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ON MEDICAL RESEARCH**

**FIRST MEETING**

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**LEPROSY RESEARCH  
IN LATIN AMERICA**

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**PAN AMERICAN HEALTH ORGANIZATION**  
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Of these articles, 82 were from Argentina; 81, Brazil; 15, México; 8, Venezuela; 4, Cuba; 3, Surinam; 2 each, Colombia, Ecuador, and El Salvador, and 1 each, Chile, Paraguay, Peru, Uruguay, and "Unclassified (PAHO)."

The relatively large number of articles on clinical aspects, lepromin testing, and general epidemiology and the small number on laboratory subjects reflect the fact that the great majority of the authors are engaged only part time on leprosy work; only a few have both time and laboratory facilities. Judging from numbers of publications, the most active senior authors were: Olmos Castro (Argentina) 22 papers; Bergel (Argentina) 9 papers; Bechelli (Brazil) 7 papers; and Jonquieres (Argentina) 6.

Latin American leprologists have been very active in International Congress of Leprology. The next (8th) Congress is scheduled to be held in Rio de Janeiro, September 12 - 19, 1963.

Importance of Leprosy. These facts do not reflect the importance of leprosy as a public health and economic problem in Latin America. Brazil is said to have more than 150,000 cases; there are more than 22,000 in leprosaria and about 5,000 healthy children of leprosy patients in pre-ventoria. More than 700 physicians in Brazil are engaged in leprosy work, most of them on a part-time basis. In Venezuela, with an estimated total of 15,000 cases and fewer than 1,000 patients in institutions, the annual cost of the leprosy program is about U.S. \$2,500,000 or 2.2 per cent of all expenditures for public health and hospitals. A much smaller country, Surinam, has only 2,500 estimated cases but more than one fifth of the patients are in leprosaria; the annual cost is about 9 per cent of the total budget for public health and hospitals. Figures for Argentina, Colombia, México, Paraguay, and Perú likewise indicate not only a

great humanitarian problem but also a sizeable economic one, especially when loss in productivity and cost of support of dependents are added.

Summary of Present Knowledge. A noncultivated acid-fast bacillus (Mycobacterium leprae) can be demonstrated in a large proportion of cases presenting the clinical syndrome of leprosy. Such a bacillus is rarely found in other diseases or in healthy persons. Leprosy has not been transmitted to any experimental animal although promising reports have recently been published by several workers.

Unsolved Problems. The mode of transmission is unknown. The present idea, accepted by most leprologists, is that the bacillus reaches the skin of a susceptible individual by indirect or direct contact with a bacilli-ferous case, usually lepromatous, and is rubbed or scratched into the skin. Whatever the method is the conditions favoring spread are far more effective in warm than in temperate climates.

Although the lepromatous case is undoubtedly more infectious than the tuberculoid it is possible that in some countries the latter type is also an important source. Tuberculoid cases may discharge many bacilli during periods of reaction and these reactions may be more frequent in some environments than in others. Another point is the potential infectivity of the indeterminate class of cases, a question which is discussed later.

Natural resistance to leprosy is apparently the most important factor which limits prevalence--and has been very inadequately studied. Increasing frequency of resistance with age is the logical explanation of the rapid fall of the attack rate after the age of 15 years in families in which lepromatous disease is present. This resistance is correlated with reactivity to lepromin but this must be only a part of the story

because patients with tuberculoid leprosy are usually highly reactive to lepromin.

Drugs of the sulfone group are universally used today in the treatment of leprosy. Controlled studies in lepromatous leprosy have shown that these drugs yield beneficial results but are slow in action. They are bacteriostatic rather than bactericidal; after three years about half the patients are still bacteriologically positive. Relapse is common if treatment is discontinued. Certain other drugs are about equal in value to the sulfones, including streptomycin and a thiourea (CIEA 1906) but these are much more expensive than the basic sulfone, 4-4' - diamino-diphenyl sulfone(DDS). Other features limiting the value of therapy--especially as a preventive--is that there is usually a long period of latency or slow progression during which the disease is unrecognized but during which the individual may be infectious to his contacts.

Faced with this situation, -inability to produce a disseminated infection in animals, lack of precise information regarding mode of transmission, knowledge of existence of resistance but ignorance of its cause, and the need of a more effective therapeutic agent, it is obvious that there are many lines of research which are scientifically justified and that a choice between them should be determined by the availability of competent scientists rather than by arbitrary administrative selection. Apparent duplication should be disregarded. Both in England and in the United States, a few investigators are working on transmission of the disease. This does not mean that the rest of the world should sit down and await the results. If it is important for the United States Government to support a considerable number of projects dealing with the desalinization of sea water, it is equally logical not to depend exclusively

on any single group to solve basic problems of leprosy.

## II. SPECIFIC RESEARCH NEEDS

### A. Single Country Projects Needing External Financial Support.

As a result of our visit we have encouraged four groups of investigators to prepare definite plans for research work and to try first to obtain financial support directly from the National Institutes of Health of the United States Public Health Service. A fifth investigator was encouraged to seek renewal of a current grant. The Organization might see fit to support these requests and in the event that the National Institutes of Health do not give support to try to find other sources. A sixth project on the therapy of leprosy is soon to get under way in Venezuela with support from the World Health Organization.

1. "Direct Cytologic Study of the Skin in Leprosy." Investigators: Dr. Rene Garrido Neves, Lygia Madeira Cesar de Andrade and Dr. Candido de Oliveira de Silva, Instituto de Leprologia, Servico Nac. de Lepra, Rio de Janeiro, Brazil.

"With a view to throwing light on the mechanism of resistance to leprosy the types of cutaneous cellular response to natural infection and to the injection of lepromin are to be studied. A direct method of examination will be used, similar to that of modern studies of exfoliated cells in the diagnosis of cancer. Instead of the usual biopsy specimen, scrapings from the corium will provide the materials for study. One interesting possibility is that in patients under treatment with sulfones and whose lepromin tests are negative, microscopic examination of cells scraped from the site of infection of the lepromin may give evidence of a favorable cellular response."

The amount of support requested is less than \$10,000 for the first

year and substantially less for 4 following years. We are convinced that a grant to these workers would have an effect far beyond the immediate objective in encouragement of leprosy research in Brazil (See Annex I, RES 1/11).

2. "Cultivation of M. Leprae in Human Phagocytes." Investigators: Dr. Murilo Paca de Azevedo, Instituto de Pesquisas Leprológicas and Dr. Ednir Antonina Lehmann Wanderley of the same institute, Dept. de Profilaxis de Lepra, São Paulo, Brazil.

"The purpose of the proposed work is to cultivate the leprosy bacillus in human macrophages obtained from patients and from normal persons, including cells from human histiocytic tumors. The reason for using macrophages is that they are considered to be of the same lineage as the epithelioid cells of the leproma. The patients and normal persons chosen as donors of tissue cells will be lepromin-negative because this is considered to indicate lack of cellular resistance to leprosy.

"The facilities at São Paulo are excellent; a new 9-story building for the leprosy department will be completed by the end of 1962 and many patients are available for study."

Both Drs. Azevedo and Wanderley have had experience in tissue culture and in efforts to grow M. leprae in tumor cells. The financial request is for \$9,680 for the first year, \$7,767 the second and \$5,203 the third, to be used for purchase of equipment and to provide a short term consultant at intervals from the United States. (See Annex II, RES 1/11).

