accomplish this twin study we will use local resources to establish a population-based registry of twins, select a random sample of twins from this registry, selected a age/sex stratified random sample of singletons from this registry, evaluate the genetics of blood pressure and salt intake while using the singleton sample to control for possible confounding variables. As Ward remarked "twins studies represent the ideal strategy for gaining an initial impression" of genetic influences. (3) *Although the twin model has been widely utilized in the developed world, we are not aware of any studies (except our pilot project in Barbados) using this model in less developed countries. This means that most estimates of heritability are based on non-black populations in developed countries. The situation may be much different in the Third World. This proposed study will be the first to examine the important question of heredity/environment in a representative sample of blacks living in a less developed country.* Following the completion of this study we will have laid the foundation for several different study designs: a long term cohort study to estimate the incidence of hypertension and the factors that will predict disease onset, or familial aggregation study. Twins will be especially useful in such a longitudinal study to partition out the role (s) of nature and nurture to the development of illness in Dominica.

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## **B. RELEVANCE OF PROPOSED WORK TO AID**

### **1. Discussion of the Problem and Pertinence of the Proposed Solution**

The project is conceived in the spirit of the historically Black colleges and Universities (HBCU) program in that HBCU investigators are in collaboration with the medical Director of the Princess Margaret Hospital. Dr. Grell, the co-investigator in Dominica, also has written extensively in the hypertension field in the West Indies. This proposal is submitted to the USAID research program for HBCU under the main program area, "health" and the sub-topic, "epidemiology, and the integration in primary health care services. of the control of non-infectious diseases, especially those related to the aging population of developing countries (4). If successful in Dominica, this program could be easily transportable to other LDCs

The proposal is in-line with the primary "mission" of the USAID--"child survival"--in that it will also help understand the contribution of maternal chronic hypertension to maternal and child health as well as to the etiology of pregnancy-induced hypertension both of which are the major reasons mothers die during pregnancy and both of which are the major contributors to small birth weight infants, which is more common among blacks than other ethnic groups (5). As small birth weight babies are the most likely to die, hypertension, whether chronic or pregnancy induced, is a major contributor the high perinatal mortality in black populations.

### **2. Collaboration of developing country personnel or Institutions**

1. Dr. Gerald Grell, Director of the Princess Margaret Hospital, Dominica.
2. *Dr. Clarissa Etienne, director of the Dominica Primary Health Care Service.*
3. A research associate (to be named by Dr. Grell), to research birth records, A laboratory worker (to be named by Dr. Grell) to assist with study, a phlebotomist, and two health care workers..

### **3. Developing country contribution**

Developing country will provide the labor of Dr. Grell, facilities for the study, laboratory facilities, and assistance with publicity for the study.

## **C. SCIENTIFIC ASPECTS OF PROPOSED WORK**

### **1. Statement of Specific Research Question/Hypothesis to be tested**

1. Heritability of blood pressure controlling for age, sex and other variables..
2. Heritability of salt intake controlling for age, sex and other variables.
3. **Heritability of diabetes-related measures controlling for age, sex and other variables.**
4. Estimate the prevalence of high blood pressure in Dominica using a random sample of singletons.

#### **1a. Background to Research Questions and Hypotheses**

##### ***Hypertension***

The World Health Organization recently predicted that hypertension will soon be the major killer throughout the developing world, far surpassing deaths from infectious diseases (5,6).

If a hypertensive case goes undetected the cost to the unborn children, to the family, to society and to the health care resources will be devastating in LDCs as elsewhere. The unborn child suffers from reduced blood flow from the mother who has undiscovered or untreated hypertension, the family suffers by premature death and disability of parents of small children due to stroke, heart attack or kidney failure. Society suffers from the sudden death and disability of the work force and from critical community leaders and health care resources are drained by the costs of preventable morbidity of enormous expense. For example, hypertensive patients with renal failure need to undergo the very expensive process of kidney dialysis. In Barbados the annual cost of disposable items alone is over \$3000/year (6, 7). Patients with uncontrolled hypertension may need hospitalization as the result of a stroke, heart attack, or kidney disease: the average cost of a hospital stay is about \$125/day.

Much of this expensive tertiary treatment can be avoided if hypertensives or those at risk of hypertension are detected and controlled early in their disease. Commenting on the West Indies as a whole, Grell wrote in 1988 that "it is estimated that rigid control of the disease and especially early treatment could reduce the need for these expensive programs by some 50%" (8). Although pharmacological control can be achieved at a relatively low cost (as little as 1¢ US per day for 50% of the patients), WHO argues that even inexpensive drugs may be too expensive for many developing countries. As the demographic pyramid becomes more top heavy, i.e. a greater relative percentage of "elderly" (>60 years age) in the population, the cost to society of hospitalization, renal dialysis and anti-hypertensive drugs will accelerate. It should be noted that the WHO expert committee on Health in the Elderly notes that by the year 2000--the year Alma Ata conference calls for "health care of all"--there will be 600 million elderly in the world, two thirds of them will be in LDCs (9)! In black communities over 2/3 of these will have hypertension if today's trends continue!