3. "Study of Ecological Factors in Leprosy." Investigators: Drs. Carlos Sisirucá Quintero, Enrique Rasi Bellabene and Jacinto Convit, División de Dermatología Sanitaria, Ministerio de Sanidad e Asistencia Social, Caracas, Venezuela.

"The principal objective of the proposed study is to search for environmental factors associated with the frequency or severity of leprosy in Venezuela. Two principal rural zones in which the disease is endemic, one mountainous in Western Venezuela, the other at a lower level in the Eastern section of the country are to be selected for study. Each zone will be divided into sectors according to known prevalence rates. A complete house-to-house

census and sanitary inspection will be made followed by physical examination of all inhabitants. Careful diagnosis studies will be made in every case or suspected case of leprosy. A special investigation will be made of the arthropod population in the search for a possible vector."

Under the proposal, the cost of this project would be divided between National Institutes of Health of the U.S. Public Health Service and the Venezuelan Government, the United States meeting the cost of a full-time epidemiologist and per diem expenses for all personnel and the Venezuelan Government providing salaries of 12 trained inspectors and automobiles. The cost to the National Institutes of Health would be about \$33, 000 per year for a period of 5 years. (See Annex III, RES 1/11).

In all three of these projects the investigators are capable, deeply interested and have contributed to the subject. The principal investigator will work full time in each instance.

4. "Biochemical and Bacteriological Properties of Mycobacteria Isolated From Leprosy Patients." A group of investigators of the Seccion de Bacteriología, Unidad de Patología, Hospital General, México, D.F., under direction of Dr. Luis J. Bojalil, has been encouraged by us to send an application to the N.I.H. for support. The Organization may wish to recommend this request or attempt to obtain support elsewhere. The investigators, especially Dr. Bojalil and Dr. J. Cerbón are international authorities on the properties of rapidly growing mycobacteria. During Dr. Hanks' visit it was disclosed that in five patients with lepromatous leprosy recently studied, mycobacteria which grew slowly on Sula's medium for tubercle bacilli were isolated from three. Dr. Hanks was greatly impressed by the interest in leprosy and by the capacity of these investigators.

5. "Studies on Chemotherapy of Leprosy." Investigators: Dr.

Jacinto Convit and others. Division de Dermatología Sanitaria, Caracas, Venezuela.

A controlled study of drugs initiated by the WHO Leprosy Section at Geneva is still in the planning stages. As a preliminary step the sulfone blood levels have been measured, following intramuscular injection of DDS suspended in oil prepared by the Bristol Laboratories. The effective "sulfone level" for treatment of leprosy is unknown.

This study is recommended for continued support by the Organization.

6. "Serologic Control of Leprosy Patients Under Treatment."

Investigator: Dr. José Oliveira de Almeida, Fac. de Medicina, Univ. de São Paulo, Ribeirão Preto.

This project is now receiving support from the National Institutes of Health, U.S. Public Health Service. This relates to the complement fixing titers of sera from leprosy patients and healthy persons using as the antigen and extract of M. tuberculosis containing the polysaccharides and mucopolysaccharides. This is an example of a research grant, given to a capable scientist of long experience in serology, which is just beginning to show interesting results. This project was designed originally for a short period; it obviously should be extended and an application for renewal is being submitted by Dr. de Almeida.

B. Single Country Projects Not in Immediate Need of External Support An example of this kind is seen in the excellent work being carried on by Dr. Tomasu Imaeda of the Instituto Venezolano Investigaciones Cientificas (IVIC) in electron microscopy. In cooperation with Dr. Jacinto Convit, head of the Venezuelan Leprosy Service, Dr. Imaeda is studying specimens from human leprosy of different types and also from murine leprosy. In the latter, he has observed what appears to be multiplication

by lateral division. He has also found that in the dimorphous class of human leprosy cases the bacillary form is solid, that is, without evidence of plasmolysis; in the lepromatous type however, plasmolysis is common.

The Leonard Wood Memorial is considering asking the Institute to take one of its workers for several weeks' instruction under Dr. Imaeda.

C. Multi-Country Projects. 1. "The Pathogenesis of Indeterminate Leprosy." Principal Investigator: to be obtained by PAHO.

At the 7th International Congress of Leprology, Madrid, 1953, the present definition of "indeterminate" leprosy was adopted: "A benign form, relatively unstable, seldom bacteriologically positive, presenting flat skin lesions which may be hypopigmented or erythematous; the reaction to lepromin negative or positive. Neuritic manifestations, more or less extensive, may develop in cases which have persisted for long periods. The indeterminate group consists essentially of the 'simple macular' cases. These cases may evolve toward the lepromatous type or the tuberculoid type, or remain unchanged indefinitely". In spite of the apparent clarity of this definition, there are at present wide differences of opinion among leprologists in respect to indeterminate leprosy. This is reflected in the wide variations in the proportion of cases so classified. A second fact is of great practical importance. No one knows how frequently indeterminate cases "evolve towards the lepromatous type". As a consequence, patients whose disease is classified as indeterminate are regarded by some countries as potentially dangerous to the public health whereas in other countries little attention is paid to them.

The object of this study is to determine the outcome of a fair sample of new cases originally classified as indeterminate--treated and untreated. A pool of cases would be established in each of several count-

tries, e.g., Brazil, Colombia, (two areas) Mexico, 2 areas, and Venezuela. The plan of research would define the common characteristics of the group, including dermatologic, bacteriologic, immunologic (lepromin) and histologic criteria. The manner of selection of groups to be treated with a sulfone and a control preparation would be laid down, together with the frequency of reexaminations and statistical measurement of results. This project was discussed with leprologists at Rio de Janeiro and at Caracas, and was considered to be essential and entirely feasible. The holding of a control group, under supervision but not given a sulfone was considered to be an ethical procedure in this class of case.

This study is recommended for consideration by the Organization. Central planning and supervision would be essential but the actual field work could be carried out, with local assistance by a competent leprologist working about six months annually for 3 to 5 years. Estimated annual cost of field work about \$15,000.

2. "A Study of Administrative Methods of Control of Leprosy."

To be carried on by PAHO.

Since the principles upon which control methods must at present be based are generally agreed upon, nevertheless there are wide differences between the American Republics in this respect. It would be worthwhile to make a detailed study of current practice in each country with a view to obtaining greater uniformity. For example, the trend to out-patient care is evident everywhere, but the best future use of existing leprosaria should be examined. The duration of surveillance and the frequency of examination of contacts are matters which affect the use of staff and expenditure of funds. Yet there is no agreement on these points; even the definition of a "contact" is not uniform.

It is recommended that a study be initiated at headquarters of PAHO on these points. An estimate of cost has not been made.

### III. TRAINING

First it should be emphasized that there are no fixed lines for training in leprosy research. Leprosy problems require investigation by biologists, biochemists, biophysicists, and other specialists. The small research staff of the Leonard Wood Memorial includes bacteriologists; biochemists, general and specialized in enzymology; epidemiologists, and clinicians. There is a great scarcity of such research workers in Latin America. Our problem is intensified by the fact that there are few attractive positions in leprosy research available even to the small number who may become interested in the subject.

It seems to us that the first step should be to assist certain countries in strengthening the staff of institutions in which leprosy research is now going on. In particular, a nucleus of research workers in each of these places should be supported on a full time basis.

A second and equally important step should also be taken. There are in Latin America a number of institutions in each of which it might be possible to interest one or more staff members in certain facets of the leprosy problem. If this could be done young research workers would be attracted to these places for training. Among the institutions which we have in mind are the following:

ARGENTINA - Instituto Nacional de Microbiología, Buenos Aires.

The prospects at this institution are for the moment discouraging but

we are convinced that better days will come. In the past there was excellent research in the epidemiology of tuberculosis. The future interests however remain to be determined.

BRAZIL - The Institute of Tropical Medicine, University of São Paulo.

A number of students from other Latin American countries are now studying at this institute.

The Oswaldo Cruz Institute, Rio de Janeiro.

With the retirement of Dr. H. C. de Souza-Araujo, now 75 years of age, leprosy investigations at this institute will end unless further interest is generated.

COLOMBIA - It might be possible for the Organization to promote interest in leprosy research at the International Center for Medical Research and Training at the Universidad del Valle, Cali, which is affiliated with Tulane University (School of Medicine) and supported by a grant from the National Institutes of Health. This has not been explored by us.

MEXICO - Training for research with leprosy in mind might be initiated at the School and Institute of Health and Tropical Diseases Training Station at Boca de Río, Veracruz.

VENEZUELA - The interest in leprosy on the part of Dr. Imaeda of the Instituto Venezolano de Investigaciones Científicas has been mentioned. There may be others in the staff of this research institute who would become interested in leprosy problems if they were presented to them. If this were to occur, and opportunities were extended to a few assistants from other countries, this institute might become invaluable for our purpose.

There should, in our opinion, be a greater interchange of graduate students than now exists between Latin American medical institutions both

in research and practice. Recently with assistance from PAHO and financial support from Venezuela, a number of leprologists from other countries were given several weeks' training in Venezuela. A few post graduate students from other countries could be given research experience at the Institute of Leprology at Rio de Janeiro. The new building of the Institute for Leprosy Research at São Paulo should provide excellent facilities for instruction in bacteriology, pathology and epidemiology of leprosy.

In the United States there are likewise few opportunities. The Johns Hopkins-Leonard Wood Memorial Leprosy Research Laboratory is limited in space but can take a few research workers.

#### IV. PHYSICAL MEDICINE

There is need also for provision in the Americas for a center for teaching and research on Physical Medicine as applied to leprosy, along the lines of that at Vellore, India. A beginning is being made at Caracas by Dr. Arvelo and at São Paulo by Dr. Faggin. Special request for training of orthopedic surgeons are given in (Addendum I (Res 1/11), Argentina and Colombia, but the needs have generally been overlooked.

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Supplemental notes on leprosy activities and opportunities for research are given by countries in Addendum I, RES 1/11, on page 14.

SUPPLEMENTAL NOTES ON RESEARCH RESOURCES  
IN LEPROSY IN LATIN AMERICA

ARGENTINA. Director of Leprosy Work: Dr. Armando Zavala Sáenz.

Dr. Hanks' observations of the leprosy control program were most discouraging because of the difficult political situation.

1. At Rosario, Dr. Meny Bergel is carrying on experiments in transmission of human leprosy to rats on a prooxidant diet (deficient in vitamin E). Efforts to confirm the published results of Dr. Bergel are being made by Dr. Karl E. Mason and his associates at the Rochester (N.Y.) School of Medicine, and by Dr. Barkulis et al. at the Laboratories of Ciba Pharmaceutical Products, Inc., Summit, N.J. Although Dr. Bergel is a devoted individual whose industry and sincerity greatly impressed Dr. Hanks it is not recommended that he should receive external financial assistance at this time.

2. The work at the Central Leprosy Dispensary, Buenos Aires, directed by Dr. E. D. L. Jonquieres is highly regarded. Pathological specimens from the Baldemar Sommer Sanitorium are examined here.

3. Sanatorio Baldemar Sommer. The Director, Dr. R. O. Manze is the only physician on a full-time basis yet much good research has been accomplished at this institution. Dr. Masante is studying amyloidosis and Dr. Melamed the effect of various drugs on lepra reaction. There are small laboratories for bacteriology and protein chemistry. Attempts are being made to transmit leprosy to mice and rats and Dr. Bergel visits once weekly. The hope that this institution would become a research institute must temporarily be laid aside.

4. Carrasco Hospital, Rosario. The dermatological staff is good but all (Fernández, Carboni, Mercau and others) are on a part-time basis and time does not permit carrying on studies for which they are capable. A request was made for a grant to send an orthopedic surgeon (Dr. Borsani) to Vellore for four months training under Mr. Brand.

5. Department of Dermatology, University of Rosario School of Medicine. Dr. J. M. M. Fernández, Director. Research work in progress includes attempts to transmit leprosy to laboratory animals and the fractioning of Rongalite in a search for the active principle. Junior personnel are inadequately trained for the work and the senior members cannot devote adequate time to it.

6. Department of Bacteriology, School of Medicine, University of Buenos Aires. Director, Dr. Armando S. Parodi. Associates: Drs. Wilkinson, Balina and others. Research under way includes attempts to confirm Bergel's results (unsuccessfully): study of a gram-positive, spore-bearing bacillus, said to have been isolated from all lepromatous patients and from contacts; Immuno-electrophoresis of leprosy sera versus mycobacterial antigens, and immuno-fluorescence of juices from leprosy lesions. Staff should have more training because of complexity of the projects which are being attacked.

7. Dr. Olmos Castro's laboratory in Tucumán was unfortunately not visited because of lack of time. He should be kept in mind because of his fertility and technical skill in immunologic experiments.

8. Instituto Nacional de Microbiología, Buenos Aires. The disruption of the staff of this institution makes recommendations undesirable at this time. The Institute was formerly under the

direction of Professor Ignacio Pirotsky and had a staff of 300.

BRAZIL. Director of the Leprosy Service: Dr. Castelo Branco.  
Director of the Instituto de Leprología; Dr. Candido Oliveira e.  
Silva.

1. Instituto de Leprología, Rio de Janeiro. The present and the next few years constitute a critical period at this institution. Able scientists now on the staff are likely to be forced out by sheer necessity unless the government takes steps to remedy the situation. Dr. Candido has recently elected to go on a full-time basis.

In addition to the project for which we encouraged an application to the N.I.H. ("Direct cytologic studies of the skin in leprosy") we found interesting investigations being made in the clinical, bacteriologic and chemical sections.

Drs. Tuma and Marjeta of the Section on Bacteriology are able workers. They are engaged in a number of microbiologic problems including cultivation of M. leprae in a variety of cell culture systems and in various media to which are added extracts of other mycobacteria. At present their work is handicapped by burden of routine and the part-time system.

In the chemistry department Dr. Anelis and his associates have developed simple spot tests for detection of sulfones (or sulfonamides) and thioureas in the urine.

2. Oswaldo Cruz Institute, Rio de Janeiro. The relationship between this institute and the Instituto de Leprología is fortunately close and harmonious. Dr. N. C. de Souza-Araujo has a large collection of cultures of mycobacteria, most of which he obtained from human or

murine leprosy. These cultures should be studied and classified and Dr. Souza-Araujo was given names of U.S. scientists who would assist him in this matter.

3. Instituto de Pesquisas de Lepra, São Paulo. This institute should be investigated again after the new building is completed. There are opportunities for field study which are apparently not being taken advantage of. One project is recommended for immediate support, namely: "Cultivation of M. leprae in human macrophages."

4. Instruction in leprosy for medical students is carried on in all countries which we visited.

An example of an opportunity in training of medical students which might eventually have a wide effect throughout South American research on leprosy, Chagas disease and other fields, and which therefore should be of interest to the Organization is seen at Ribeirão Preto.