Based upon several population based surveys in the West Indies, Dr. Grell estimates that 10-12% of blacks have hypertension. He emphasizes that many of these surveys were not random samples and that Ministries will have an impossible time assessing the health burden of this disease. He recommends population-based random surveys to help the Ministries plan health programs for the future. In Dominica itself, cause-specific mortality rates suggest that hypertension is the major underlying cause of death already. Up to 61% of the total deaths could be attributed to underlying hypertension: these include death from heart disease, kidney disease, cerebrovascular, diabetes, arteriosclerosis, diseases of the perinatal period, and urinary tract diseases (See Table 1).

Table 1 Cause-specific Mortality Rates, Dominica, 1987. Per 100,000

1.	*Heart Disease [Hypertension]	156.70:100,000 [75.95:100,000]
2.	Malignant Neoplasms	88.20:100,000
3.	Respiratory (other)	41.65:100,000
4.	*Cerebrovascular	36.75:100,000
5.	Pneumonia	30.62:100,000
6.	*Diabetes	29.40:100,000
7.	*Conditions of Perinatal period	22.05:100,000
8.	*Diseases of Urinary Tract	14.70:100,000
9.	Transport Accidents	8.75:100,000
10.	*Arteriosclerosis	8.57:100,000

\*Hypertension is a risk factor in these conditions. The conditions comprise about 268:100,000 deaths out of a total of 437.39:100,000, or over 61%.

Source: Annual Report of the Chief Medical Officer of Dominica for 1987

To decrease the overwhelming contribution of hypertension to death in Dominica requires detection and treatment of the disease, or, preferably, the implementation of preventive measures to the total population or to those at risk of developing hypertension. Both actions can benefit for a study which assesses the genetic and environmental contribution to blood pressure variability.

### **Salt Intake**

Since the late 19th century, salt and elevated blood pressure have been linked (10). A massive international study reported in 1988, demonstrated a statistically significant positive relationship between salt intake and blood pressure. Indeed the West Indian site, Trinidad and Tobago, demonstrated a clear cut increase in blood pressure in the population as salt intake increased. Lowering salt intake has been shown to be an effective non-pharmacological treatment in itself. Thus, salt intake may be both a crucial risk factor for the development of hypertension in black LDCs. The strongest evidence for this comes again from the Intersalt study in which those populations that did not consume over 70 mM of sodium a day did not have hypertension and did not get a rise in blood pressure as they aged. One of these was a population in Kenya. This strongly suggests to us that hypertension could be eliminated in those populations of African descent if sodium intake could be reduced to these levels. Obviously this has tremendous implications for countries with limited resources in which it may be possible to decrease the populations blood pressure by changing the habitual intake of salt. One of us (CEG) has been a key investigator in the interaction of sodium and blood pressure in African-Americans (11). He (and others) have been suggested that a greater proportion of blacks (in the US) have salt sensitive hypertension. This conclusion is based on several studies that reveal a higher rise in blood pressure among normotensive blacks than in normotensive whites under an intravenous salt load, as well as a slower rate of sodium excretion than whites under the same salt load (12). In addition, the better responsiveness to diuretics in black hypertensives in lowering blood pressure--in both the United States and the West Indies--supports this "salt sensitive" hypothesis (13).

The singleton sample--*designed to be a representative sample of the population--* will enable us to assess the relationship between salt intake and blood pressure in this population *(as well as acting for a precise measure the relationship between age/sex in the population which will be use to correct the possible bias in these measures within the twin sample)*. The twin sample will enable us to assess the heritability of "salt intake" as a surrogate for salt craving. Denton has noted a strong craving for salt may be associated with high blood pressures (14).

### **Diabetes-related risk factors**

*The USAID reviewers have suggest we add items to the study that assess diabetes related factors. We are fortunate to have enlisted the cooperation of Dr. Richard Eastman, of NIH-NIDDK to assist with the assessment of biological variables related to diabetes. The variable of greatest interest in insulin resistance. Although hyperinsulinemia has been reported in hypertension since 1966 there has been a recent surge of interest with new observations that one of the metabolic correlates of increased blood pressure is insulin resistance. This has led Reaven and Hoffman to suggest a role for insulin in the hypertensive process as insulin stimulates the SNS, increases renal sodium retention, and may stimulate hypertrophy of vascular smooth muscle and modulate cation transport (15). A major argument against this hypothesis is that insulin resistance is neither a necessary nor a sufficient condition for increased blood pressure as evidenced by the Pima*

Indians who have a high rate of insulin resistance and a low rate of hypertension. Few studies, however, have been done on blacks in the United States or elsewhere.

One of the earliest reports of the blood pressure-insulin relationship in blacks was by Voors (16) who studied black children in Bogalusa, LA and showed no relationship between blood pressure and insulin resistance. Later Falkner et al (17) reported a significant relationship between the two variables. They studied insulin resistance and blood pressure in eight young black men with normal blood pressure (<135/85 mmHg) and eight with borderline hypertension ( $\geq 135/95$ ) using the euglycemic hyperinsulinemic clamp technique. Borderline hypertensives had higher fasting insulin levels (8.67 v 18.5 mU/ml) and lower insulin directed exogenous glucose metabolism (8.2 mg/kg/min vs. 6). Overall, there was a significant inverse correlation ( $r = -0.61$ ) of glucose infusion rate and systolic blood pressure. The insulin glucose ratio  $\times 100$  was 10 v 18. Falkner's preliminary results suggested a role for insulin in blood pressure in blacks. In sharp contrast Falkner's report, Saad et al (18) have recently reported no relationship on insulin resistance to blood pressure in blacks or Pima Indians in contrast to whites. Mean blood pressure was correlated ( $r = -0.41$ ) with fasting insulin levels in whites, but in blacks and Pima Indians the correlation coefficient was not significant (blacks:  $r = -.10$ ; Pima Indians:  $r = .15$ ).