The medical school at Ribeirão Preto, a division of the University of São Paulo is unique in Brazil in that the full-time system prevails throughout all departments. It is excellently staffed and equipped. The entering classes now number 80 selected by competitive examination. About 20 of each class are from other South American countries and this policy will be continued if qualified students with external source of support continue to apply for admission. Plans are being made for a new hospital to be constructed on the campus outside the City of Ribeirão Preto. There is a strong Department of Preventive Medicine headed by Dr. José Lima Pedreira de Freitas. A teaching and research district should be developed with the new hospital as the center, in which the medical students will be brought face-to-face with the problems of health and disease in an entire community. Diseases

which urgently demand study and control include Chagas disease, leprosy, syphilis and tuberculosis. In our opinion the PAHO should encourage such a development because this medical school could become a source of young physicians excellently trained in both clinical and preventive medicine who would have a great and expanding influence.

COLOMBIA Director of Dermato-Leprosy, Dr. Fabio Lordoño, Professor of Dermatology, Universidad Nacional de Colombia, Bogotá.

1. Status of Program. Country is divided into areas in each of which there is at least one dermatology dispensary and mobile unit. The plan is to reach infectious cases and bring under treatment without cost to the patients. Contacts are registered and followed, and vaccinated with BCG until a two-plus Mitsuda reaction is produced. An attempt is being made to get old patients out of leprosaria.

Dr. Fabio Lordoño, Universidad Nacional de Bogotá wishes to have Dr. Fuad Muvdi (orthopedic surgeon) trained in surgery and rehabilitation under Paul Brand or one of his disciples rather than in New York City because of modest budget and facilities in Bogota.

2. Sanatorio de Agua de Dios, Tacaima. Director: Dr. Wilfredo Davilla. Patients: more than 4,000. Good routine clinical work, minor surgery and some radiology. Laboratory old, almost without equipment and not active interest evident.

There is a special opportunity for epidemiologic study. Along with the patients there are about 10,000 healthy (?) persons, mostly relatives attracted by the previous pesos 1.50 per day allowance for patients.

3. Centro Dispensario Dermatológico, Buramanga. Dr. Alvaro Sabogal, Chief of Dispensaries. A mobile unit under Dr. Virgilio Rod-

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## LEPROSY RESEARCH IN LATIN AMERICA\*

## I. INTRODUCTION

Scientists of the Latin American Republics have made notable contributions to our knowledge of leprosy. Selected examples are the early account of the Lucio phenomenon in Mexico by Lucio and Alvarado and its modern description by Latapi and Chavez Zamora; the description of the early cutaneous reaction to lepromin by Fernández of Argentina and the demonstration by him and by the São Paulo group of leprologists of the effectiveness of BCG in producing reactivity to lepromin, and the excellent study by Pardo-Castello and Tiant of Cuba correlating the clinical, pathologic, immunologic and bacteriologic aspects of the disease.

From January 1958 through September 1961 we have counted 204 articles on leprosy and related subjects by Latin American authors which have been published in original or abstract form in international journals. We have classified these by subject as follows:

Bacteriology	3	Lepromin	64
Biochemistry	10	Serology	4
Clinical (including therapy)	49	Rehabilitation	7
Education	5	Prevention BCG	5
Epidemiology	23	Prevention, other	18
Experimental Transmission	6	Murine leprosy	5
Pathology	7		
		TOTAL	204

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\*Prepared for the first meeting of the PAHO Advisory Committee on Medical Research, 18-22 June 1962, by Dr. James A. Doull, Medical Director, and Dr. John H. Hanks, Bacteriologist, of the Leonard Wood Memorial (American Leprosy Foundation) acting as PAHO Consultants in Leprosy Research. Dr. Hanks visited Argentina, Brazil, Colombia, Mexico and Peru April 3 - May 3, 1962 and Dr. Doull visited Brazil and Venezuela April 19 - May 19, 1962 on behalf of the Organization.

riguez was visited. Excellent work was being done.

4. Dispensario Dermatológico, Socorro. Director: Dr. Saul Rugeles Moreno. This is a base for two mobile units and the center of an endemic focus of leprosy. Dr. Moreno has shown research ability by a study of effectiveness of BDG vaccination in producing lepromin reactivity in children (See Rev. brasil. de Leprol. (1959) 27:183-197). The high endemicity of leprosy on the Chicamoso plateau and basin, between Pescadero and Socorro excites interest in unstudied ecologic factors.

5. Instituto Nacional de Salud. Director: Dr. Fernando Serpa Flores Bogotá. Functions of the Division of Bacteriology are strictly practical, e.g., production of vaccines. The Division of Epidemiology under Dr. Federico Lleras shows promise. A new building for the Institute is being built.

6. Other institutions. Dr. Muñoz Rivas private investigator, suspects insect transmission and has published many papers to support this view. His broader interest is the distribution of mycobacteria in nature.

Dr. César Gómez Villegas who has a private laboratory in Bogotá was trained at the University of Michigan and could be used as a consultant in microbiologic problems.

Dr. Arturo O'Byrne, Cali, is no longer in charge of leprosy work in that region. He came to attention by his theory that the distribution of leprosy in Latin America is related to iodine: high iodine = susceptibility; endemic goiter = low prevalence. Hence the value (?) of Tapazole, an anti-thyroid drug in treatment of leprosy.

MEXICO.

1. Status of Program. The country is divided into two endemic zones: México, D.F. (Dr. A. Saúl) and Guadalajara (Dr. Gloria Pérez Suárez). Control measures are based on the principle that leprosy is fundamentally a familial disease. Efforts are made to find all cases primarily by examination of household contacts but also by educational measures designed to bring cases to local physicians or clinics. Mobile units are used. All contacts are registered. No immunizations (with BCG) are undertaken as a preventive measure. Public relations and relations with physicians are excellent. Emphasis has been placed on educational courses supported by the National Government for physicians and public health personnel.

2. Status of Supporting Institutions. At the Centro Dermatológico "Pascua," Prof. Latapi, Director, in Mexico City, good clinical work is done. The facilities are modest and badly crowded. The laboratory is scarcely sufficient for routine work and for teaching. The staff are part-time.

At the Institute of Dermatology at Guadalajara (Prof. J. Barba Rubio, Director) the facilities are much better and less crowded than those of Prof. Latapi. The clinical work and teaching of medical students appear to be excellent. In the bacteriological laboratory there are two Leitz and two phase microscopes but the mycology laboratory is meagerly equipped. Dr. Jesús Mayorga has had excellent training and with encouragement would develop further. If support could be obtained he might accept an invitation to work at the JHU-LWM Leprosy Research Laboratory; or a period in Rio de Janeiro with Drs. Tuma and Marjeta at the Institute of Leprology would be useful to him.

Similarly, Dr. Pedro Lavalle of the Pascua Clinic, Mexico City, who is an able student of the Mycobacteria associated with skin ulceration would do excellent research work if he could find support in a full-time laboratory and clinical position. He is an authority on the etiology of Mycetoma. He was trained at the Institut Pasteur and has a mind well adapted to continue research.

PERU. Director of the Leprosy Division: Dr. Hugo Pesce.