The contradictory reports may be explained by admixture. That is, if the blood pressure-insulin association is genetic and specific to whites or Amerindians, then blacks showing this association may be admixed with whites or Amerindians, i.e. the white/Amerindian pattern appears in black populations by virtue of admixture with white/Amerindian genes for a BP-insulin correlation. Thus, the small study by Falkner et al may have included a more admixed population of blacks than was studied by Saad. It will be important in future investigations of this phenomenon in blacks to obtain a detailed family genealogy as well as to use genetic markers such as Duffy and mt-DNA that will allow an estimate of racial admixture.

This study would be a great opportunity to assess the heritability of insulin resistance in a black population with a probable, significant admixture with Amerindians (there is a Carib Indian reserve on the island and some estimates place the black/Indian admixed proportion of the population around 10%) however, unless other resources are available we will be unable to directly measure this. But we will have added some biological variables to the study: fasting glucose, insulin, glycosylated hemoglobin, and, if resources are available, a glucose tolerance test. These can give some indication of insulin resistance. These assays will be done in Dr. Eastman's laboratory in Bethesda at no charge to the grant. Statistically, we will assess these variables as both independent risk factors for elevated blood pressure and as potential confounders. (We wish to thank Dr. Eastman for finding the resources to transport a few twin sets to his laboratory to conduct complete tests of insulin resistance. These tests will give us some important preliminary findings as to the heritability of insulin resistance.)

#### Twin analysis

It has long been assumed that the variability of blood pressure in a population is due to environmental factors. However, the reporting of familial aggregation of hypertension suggest that genetic factors play a very significant role. Family aggregation of blood pressure in the Caribbean was first reported by Miall et al in 1962 in the twin families in Jamaica (19) and in the US by Zinner et al (20). Evidence from twin studies support the hypothesis that the

major reason for familial resemblance of blood pressure is due to genetic factors. The power of the study of twins allow one to assess the relative influence of genetic (nature) and environmental (nurture) factors on blood pressure (and related measures) in the entire population--twins and non-twins alike. It is known, for example, that any difference in blood pressure levels between identical or monozygotic (MZ) twins must be due to environmental factors. Thus MZ twins, who are genetically identical, provide a natural bioassay for the influence of environment on health and diseases. On the other hand any difference in blood pressure levels between non-identical or dizygotic (DZ) twins are due to either genetic or environmental factors. Using formulas, which are discussed in the methods sections, the heritability of blood pressure variability, salt intake and other variables (*controlling for age and sex blood pressure slopes which will be generated from a representative sample of singletons*) can be calculated (21,22,23). The Barbados twin study developed by Dr. Wilson and Dr. Grim in conjunction with three Barbadian physicians was an important pilot study which determined that, in fact, a twin study could be a successful venture in less developed countries. Some of the problems of that study, i.e. the a non-representative sample of the population and the lack of an adjunct singleton sample are corrected in this study design. It should be noted, that among a small sub-sample of males, a strong "heritability" (70%) to blood pressure was calculated (31).

## 2. Bibliography

1. Nissinen A, Böthig S, Granroth H, and Lopez AD: Hypertension in Developing Countries. World Health Statistics Quarterly. 1988; 41: 141-154.
2. Manton KG: The global impact of noncommunicable diseases: estimates and projections. World Health Statistics Quarterly. 1988; 41: 255-266.
3. Ward R: Familial aggregation and genetic epidemiology of blood pressure in Laragh JH, Brenner BM. [eds.] Hypertension: Pathophysiology, Diagnosis, and Management. New York: Raven Press, 1990: 81-100.
4. USAID "Guidelines for Submitting Proposals under the AID RESEARCH PROGRAM for Historically Black Colleges and Universities. Effective January 1, 1990 (2nd revision). p. 5.
5. Chesley LC In Hellman LM, Pritchard JA (eds): Hypertensive disorders in pregnancy. NY: Appleton-Century-Drofts, 1978.
6. Nicholson GD, Bailey N, Evelyn VS, Sivarajan S, Vaughan E: "Regular haemodialysis in Barbados: the first five years," West Indian Medical Journal 1985;34: 184-189.
7. Nicholson George D.. Morbidity and Mortality from Hypertension in the Caribbean. in The Control of Hypertension in the Caribbean Community. PAHO/WHO, 1988: 32-37.
8. Grell Gerald AC. Hypertension in the Caribbean: An Overview. in The Control of Hypertension in the Caribbean Community. PAHO/WHO, 1988: 22-31
9. Report of a WHO expert committee: Health of the elderly. WHO, Geneva (Technical Report Series 779, 1989
10. Dahl, L.K. "Salt and Hypertension." American Journal of Clinical Nutrition 25 (1972): 231-244.
11. Grim CE, Luft FC, Weinberger MH, Miller JZ, Rose RU, and Christian JC. Genetic, Familial and Racial influences on Blood Pressure Control Systems in Man. Aust NZ J Med 1984;14:453-457.
12. Luft, F.C., C.F. Grim, J.T. Higgins Jr, and M.H. Weinberger "Differences in Response to Sodium Administration in Normotensive White and Black Subjects." Journal of Laboratory and Clinical Nutrition 90 (1977): 555-562.
13. Freis ED, Reda DJ, Materson BJ: Volume (weight) loss and blood pressure response following thiazide diuretics. Hypertension 1988;12:244-250.
14. Denton D (1982) The hunger for salt: an anthropological, physiological and medical analysis. Berlin: Springer-Verlag