1. Status of Program. Perú is divided into three zones: Coastal, Sierra, and Eastern, each of which is subdivided into three regions, North, Central and Southern. There is a "Centro Anti-leproso" in each region. Three of these centres are each associated with a leprosarium. The major focus of leprosy is along the valley of the Río Acayali, Loreto (Eastern Zone). Case finding is done as well as possible in this difficult jungle. Infectious patients are put in leprosaria. Dr. Bresani Silva has been comparing the present cost with that of a clinic plan (ambulatory service).

2. In the Division of Leprosy, Dr. Pesce maintains a national register with adequate files.

3. Research possibilities are primarily those related to public health administration.

VENEZUELA. Excellent work on transmission of leprosy to hamsters and other rodents is being carried on at the Division of Public Health Dermatology in Caracas under the direction of Dr. Jacinto Convit. Although other workers have not succeeded in reducing resistance by radiation or other means, Dr. Convit has thus succeeded in producing disseminated disease in one hamster.

PRIORITIES IN LEPROSY RESEARCH IN LATIN AMERICA

In the type of research which opens new vistas, there seem to be no assignable priorities aside from the recognition of major problems. In the case of leprosy, these are known to informed laymen.

Scientific priorities consist largely of two elements:

a) Among various proposals, the support of those made by persons with training, imagination and ability to produce and, further, the support of proposals which are concerned in appropriate degree with major questions. (Insofar as possible, these matters have been covered in preceding sections of the report.)

b) The examination of trends and potentialities to see if there are possibilities which have not been widely discussed, but which may open new avenues for progress.

In the present Section, a few suggestions related to " b " will be offered. They are classified under the disciplines which seem useful in the study of leprosy.

1. Public Health Administration: The following questions might be asked:

a) How do we learn to manage leprosy as any other infectious disease?

b) How to bring greater resources to bear on the problem when there is no real increase in funds?

The first question concerns sociology and the art of PH administration. Answers doubtless will be obtained by further study and experience. One step would be to get leprosy more closely associated in the public mind with some other "manageable" infectious disease (see below).

The second question generated the following suggestion:

THE STUDY AND MANAGEMENT OF LEPROSY REQUIRES TWO TYPES OF CONNECTIONS:

A. General dermatologic clinics and local health centers, for example, for diagnosis, classification, ambulatory treatment, study of contacts, and certain aspects of PH administration.

B. Closer association with tuberculosis hospitals, services and research.

For example:

a) Services: based on Tb hospitals and associated organizations:

The required laboratory facilities and diagnostic skills are identical; in radiology: Dr. Davilla, Agua de Dios, says that radiology of the feet can assist in early diagnosis. Radiology is useful in rehabilitation, in studies on the physiology of bone absorption, etc.

There is an excellent pool of knowledge concerning the chemotherapy of mycobacterial disease.

There is an excellent pool of resources for PH nursing, social work, follow-up, and psychologic and social rehabilitation.

b) Research: examples drawn from three disciplines:

Microbiology: anonymous mycobacteria are associated with both diseases. Are they similar types of strains? This gives the student of tuberculosis an excellent series of questions, and occasion to deepen his knowledge and interest in leprosy. Immunology: cross-fertilizations needed:

Students of tuberculosis do not know the usefulness of interpreting skin reactions to intact mycobacteria in terms of prognosis or measuring potential resistances in populations. Leprologists are skilled in this.

There is a joint concern about the principles involved in prophylactic immunization. In populations, any pilot study which determines the effect of immunization on only one disease brings a reward equal to +50% of the investment.

Epidemiology:

Because of cross-sensitizations, the factors stimulating Mitsuda reactivity or low-grade tuberculin reactivity (e.g., anonymous or atypical mycobacteria) are of equal interest and are suspected to influence the distribution of susceptibilities in populations. Students of tuberculosis know that there are many infected (tuberculin positive) persons, clinical case. Leprologists, being unable to obtain similar information (and being able to recognize what are known as "infectious" cases), have often tended to suppose that new infections arise only from recognized, bacteriologically positive cases (see no. 4, Epidemiology, below).

Finally, to look still further ahead:

Association with tuberculosis = association with a "manageable" disease. Tuberculosis in some places may be brought under control more rapidly than leprosy (?). The skills in tuberculosis need not be lost to other activities. They can in large part be transferred to the study of a related and equally challenging disease.

2. Clinical (+ laboratory): Dr. Muñoz Rivas raised the question whether leprosy is in fact primarily a disease of peripheral nerves and cutaneous tissues. He suspects that leprosy, like certain other infections, may develop in the sequence: local, systemic, focal. This implies that even when we find only a single lesion it may be focal and not always primary.

It may be noted that the modest number of lesions in early indeterminate and tuberculoid could be explained on the basis of a bacteremia which succeeded in producing only a small number of successful "takes". It has been stated that Dr. Campos, Perú, has demonstrated visceral lesions in indeterminate and tuberculoid leprosy.

It is also evident that the large number of lesions which may appear at the onset of lepromatous leprosy are indicative of a prior septicemia.

Re-examination of the true character of the disease might lead to new premises concerning principles for the detection of presently unrecognizable forms of infection.

3. Rehabilitation: A better understanding of psychologic trauma and of the prevention of disabilities is recognized to be important.

4. Epidemiology (+ control): The question is revised:

Are we in a rut of doctrinal application?

The trip to Latin America revealed that, although there are healthy differences of opinion, all leprologists seem to entertain similar proposals, as follows:

a) insofar as possible, we should cease placing patients in leprosaria,

b) the disease can be brought under control by: dermatologic surveys and clinics, treatment of cases, surveillance and/or immunization of contacts, etc. However, two major facts demonstrated by epidemiologic studies are: a) that while the greatest likelihood and concentrations of new cases are associated with recognized cases, b) the greatest absolute number of new cases arises without demonstrated contact with a previous case.

Thus, there is strong evidence that we cannot continue:

a) to ignore the infectiousness of cases which show only periodic bacteriologic positiveness (major or reactional tuberculoid, etc.,) but may be quiescent when discovered.

b) to forget that there may be unrecognizable carriers. (Children,

with their inclination to Mitsuda negativeness, may develop considerable numbers of bacilli and subsequently destroy them without manifest lesions?) (Dr. Latapi said that some "difusa" cases in adults may be healed without the Lucio phenomenon or transformation to nodular lesions.)

The problem of carrier states, therefore, deserves the closest attention of all persons interested in leprosy, irrespective of discipline. It would seem desirable for clinicians and pathologists to re-examine the premise of Munoz Rivas; for epidemiologists to re-examine the implications of their data in order to calculate the number of cases to be expected without demonstrated association with previous cases; and for bacteriologists and immunologists to dream of new principles which might be applicable.

5. Bacteriology: There is no hesitation about assigning a priority to cultivation of M. Leprae. This problem, however, has long been confused by the fact that cultivable types of mycobacteria are recoverable from lepromatous tissues, even after presumed adequate disinfection of skin surfaces. The association of cultivable strains with lepromatous ulcers has been emphasized by Hanks. Ulcers of mycobacterial (non-leprosy) origin have since been described by a series of investigators. Interesting questions remaining are: what types of strains are recovered due to failures to sterilize skin surfaces; what types are associated with ulcerations originating because of leprosy; do some strains reside in skin after its sterilization and irrespective of ulceration; are some of these strains similar to the atypical or anonymous groups causing pulmonary disease or recovered from nose, throat and tonsils?

Study of these questions could bring several rewards: more adequate knowledge of the ecology of mycobacteria in relation to "natural"

stimulations of Mitsuda and tuberculin reactivity; discovery of unknown factors which may precipitate or complicate the lepra reaction; a convenient introduction of students of the tubercle bacillus into the problem of cultivating M. leprae.