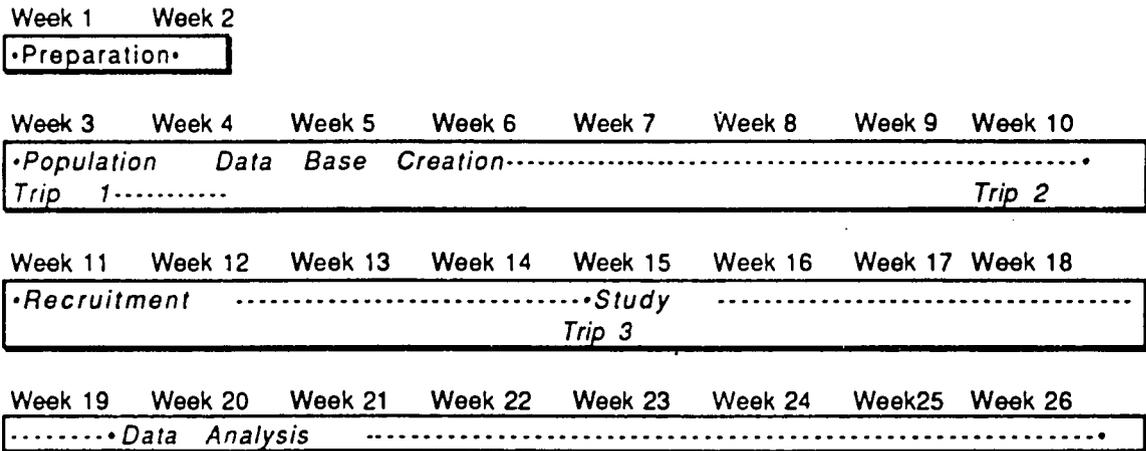
15. **Reaven GM, Hoffman BB. A role for Insulin in the aetiology and course of hypertension? Lancet 1987; 2:435-7.**
16. **Voors AW, Radhakrishnamurthy B, Srivinasan SR, Webber LS, Berenson GS. Plasma glucose level related to blood pressure in 272 children, ages 7-15 sampled from a total biracial population. Am J Epidemiol 1981;113:347-56.**
17. **Falkner B, Hulman S, Tannenbaum J, Kushner H. Insulin resistance and blood pressure in young black men. Hypertension. 1990; 16: 706-711.**
18. **Saad MF; Lilloja S; Nyomba BL; Castillo C; Ferraro R; De Gregorio M; Ravussin E; Knowler WC; Bennett PH; Howard BV; et al. Racial differences in the relation between blood pressure and insulin resistance. New England Journal of Medicine, 1991;324 :733-9.**
19. Miall WE, Kass EH, Ling J, Stuart KL: Factors influencing arterial pressure in the general population in Jamaica. British Medical Journal 1962; 2:497-506
20. Zinner DK, Margolius HA, Rosner B, Keiser HR, Kass EH. Familial aggregation of urinary kallikrein concentration in childhood: relation to blood pressure race and urinary electrolytes. Am J Epi 1976; 104:124-132.
21. Christian JC, Kang KW & Norton JA: Choice of an Estimate of Genetic Variance from Twin Data American Journal of Human Genetics 26: 154-161, 1974.
22. Grim CE, Cantor RM. Genetic influences on blood pressure in blacks: Twin studies. Clin. Res.1986;34:98A
23. Schieken RM, Eaves LJ, Hewitt JK, Mosteller M, Bodurtha JN, Moskowitz WB, Nance WE: Univariate Genetic Analysis of Blood Pressure in Children (The MCV Twin Study): Genetics of Children's Blood Pressure. American Journal of Cardiology (in press)
24. Christian JC, Kang KW & Norton JA: Choice of an Estimate of Genetic Variance from Twin Data American Journal of Human Genetics 26: 154-161, 1974.
25. Intersalt Cooperative Research Group: Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. British Medical Journal 297 1988: 319-328.
26. **Duncan BB, Helss G. Nonenzymatic glycosylation of protein--A new tool for assessment of cumulative hyperglycemia in epidemiological studies, past and future. American Journal of Epidemiology 1984; 120: 169-189.**
27. Grim CE, Grim CM, Klimazewski DL, Wolde-Tsadik G: An audiovisual system for monitoring quality control in blood pressure observers. CV Epidem Newsletter1985;37:50
28. Grim CE, TW Wilson, GD Nicholson, TA Hassell, HS Fraser, CM Grim, DM Wilson: Blood Pressure in Blacks: Twin Studies in Barbados. Hypertension 1990 15: 803-809.
29. King MC, Friedman GD, Lattanzio D, Rodgers G, Lewis AM, Dupuy ME, Williams H: Diagnosis of Twin Zygosity by Self-Assessment and by Genetic Analysis Acta Genet Med Gemellol 29 1980:121-126.
30. **Kraemer HC, Thiemann S. How many subjects?: statistical power analysis in research. Newbury Park: Sage Publications, 1987.**
31. **Cohen, Jacob, Statistical power analysis for the behavioral sciences. Hillsdale, N.J.: L. Erlbaum Associates, 1969.**
32. Christian, JC. Testing twin means and estimating genetic variance. Basic methodology for the analysis of quantitative twin data. Acta Genet Med Gemellol 28:35-40, 1979.
33. Falconer, DS: Introduction to Quantitative Genetics. 3rd ed. New York, Roland Press Co., 1989.
34. Heath AC, Neale MC, Hewitt JK, Eaves LJ, and Fulker DW: Testing Structural Equation Models for Twin Data using LISREL. Behavior Genetics. 19 (1989) :9-35.
35. Neale MC, Heath AC, Hewitt JK, Eaves LJ, and Fulker DW: Fitting Genetic Models with LISREL: Hypothesis Testing Behavior Genetics. 19 (1989): 9-35.