6. Immunology: a) Dr. Motta (PAHO), Colombia, raised the question of how to secure more effective and specific immunizations against M. leprae in contacts of leprosy patients. This is an interesting question, particularly when the following factors are considered: - The desirability of reducing the likelihood of infection and also the number of negative or weak Mitsuda reactors which require surveillance, - the limited supply of M. leprae antigen and the fact that this organism seems not to excite strong immune responses.

It would seem desirable to conduct careful studies of the successes obtainable by employing very small amounts of M. leprae while using an accompanying adjuvant (eg., BCG, vaccinia virus, or oil and synthetic polymers) to elevate the immune response. The principle is readily stated: while giving a small amount of antigen, give the immunologic systems a kick in the pants.

b) The Rebuilt Engine: Even after taking into account the advantages to be gained by the principle just discussed, there will remain a small proportion of persons who fail to achieve an adequate specific response. There are also the persons who remain Mitsuda negative or liable to relapse in spite of successful treatment, and the stimuli afforded by infection.

In the long run, however, there is no reason for despair. From studies on genetic inefficiencies and on the immunology of tissue transplantation, we may foresee the time when serious deficiencies toward

leprosy can be corrected in either of two ways; - by repopulating persons with known competent lymphocytes, or by transfer of DNA nucleoproteins which can confer the required templates on existing lymphocytes. It is not premature to aid general progress in this direction, in order to know the day when such principles become applicable to infectious disease and to leprosy.

7. Pathology: a) One valuable new proposal, to employ direct cytologic samples as a means of improving bacteriologic indices and of replacing the expense and tedium of preparing tissue sections, has been mentioned (Brazil, Dr. Rene).

b) Other interesting questions have been posed in this section of the report.

8. Other: The extent to which susceptibility or resistance to mycobacterial diseases can be modified by diet remains surrounded by some uncertainties. However, there is no question that the lipid stores of the body are amenable to experimental modification and that such items are among the important nutriments of mycobacteria.

Evidence also is accumulating that there are regional associations between the occurrence of tuberculosis and of kwashiorkor. Furthermore, the amino acid requirements for normal growth are less demanding than the requirements during physiologic stress and infectious disease (Dubos).

Such considerations are applicable not only to the problem of transmitting leprosy to animals, but to the management of the human disease itself.

Comment: Any person who undertakes to make suggestions on priorities in leprosy research may state a few facets which seem to be of interest. Much more would be gained by establishing a symposium or journal solely for the expression of unorthodox ideas. Who knows where eventual answers may lie?

DIRECT CYTOLOGIC STUDY OF THE SKIN  
IN LEPROSY

Summary.-

With a view to throwing light on the mechanism of resistance to leprosy the types of cutaneous cellular response to natural infection and to the injection of lepromin are to be studied. A direct method of examination will be used, similar to that of modern studies of exfoliated cells in the diagnosis of cancer. Instead of the usual biopsy specimen, scrapings from the corium will provide the materials for study. One interesting possibility is that in patients under treatment with sulfones and whose lepromin tests are negative, microscopic examination of cells scraped from the site of infection of the lepromin may give evidence of a favorable cellular response before clinical signs of improvement are apparent.

A. Specific Aims - The specific objective of these studies is to advance knowledge of cellular resistance in leprosy. The usual histopathologic techniques will be adapted to the direct study of the cutaneous cytology in leprosy; special staining methods will permit simultaneous study both of M. leprae and of tissue cells.

In patients with different forms and in different stages of leprosy, treated and untreated, there will be studied the relationship between type of cellular response to the intracutaneous injection of lepromin and clinical or bacteriologic evidence of severity or mildness and of regression or improvement of the disease. Supplemen-

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using macrophages is that these cells are thought to be of the same lineage as epithelioid cells which are the only cells which actively phagocytize the bacilli in the natural disease. This applies also to the use of histiocytic tumor cells.

C. Significance of this Research Cultivation of M. leprae is one of the most important, if not the most important, objectives in leprosy research. Success will lead to such developments as direct screening of drugs, specific antigens for serology and for lepromin, and to a vaccine for experimental study. Study of the lipids, proteins and other components of bacilli would be feasible. Furthermore, it would allow unlimited expansion of studies in experimental transmission, now dependent on suspensions of bacilli obtained from patients.

The plan which is proposed envisages obtaining from the United States for three weeks during the first year; six weeks during the second; and three weeks during the third, a scientist well-qualified in tissue culture procedures, especially as these relate to macrophages. This expert will assist Drs. Azevedo and Wanderley and give to the technicians in our laboratory the additional training which we consider essential in this complex field.

Leprosy is one of the most important infectious diseases in Central and South America. In Brazil alone there are more than 150,000 cases and no decrease in incidence has been detected. The PAHO/WHO, recognizing these facts, have adopted the control of leprosy as one of the Organization's major objectives. The proposal herewith submitted is one which will benefit not only the Americas but all countries where leprosy is an important problem.

with M. leprae. Nevertheless, the degree and character of the cellular response are known to vary greatly and have been used for many years as a means of differentiation between the major types of disease--lepromatous and tuberculoid. Virchow's cells and globi are the mark of the lepromatous type; accumulations of epithelioid cells and giant cells of the Langhans type the mark of the tuberculoid type. Similarly, the reaction to intradermal injection of lepromin (which contains large numbers of M. leprae) is pronounced in the less severe type of the disease (tuberculoid) and macroscopically absent in the lepromatous type. So called "natural reactivity" to lepromin is common among healthy persons, both in endemic areas and in places where leprosy is unknown; increases rapidly in childhood and adolescence as age progresses and is considered to represent some degree of resistance to the disease.

Changes in the cytology of the skin in leprosy may be of great significance in prognosis. They may indicated the effect of therapy before gross appearance of the lesions gives any sign of change. They may indicate in tuberculoid or indeterminate lesions evidence of change to the lepromatous type whereas at present successive biopsies, each requiring several days and discomfort to the patients are required to detect these changes. The method which is proposed, if successful, will give results within an hour. In short, the principal significance of this project, if it results in staining of bacilli and excellent preservation of tissue cells, is that:

- 1) Immediate and direct staining will replace the much more tedious and expensive preparation of tissue sections.

- 2) Application of this relatively simple procedure to leprosy cases of different classes will permit some quantitative evaluation of the ratio of bacilli to tissue and perhaps aid in prognosis.
- 3) Application to the reactions induced by the intracutaneous injection of lepromin (Mitsuda reaction) may detect in lepromatous cases signs of an immune tissue response in cases where palpation indicates a doubtful or negative result. In general, studies of carefully fixed and stained cells obtained by slitting the skin at the site of the lepromin injection should give the trained cytologist much information concerning the type of cells entering into the reaction and the way in which these cells are handling the killed bacilli used in the test - i.e., should be an indication of cellular immunity.

D. Facilities Available - All the facilities of the Institute of Leprology are available. The Institute located on the Rua São Cristóvão 1298, Rio de Janeiro, State of Guanabara, Brazil, is an organ of the National Leprosy Service of Brazil which is a principal division of the National Department of Health of the Ministry of Health. The Institute was established in 1944. The budget of the Institute which was approximately nine million cruzeiros (\$30-40,000) in 1961 is included in that for the National Leprosy Service. There are currently 13 members of the scientific staff; 1 Director, 2 in the Section of Bacteriology and Immunology;

3 in Anatomic Pathology; 2 in Biochemistry and Pharmacology; and 5 in Clinical Investigation. The total will be 14 when a vacancy in chemistry is filled.