- 36. Cantor, RM, Nance, WE, Eaves, LJ, et al. Analysis of the covariance structure of digital ridge counts in the offspring of monozygotic twins. *Genetics* 103:495-512, 1983.
- 37. **Martin NG, Eaves LJ, Kearsley MJ, Davies P. The power of the classical twin study. *Heredity* [Edinburgh]. 1978; 40: 97-116.**
- 38. US State Department: US civilian personnel pro diem rates, Feb. 1989 version
- 39. Frerichs RR, Miller RA: Introduction of a microcomputer for health research in a developing country--the Bangladesh Experience. *Public Health Reports* 1987 100: 638-647.

**3. Detailed Description of Time Plan and Method**

The project will take approximately 6 months to complete-*although we may change this time line as a result of our planning meeting.* During much of this period workers from Drew/UCLA hypertension center will be on site in Dominica to co-coordinate the project.

**Time Line for Project:**  
6 months for project



**Travel Plan**

Four separate international trips are necessary for the proper completion of the study, as well as travel expenses for the local research worker. 1) Dr. Wilson and the Drew research associate will travel to Dominica at the beginning of week 3 of the study to train the local research associate in population data base creation. In addition, logistical support will be discussed. 2) Dr. Grell will travel to Los Angeles for a planning and training session during week 10 of the study and will meet with Dr. Wilson, Dr. Grim, and Dr. Cantor. He will be trained in twin analysis and the protocol of the study, review of the sample selection, scheduling, and recruitment. 3) Three members of US investigative team--Dr. Wilson, Dr. Grim, and a research associate will arrive in Dominica week 14 of the study to assist in last minute recruitment and study. 4) In line with the recommendation from the proposal guidelines--"travel to formally present results at professional meetings is encouraged" (p. 8)-the project team has requested travel expense for Dr. Wilson and Dr. Grell to present findings from the Dominican project at the 1992 meeting of the International Twins Association, tentatively scheduled to be held in Tokyo in June 1992. 5) Local research worker. Will need to travel to the various villages on the island during his recruitment effort. We estimate he will need to rent a car for 22 days.

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## Methods

### 1) Overall Study Design.

This is fundamentally a twin study design. We will also draw a stratified random sample of singletons to generate age/sex curves. This will permit the development of regression equations to regress out these effects in the twin sample.

We will attempt to recruit *at least* 75 monozygotic and 75 dizygotic sets of twins for the study; if we are unsuccessful we can still estimate that the heritability is more than zero with as few as about 30 MZ and 30 DZ sets. If fewer than 30 sets of each type are willing to participate, we can still estimate the relationship between blood pressure and other variables through the stratified random sample of singletons. In other words, something useful will emerge from the Dominican study.

### 2) Creation of population-based registry.

Twin births are about 1% of all births, or about 2% of any population, this twinning rate is even higher in black populations (24). Therefore, in the Dominican population of 80,000 we estimate there are about 800 twin sets or 1600 twins. About 2/3 should be dizygotic (DZ) (1/2 same sex, 1/2 different sex) and 1/3 should be monozygotic (MZ). That means about 266 MZ sets and 532 DZ sets in Dominica. Over 50% of the twins on the island are in the age group 15-54. To be more precise we estimate there should be about 444 twin sets in this target age group, this would break down to 148 MZ sets and about 296 DZ sets. Thus, we are all confident that 75 MZ and 75 DZ sets will be willing to participate in the study. It goes without saying that 100 singletons will be available as well. A estimated age breakdown appears below.

**Table 2: Population Estimate and twin set estimate in Dominica: Based upon 1987 Dominica population estimate**

Age Range	Population	Estimated Twin Sets	
<1	1,334	13	
1-4	6,004	60	
5-15	18,815	188	
15-24	19,444	194	194
25-34	12,756	127	127
35-44	7,130	71	71
45-54	5,171	52	52
55-64	4,572	46	
65-74	3,779	38	
75-84	2,420	24	
<u>85+ &amp; unknown</u>	<u>211</u>	<u>2</u>	
<b>Total</b>	<b>81,636</b>	<b>815</b>	<b>444</b>

Source: Annual Report of the Chief Medical Officer of Dominica for 1987

With the assistance of a competent university student or other person, selected by Dr. Grell and Dr. Wilson, a population data base of twin sets and singletons will be created. To accomplish this goal, this worker will be trained during Week 3 and 4 of the study in lap-top computer technology, computer data base software, procedures for careful entry of data into the computer, and subject recruitment strategy and techniques. This training will take about

two weeks. This student will work for a total of four months (from Week 3 to Week 15) and will be paid by the project. Specifically, this individual will a) select all multiple births in the birth records in the target age groups. Thus will be accomplished the same as was done in Barbados, by examining the records for births on the same day with the same mother. The worker will select 4 names adjacent (2 above and 2 below) to the multiple birth listing for singleton subjects. c) Enter all names into the laptop computer purchased for this study...d) Give all names will be given a random number. (25).

This data base of twins and age-matched singletons will be unique in the world and can be utilized by other researchers interested in other cross-sectional or cohort or longitudinal studies, especially if the researcher has an interest in the genetic and environmental contribution to a specific disease.

### 3) Sampling techniques/methods

A sample of twin sets from the population data base will be selected. Our target is at least 75 MZ sets and 75 DZ sets between the ages of 15 and 44 without regard to sex.

To generate curves for age and sex we will draw a stratified random sample of singletons. There will be eight strata based on age and sex. The target number of subjects for the 8 age-sex strata for Dominica study appear below..

	Males	Females
15-24	20	20
25-34	20	20
35-44	20	20
<u>45-54</u>	<u>20</u>	<u>20</u>
Total	80	80

By doing this we can estimate the stratum specific blood pressure means, standard deviations, and standard errors in the population from, which the twins are drawn and thereby eliminate any possible age/sex: blood pressure bias in our twin sample. In other words we remove the effects of the possible confounding effect of sex and age on our heritability estimates..

Under supervision of Dr. Grell and Dr. Wilson, the Dominican research assistant will be in charge of this recruitment effort. Attempts will be made to find the present address/phone number of the selected names by examining telephone books, voter registries, birth registries, through the distribution of flyers, and advertising the names in local newspapers, radio, and television. The ministry of health has agreed to donate office space at Princess Margaret Hospital. This office will be the command post of the local research associate throughout the duration of the project. The local research associate will attempt to contact the subject by all means possible. If necessary, he or she will visit the communities of Dominica by a rented automobile to contact each potential subject personally. If the attempt is unsuccessful on any single name or the subject unit refuses to participate a new subject will be drawn. This will continue until the proper number of subjects have been recruited and screened for the study. If we exhaust the first 200 sets, we will draw a random sample of 200 and make the attempt to contact them. This will continue until we have exhausted the registries or we have recruited at least 150 sets of twins. If we cannot get 150 sets of twins we will expand our age category and advertise for twin sets from other age groups. This will enable us to increase precision in the estimate of heritability.

Twin sets will be asked if they are identical or non-identical. It will be required that both members of the twin sets come to the study site the same day and time to be interviewed.

environmental factors associated with blood pressure variation (i.e. temperature, time of day, etc). The principal investigator will coordinate the research assistant in this sampling and recruitment effort.

#### 4. Protocol

The actual study will last up to 5 weeks. We will attempt to schedule 12 subject units (12 singletons or 6 twin sets) per day. We will schedule all twin subjects in the morning, with a request that they arrive after fasting for 12 hours to minimize food intake on blood pressure. The steps outlined below will be followed for both the twin and the singleton sample, except we will not draw blood for the singletons.

##### STATION #1

1. At this station the protocol will be reviewed with the subject and informed consent will be obtained. The participant will fill out a questionnaire with questions on medical health history and alcohol consumption. (*Investigator 1*)

##### STATION #2

The purpose of this station is to obtain anthropometric and blood pressure measurements.

2. Anthropometric station: (*Investigator 2*)  
Standard anthropometric measures including height, weight, skin folds will be taken by a trained Drew/UCLA observer.
5. Blood Pressure #1 (*Investigator 2*)  
Pulse rate will be measured. Blood pressure will be taken in the right arm in the seated position three times by a Drew/UCLA trained observer waiting about 30 seconds between each measure
6. Blood Pressure #2: (*Investigator 3*)  
Pulse rate will be measured. Blood pressure will be taken in the right arm three times in the seated position by a second Drew/UCLA trained observer waiting about 30 seconds between each measure.
3. Blood Pressure #3 (*Investigator 2*)  
Pulse rate will be measured. Blood pressure will be taken in the right arm in the recumbent position three times by a Drew/UCLA trained observer waiting about 30 seconds between each measure
4. Blood Pressure #4: (*Investigator 3*)  
Pulse rate will be measured. Blood pressure will be taken in the right arm three times in the recumbent position by a second Drew/UCLA trained observer waiting about 30 seconds between each measure.

##### STATION #3

The purpose of this station is to give the subject a medical exam and to draw the blood sample.