The equipment of the Institute is adequate for the investigation proposed in this application. This includes the usual equipment for routine histopathologic examinations. There are about 70 hospitalized patients in Frei Antonio and this number is to be increased substantially soon. More than 300 patients are attending the outpatient clinic of the Institute. Also there are two large leprosaria within easy distance.

A close relationship is maintained between the Institute staff and the staff of the Oswaldo Cruz Institute and the Cancer Hospital at both of which cytology research of a high order is being carried on.

E. Previous Work - The Principal Investigator has been interested in cytologic investigations for 8 years (since taking special training at the Oswaldo Cruz Institute in 1954). Particularly pertinent is the comparative study of the macroscopic readings and microscopic findings of the lepromin reaction published in 1960. It was discovered that the cellular response in many reactions read as negative because the size was below the standards set by the WHO and the International Leprosy Association, and even in some instances when no reaction whatever could be palpated, nevertheless presented the same characteristics as did the cells observed in definitely positive reactions. This finding has led

to a great deal of experimental work on leprosy patients and normal persons, only a fragment of which can be satisfactorily completed because of lack of staff.

BUDGET.-

Personnel.-

1 Pathologist (100%)	\$4,250
1 Pathologist (Full-time in Institute 25%)	1,680
1 Pathologist (Full-time in Institute 15%)	--
Consultant (two weeks)	700
Technician (1 full-time 100%)	--
Technician (1 part-time, as necessary)	--

Permanent Equipment.-

NONE

Consumable Supplies.-

\$ 300

Not available in Brazil

Special stains \$100

Purified reagents \$200

Travel.-

Consultant from U.S.A. (2 weeks)	
per diems \$280	
fare \$1,020	

\$1,300

Consultations at Sao Paulo

150

Total Direct Cost Requirements

8,380

Indirect Cost Allowance (7.5%)

627

Total Budget

\$8,907

## CULTIVATION OF MYCOBACTERIUM LEPRAE IN HUMAN MACROPHAGES

Summary

The purpose of the proposed work is to cultivate the leprosy bacillus in human macrophages obtained from patients and from normal persons, including cells from human histiocytic tumors. The reason for using macrophages is that they are considered to be of the same lineage as the epithelioid cells of the leproma. The patients and normal persons chosen as donors of tissue cells will be lepromin-negative because this is considered to indicate lack of cellular resistance to leprosy.

The facilities at São Paulo are excellent; a new 9-story building for the leprosy department will be completed by the end of 1962 and many patients are available for study.

A. Specific Aims To continue efforts to cultivate Mycobacterium leprae in tissue cultures using, as host cells, human macrophages from normal persons and from patients, including cells from human histiocytic tumors. Cells from the human fetus will also be tried.

B. Method of Procedure Various methods are to be explored. In general, the plan is to obtain cells from blood, skin, fetal material and tumors and to inoculate them in various tissue culture systems using tubes, flasks and slides. Suspensions of M. leprae will be introduced into the systems, using heated bacilli as control. Media will be changed as necessary, using the hydrogen ion concentration as a guide. Daily observations will be made and transfers to fresh tissue culture systems and to synthetic media will be made at those times when prospects seem most promising. Lepromin-negative patients and normal persons will be used as a source of macrophages because those suffering from the bacillus-rich lepromatous type of the disease are almost universally lepromin-negative. The reason for

tal studies will be made of the cellular response in normal persons both contacts and non-contacts.

B. Methods of Procedure - To obtain specimens from the skin, a clamp is applied to exclude blood cells from the site. A slit incision is made by the technique of Wade and Rodriguez deep enough to expose the whole thickness of the corium. The lateral walls of the incision are scraped with the knife and the material (tissue cells, and bacilli if present) are transferred to a slide and fixed moist in equal parts of alcohol and ether. These specimens have been stained by us by Giemsa or by Shorrs stain - essentially as in studies in exfoliative vaginal cytology, the bacilli being stained by Ziehl-Neelsen. It is proposed to test the degree to which the tissue cells retain their integrity by Hanks methods by which the bacilli are stained first with carbo-fuchsin.

The tissue cells can be enumerated while being differentiated and classified and the ratio of bacilli to cells can be determined. This index reflects the status of the infection because the number of cells in the specimen will represent approximately the amount of tissue examined.

In the lepromin studies the antigens used will include not only the classical Mitsuda-Hayashi lepromin but lepromin from which all but minute portions of the tissue have been removed leaving an almost pure bacillary suspension. "Lepromin" containing only tissue components will also be used.

C. Significance - The character of the cellular response in the granuloma cannot measure all the factors which are involved in resistance to leprosy and which influence the outcome of infection

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bacilli; if these are found an attempt will be made to cultivate them and suspensions will be injected into mice and hamsters. In addition to the principal objective an attempt will be made to obtain data relating to a possible genetic factor (or factors) which may influence the occurrence of severity of leprosy or both. For every family in which a case of leprosy is found, and for a group of selected and comparable control families, pedigree charts will be prepared. These charts will go back wherever possible to the grandparents of the first current case and in the control families to the grandparents of a selected individual of the same sex and age. This last phase will be planned with the assistance of Professor Dario Curriel.

This study involves repetition of examinations. Each person must be seen at least three times and those in whom leprosy is suspected will have to be examined on several additional occasions. Smears will be made from suspected lesions and stained for acid-fast bacilli. At least one biopsy specimen will be obtained from each patient studied for confirmation of diagnosis and of type of disease.

All data will be transferred to simple punch cards for hand tabulation. For each zone and sector, prevalence rates for each type of leprosy will be calculated and these rates will be broken down by age, sex, color, nutritional status, presence of other skin diseases and sanitary classes (type of water supply and of excreta disposal), and results of tuberculin and Mitsuda tests. In addition the respective population groups and leprosy cases will be studied in relation to exposure to various domestic animals and to the common varieties of insects.

Preliminary studies on the selection of zones and sectors are to be made immediately. It is proposed that the services of a consultant

epidemiologist be secured to aid in the planning and to assist at intervals throughout the study.

The estimated time required to complete all phases of the study is four years.

C. Significance This inquiry will throw light upon several important questions the answers to which are of fundamental importance in the control of leprosy.

Is there any relationship between environmental conditions such as overcrowding, insanitation, or diet and the frequency of leprosy? Or between any observable environmental factor and the predominance of either major type, that is, lepromatous or tuberculoid? Great variations in the relative frequencies of the respective types have been reported in different parts of the world and we think that variations occur in different parts of Venezuela but we have no clue as to the cause. The question must be studied carefully because of its practical importance in control of this disease--the lepromatous type being by far the more infectious.

Is any environmental factor associated with resistance to leprosy in normal persons, in so far as this resistance is indicated by reactivity to lepromin? "Natural" reactivity to lepromin is gained as age increases but the cause is unknown.

Is there any evidence that an insect is involved in the transmission of leprosy? The bedbug, flea and itch-mite have been suspected of being vectors but there is no scientific evidence to support this view. Acid-fast bacilli have been reported in these insects but there are no modern studies.