5. Physical Exam (*Investigator 3*)  
Physical exam will search for *possible secondary causes of hypertension* as well as damage to target organs. The examiner will fill out a check sheet specifically looking at: General appearance (distribution of body fat), eyes (damage to fundal vessels), neck (palpation and auscultation of carotids, thyroid), heart (size, rhythm), abdomen (renal mass, femoral pulses), and extremities (peripheral pulses, edema)
- 6) Blood Drawing Station. (*local health care worker*)  
5 cc of whole blood will be drawn by the local phlebotomist. Assays for ABO Rh blood groups will be done on site in Dominica. *Blood will be drawn at fasting condition and blood glucose will be measured on site. In addition, we will measure glycosolated hemoglobin as it is good indicator of long term glucose handling by the cell and can be another useful screening tool for diabetes (26).*

In addition, an aliquot of white blood cells will be frozen locally and stored for genetic typing and/or later studies.

**STATION #1 (Investigator 1)**

**6 Exit Station**

**a) 24 hour urine station and dietary recall**

--24 hour urine collection procedure will be explained. The subject will be instructed to return the urine collection container the following day.

-Urine analysis will look at sodium, potassium, creatinine and **albumin**.

A diet diary will be given to the subject and returned the following day

**b) The subject will return to this station 24 hours later and his diet diary and 24 hour urine collection will be returned. An exit interview which will ask the subjects for their personal evaluation of the study. The local health care worker/phlebotomist will assist in the partition of urine for electrolyte analysis.**

*If resources are available subjects will also be given a glucose tolerance test and will be tested for degree of insulin resistance. These tests, if done will be coordinated by Dr. Eastman from NIDDK.*

**5) Data Collection Methods and Data Analysis**

**Informed consent**

Consent forms will be obtained from each subject. These consent forms will explain the protocol, this will include the possible harmful effects of blood drawing. If the subject refuses to sign the protocol he/she will be excused.

**Blood pressure:** All scientific investigators in the twin/singleton study measuring blood pressure by sphygmomanometry will be trained and certified by the the Drew/UCLA blood pressure certification program (27). The subject will be seated, and after resting with the cuff on for five minutes, each individual will have blood pressure measured three times by two separate observers. *The subject will then lie down and the blood pressure measuring procedure will be repeated by the two observers. The last two readings in each position will be averaged for the analysis. The analysis will be done in blood pressures in both positions.* All primary investigators are tested every 6 months with a standardized video test. All six blood pressure measurement will be averaged.

**Prevalence of hypertension estimation:**

Hypertension will be defined as in the Intersalt study: systolic  $\geq 140$  mm Hg, diastolic  $\geq 90$  mm Hg, or on anti-hypertensive medication. We will also estimate prevalence using the World Health Organization classification: systolic  $\geq 160$  mm Hg, diastolic  $\geq 95$  mm Hg, or on anti-hypertensive medication.

**Anthropometrics.**

Anthropometrics including height, weight will be conducted using standardized techniques. Body mass index will be calculated as weight (kg) divided by height (m) squared.

**24 hour urine collection:**

The subject will be given written and oral instructions as to the methods of 24 hour urine collections. The subject will be asked to void and the time of that void is recorded-this starts the urine collection. When the subject returns 24 hours later they will be asked to void into the collection container-this marks the end of the collection. The time will be recorded. The volume of urine will be measured and an 10 cc aliquot will be taken for later analysis of sodium, potassium, and creatinine. The excretion rate will be adjusted to a 24 hour period. The urine

will be analyzed in Dominica.. ***A duplicate sample may be transported to Drew Hypertension for analysis of microalbuminuria.***

Blood collection and assays:

Blood will be drawn by the local ***certified*** phlebotomist ***who will be selected by Dr. Grell.*** The blood will be analyzed for ABO blood groups to help with zygosity. ***The blood will also be measured for glucose and insulin level by Dr. Eastman, from NIH-NIDDK (see attached letter).*** The blood will be frozen and kept in Dominica in for blood tests for other assays and genotyping at a later date.. ***A duplicate sample may be taken to Drew Hypertension for measurement of glycosolated hemoglobin.***

Zygosity Determination: This will be determined initially by the answer to the following questions: When you were young were you and your twin as alike as two peas in a pod?" and "Did your parents, family members, and friends get you two confused when you were children?" If they answer "yes" to both questions they will be tentatively classified as identical or monozygotic (MZ) twins, if they answer "no" to either question they will be classified as non-identical or dizygotic (DZ) twins. These questions were 80% successful in the Barbados study (28), a bit lower than that found in other studies (29). Further zygosity breakdown will be done by ABO Rh blood group testing at the Princess Margaret Hospital in Rouseau, Dominica this will increase the accuracy of our zygosity breakdown. If any blood group measure is different between the twin and co-twin the set will be classified as DZ . Future blood group testing will be done on the frozen sample as funds become available which will increase zygosity accuracy to 99%.