Proof of relationship between any environmental factor and the

Other Expense

Excess Baggage (Consultant) 40-50 lbs.  
teaching materials (RT) \$ 150

Indirect Cost

(7.5%) 670

TOTAL \$9,680

STUDY OF ECOLOGICAL FACTORS IN LEPROSY

Summary

The principal objective of the proposed study is to search for environmental factors associated with the frequency or severity of leprosy in Venezuela. Two principal rural zones in which the disease is endemic, one mountainous in Western Venezuela, the other at a lower level in the Eastern section of the country are to be selected for study. Each zone will be divided into sectors according to known prevalence rates. A complete house-to-house census and sanitary inspection will be made followed by physical examination of all the inhabitants. Careful diagnosis studies will be made in every case or suspected case of leprosy. A special investigation will be made of the arthropod population in the search for a possible vector

A. Specific Aims To search for ecological factors associated with the frequency or severity of leprosy. The ultimate objective is to eradicate the disease by the identification and elimination of such factors. Data regarding possible hereditary factors will be obtained on families of patients and selected control families.

B. Methods of Procedure Two principal rural zones, in which leprosy is endemic, widely separated from one another will be selected, the one mountainous in Western Venezuela, the other at a lower elevation or sea level in the Eastern or the Southern part of the country. In the mountainous area the racial stock is European with some Indian intermixture; in the other area there is some Negro in addition to Caucasian and Indian blood.

Each of these zones will be subdivided into 3 sectors; high prevalence (average above 15 per 1000); lower prevalence, approximately

7.5 per 1000, and a third where, according to present information, there is no leprosy. Each of these sectors will have approximately 5000 population and the total population of both study zones will be about 30,000 persons.

In each sector a house-to-house census will be conducted by specially trained auxiliary personnel. At the same time a sanitary inspection of each house and its immediate environment will be made. There will follow a physical examination of all inhabitants by an epidemiologist with several years experience in leprosy. This examination will include inspection of the skin of the entire body and a search for disturbance of function of those peripheral nerves usually involved in leprosy. The height and weight of each person will be recorded and the hemoglobin level will be estimated with the talquist scale. The general health of the individual will be noted as well as the presence or absence of signs of physical and mental illness. A special search will be made for signs of dietary deficiencies and also for parasitic infestations (stool examinations in a sample of the population).

Each person will be tested with tuberculin ( 5 TU of PPD) and with Mitsuda-Hayashi lepromin, both of these tests being made throughout both zones with the same materials.

An important phase of the study will be an intensive search for possible insect vectors. In addition to the recording of presence of common insects on all household schedules, in every house in which leprosy is found and in a sample of houses free from leprosy insects will be collected, tagged, and sent to the Central Laboratory at Caracas for identification. These insects will be examined for acid-fast

D. Facilities Available A new laboratory building for the Institute for Leprosy Research of the State of São Paulo is now under construction and will be completed by the end of 1962. About 1,200 square meters of laboratory space will be available, exclusive of animal quarters. For tissue culture, 322 square meters are reserved. The equipment to be transferred from the present laboratory includes the usual centrifuges, a high speed refrigerated centrifuge, a Zeiss photomicroscope with fluorescence and phase attachments, incubators for operation from 25°C. to 38°C. and glassware. Items of equipment which are needed and which we hope can be obtained from the requested grant are listed below. A large number and a wide variety of leprosy patients are available for study at the Institute which gives especial pertinence to this laboratory as a place for these studies.

BUDGET.-Personnel

Leprologist	66%	\$ -
Biologist	100%	\$ -
Consultant from U.S.A. (21 days at \$50)		\$1,050
Technicians (2)	100%	

Permanent Equipment

Refrigerator, Sub-Zero, two-stage, -85° F, 6.5 cubic feet.		\$1,235
Oven-Drying, Electric, double wall range 200° C.		\$ 250
Carbon Dioxide Incubator		\$1,245
Other equipment		\$1,284
		<u>\$4,014</u>

Supplies - (First Year)

<u>Falcon Plastics</u> Tissue culture container		\$ 137
<u>Difco</u> Bovine embryo extract		\$1,050
<u>Microbiological Associates</u> NCTC 109, a protein-free, chemically defined medium. (incl. \$224 Freight and Ins.)		\$1,274

Supplies - (Second and Third Years)

<u>Difco</u> Description above (60 packages)		\$1,050
<u>Micro. Assoc.</u> Description above (300 bottles)		\$1,050

Sub-total	\$2,100
Freight and Ins.	\$ 210
TOTAL	<u>\$2,310</u>

Travel

Consultant N.Y.- São Paulo (RT)	\$ 910
Per diems 21 x \$20	\$ 420

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frequency or severity of leprosy or between any of these factors and reactivity to lepromin would be a significant step towards the prevention of the disease.

On the practical side, data obtained in this study will permit us to make reliable estimates of the prevalence of the various forms of leprosy in Venezuela. In addition to the two major types there is increasing interest in the so-called "indeterminate" group regarded by some leprologists as unstable and of potential importance in the spread of the disease. The two zones chosen will represent those parts of the country where the disease is of importance and where our major efforts at control are being made. The present estimate of total cases is 12,000 but we suspect this to be too low.

In addition to being applicable to leprosy, the data which are obtained will reveal the prevalence of tuberculous infection, in so far as this can be deduced from the occurrence of hypersensitivity to PPD (5 TU). A basis will be laid for subsequent and more intensive studies of malnutrition, and of parasitic and other diseases. The presence or absence of smallpox and BCG vaccination scars will be recorded for each individual. Information which is obtained concerning the frequency of blindness and other physical disabilities which will be of value in our new rehabilitation program, and of future public health work in Venezuela.

Currently, and as a part of the daily work of the study, all persons who are found to be suffering from leprosy or other diseases will be referred to the nearest health center for treatment.

D. Facilities Available Throughout Venezuela there are 80 health Centers. In every state (20) and in the territories (2) there is at least one principal center with a complete public health staff and laboratory. Each state and territory is subdivided into districts, each of which has a center, usually smaller than the main one but which has one physician, auxiliary personnel and a small laboratory for routine work. In addition, in most municipalities one full-time physician, a nurse and sanitary inspector are employed. Each physician is furnished with a microscope. Thus, in each sector selected, there will be at least one larger health center and several municipal centers. In each major center of each state and in some of the district centers there is a specialist leprologist whose services are available as required.

The principal laboratory of the Department of Health of Venezuela is located in a new building in Caracas. It has a large staff and ample equipment for bacteriology and serology. Histopathologic service is furnished by Laboratory of the Division of Public Health Dermatology located in the same building. In this laboratory, transmission experiments in leprosy are now actively in progress. It is therefore readily possible to furnish all additional laboratory services which are envisaged in this project.

Statistical services will be rendered by the Division of Public Health Dermatology. Transportation for all field personnel--physicians and auxiliary workers--will be provided by the Division of Public Health Dermatology. Subsistence is requested only while these men are away from their homes.

Equipment - All necessary equipment will be provided by the Division of Public Health Dermatology.

Supplies - All supplies will be furnished by the Division except that it will be necessary to purchase punch cards as mentioned.

BUDGET

1. Personnel

Leprologist (Spends full-time in Division)	20%	\$ -
Leprologist	100%	\$9,600
Dermatologist (Spends full- time in Division)	15%	\$ -
Consultant (to be selected) (45 days)		\$2,250

2. Permanent Equipment

None

3. Consumable Supplies

7,000 family punch cards )	\$2,000
35,000 individual punch cards )	

4. Travel \$19,828

Total Budget \$33,678