Statistical methods

Sample Size Determination:

***In twin studies sample size is based on the expected difference in correlation coefficients between MZ and DZ twins. In our Barbados study we found the r value for MZ twins to be 0.53 and for DZ twins 0.19. Based upon classic power analysis and effect difference of 0.34 [.53 - .19] (at p<.05 with a two sided test at power equal to or greater than 0.80) a sample size of 75 sets per zygosity group is sufficient to test for heritability of a trait. For other measured variables (e.g. fasting glucose, urinary salt excretion) a sample size of 75 per twin type will have the power (.8) to test for a significant (p<.05) heritability with a number of rMZ and rDZ combinations including rMZ=.5 & rDZ=.1 and rMZ=.9, rDZ=.4. To determine effect size differences in correlation coefficients the following formula was used: effect difference= (MZ<sub>r</sub> - DZ<sub>r</sub>)/(1-(MZ<sub>r</sub> · DZ<sub>r</sub>)) 2). The rationale for this method can be found in Kraemer & Thiemann (30) and Cohen (31).***

For the age/sex stratified sample, Dr. Cantor's sampling guidelines suggest we use at least 20 subject per each eight age/sex strata ***to adjust for the well-known rise in blood pressure with age (In most populations measured thus far) is different in males than in females. In the Intersalt study 20-25 individuals per sex/age group were enough to generate sufficiently precise estimates of the standard error of age/sex stratum specific blood pressure For example in the Trinidad and Topago site the 10 year age/sex specific blood pressure ranged 115 mm Hg to 134.3 mm Hg), the stratum specific sample size ranged between 19 and 25, and the standard errors averaged about 3.0 (range 1.8 - 4.8).***

**Data Entry**

Data will be entered on each day on the Toshiba Laptop computer purchased from the project. The software--Survey Mate--is already owned by the Drew/UCLA hypertension center. We hope to see the subjects each morning and will enter blood pressure, weight, height, age, and gender data each afternoon. All data will be printed out each day by the Dionix printer and will be proofread against the original data entry sheet by two of the investigators.

**Descriptive and Inferential Statistics.**

For all studies descriptive statistics of all variables will be calculated. Correlation coefficients and linear regression modeling will be used to determine the strength and direction of the association between the dependent and independent variables.

**Twin Model:** Dr. Rita Cantor, consultant on this project, has been instrumental in the development of the twin data analysis techniques. The analysis of twin data has been used previously by Dr. Grim and Dr. Wilson as well. Prior to analysis all variables will be transformed to normality if necessary. The twin design analysis then tests the assumption of equal variances and equal means between the two twin types. (32). Heritability is then calculated using the simple formula of Falconer (33):  $Heritability = 2(r_{MZ} - r_{DZ})$ , where  $r$  is the intraclass correlation coefficient for the trait in MZ and DZ twins respectively. Because MZ twins share all genes a perfect genetic trait would have a  $r_{MZ}$  of 1.0. DZ twins share, on the average 1/2 of their genes so that a  $r_{DZ}$  for a perfect genetic trait would be 0.5. Thus heritability can vary from 1.0 (a perfectly genetic trait) to 0.0 a trait with no genetic influences on it. Heritability is also written as  $h^2$ . Then using multivariate analysis of variance on the MZ and DZ twins separately, variance-covariance matrices will be obtained. Using linear structural modeling with maximum likelihood estimation as performed by the LISREL program, tests of various genetic and environmental models will be conducted by Dr. Cantor, and estimates of heritability obtained. The application of the LISREL program (34, 35) to similar analyses on the offspring of MZ twins are explained in detail by the Cantor (36). *At the anticipated sample sizes we will test basic hypothesis on the variables, i.e. that genetics (or environment) is a factor in blood pressure variability in Dominica. In addition, as we are collecting information on other variables as well, we can test the hypothesis that environment (or genetics) is a factor in the variability on these variables as well. (37)*

**4. Statement on Logistic Feasibility**

The Minister of Health and the Medical Director of Princess Margaret Hospital have endorsed the project (see attachment) and have assured the PI that the project is feasible.

**D. COMPLIANCE WITH FEDERAL GUIDELINES AND REGULATORY PROCEDURES**

Since this proposal is not related to biotechnological activities, this section does not apply.

**E. QUALIFICATIONS OF PRINCIPAL INVESTIGATORS****C.E. Grim, M.D.**

See attachment

**T.W. Wilson, Ph.D.**

see attachment

**G.A.C. Grell, M.D.**

See attachment

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**R. Cantor, PhD** (consultant)

See attachment

**Richard C. Eastman, MD**, Clinical Director, Diabetes Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Health will act as an unpaid consultant on the grant. He will be in charge of the blood assays for diabetes-related assays. In addition, he is considering to transport a few twin pairs to his lab for extensive studies of insulin resistance to calculate preliminary information on heritability of this variable.. (see attached cv for further information)

**Dr. Carlissa Etienne, MD** is the director of the Dominica Primary Health care services and is actively involved in community projects within the Ministry of Health. Dr. Etienne has developed a Dominican data base with her household surveys and has agreed to co-ordinate the activities of the local health personnel in the island as part of the proposed study.

2. Prior experience of PI in a similar environment (is highly desirable)

Dr. Grim and Dr. Wilson have been involved in a study which followed a similar protocol in Barbados, another West Indian island. The major purpose of this study was to determine if a twin study was feasible in a less developed country. We knew the value of a twin registry in the US and wished to develop a "black" twin registry outside of the United States. This part of the study was successful 84% of the twins we scheduled appeared for the study and every participant agreed to participate in another study (30). The major weaknesses of this study-- lack of a random sample from the population and small sample size are corrected here.

In addition, Dr. Wilson has recently returned (Jan 1990) from an very successful study on blood pressure and salt intake in Nigeria. A random sample of 140 males between the ages of 20 and 59 was selected from a population-based registry. This registry was developed for the study. 120 of the scheduled subjects participated in the study. This was one of the first random samples studies in rural West African and the first to estimate salt intake using reliable measures.

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