

**PAN AMERICAN HEALTH
ORGANIZATION**

**ADVISORY COMMITTEE
ON MEDICAL RESEARCH**

FOURTH MEETING

14-18 JUNE 1965

Washington, D.C.

**RESEARCH ACTIVITIES OF PAHO
IN SELECTED FIELDS
(1964-1965)**

-B-

Ref: RES 4/2B

30 April 1965

PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
WORLD HEALTH ORGANIZATION

WASHINGTON, D.C.

RESEARCH ACTIVITIES OF PAHO
IN SELECTED FIELDS

Table of Contents

	<u>Page</u>
1. Endemic Goiter.	1
2. Nutritional Anemias	9
3. Malaria	19
4. Mental Health Information Center on Latin America . .	25
5. Population Dynamics	27
6. Research Training and Medical Education	31
7. Dental Public Health.	35
8. Radiation and Isotopes.	37
9. Tuberculosis.	43

Appendix I

Biological Effect of Background Radiation in Man

Appendix II

Manganese Poisoning in Chile

Appendix III

Biodynamics of the Vitamins D and Certain Trace
Metals in Osteomalacia

Appendix IV

Investigation of the Biology and Ecology of Rhodnius
prolixus

RESEARCH ACTIVITIES OF PAHO
IN SELECTED FIELDS*

1. Endemic Goiter **

The investigators participating in the PAHO sponsored research program in endemic goiter have continued their research activities in the laboratory and in the field. During the year, the PAHO Reference Laboratory and Training Center for Iodine Determinations in the Study of Endemic Goiter was established in Santiago in the Department of Medicine, University of Chile, Hospital del Salvador, under the direction of Dr. José Barzelatto, through a grant of \$32,700 from the Williams-Waterman Fund. The grant made possible the initiation of activities of the control laboratory and will provide for its operations during the first three years. The Reference Laboratory has two main purposes: to serve as a center for the standardization of iodine determinations in samples of serum, urine, feces, soil, water and food; and to serve as a training center for technical personnel from the participating laboratories in the PAHO research program in endemic goiter, and for the training of other investigators in Latin America who wish to acquire chemical techniques for iodine determination and establish these in their home laboratories.

*Prepared for the Fourth Meeting of the PAHO Advisory Committee on Medical Research, 14-18 June 1965, by the Office of Research Coordination from reports submitted by PAHO staff and consultants.

**Based upon a report from Dr. J. B. Stanbury, Massachusetts General Hospital, Boston, Massachusetts.

The Reference Laboratory does not have a service function but will serve as a control of other laboratories. It will in turn have spot samples controlled by the Boston Medical Laboratory and by Professor Kellershohn's Laboratory in Paris, using the technique of activation analysis.

The chief technician who will be the full-time senior technical person in the Reference Center, has undergone an eight-week training period at the Boston Medical Laboratory and has returned to Santiago to establish the methods. A consultant from the Boston Medical Laboratory will visit the Reference Laboratory late this year to insure its optimal operation. The first trainee is expected late in 1965. It is anticipated that approximately four trainees from Latin American countries will be in residence for a period of about two months each during each year.

The equipment is being purchased and the purity of the reagents is being controlled by the Boston Medical Laboratory before being sent to Santiago.

1.1 Chile

The group, under the direction of Dr. José Barzelatto, has been active in research on the endemic goiter of the Pewenche Indians of the Pedregoso reservation in southern Chile. In the past two years they have made three expeditions to this area. A survey of the entire community for thyroid and other diseases focussing on the nutritional problems has been made. The thyroid function studies have included P.T.C. taste sensitivity, reflex relaxation

time, radioiodine uptake and turnover, circulating iodoproteins, perchlorate and methylmercaptoimidazole effects, TSH and triiodothyronine effects on iodine metabolism, and other studies related to iodine metabolism. These findings are scheduled for publication as follows: one paper is in press (NATURE), one has been submitted for publication, and two are in preparation.

To date the data are consistent with iodine deficiency as the sole cause of the endemic, but the possibility of a goitrogenic agent in the araucaria nut, the principal item of the diet of the Indians in the area, has not been eliminated. Studies in rats to determine whether this nut contains a goitrogenic substance are in progress. Further studies among these Indians are planned.

1.2 Ecuador

A field study of eight villages with varying goiter incidence has been completed by Dr. Rodrigo Fierro and his group. A total of 4,929 persons have been surveyed and interviewed. Volunteers from each of these villages have been taken to Quito for intensive thyroid function studies. The water, soil, salt, and food from these villages have been measured for iodine. Ten extremely retarded subjects from two of these villages were taken to Quito for study of their cretinism, their development and state of health. They are now receiving full doses of thyroid hormone and will be returned to Quito after a year of therapy for a re-evaluation.

A maternal and child health service has been established in two of these villages with the specific purpose of obtaining more information about the relationship between iodine metabolism and the occurrence of cretinism. The incidence of cretinism in these two villages is high. As much information as is possible regarding thyroid state in mother and neonate is being obtained.

Dr. Fierro will present some of his data at a meeting in Madrid and at the Fifth International Thyroid Conference, 24-29 May 1965, in Rome. He will then spend a month in the Thyroid Laboratory of the Massachusetts General Hospital preparing his findings for publication before returning to Quito.

1.3 Brazil

Because of the necessity of preparing material for publication, field studies by Dr. Yaro R. Gandra's group in São Paulo were interrupted towards the end of 1964. Up to that time, clinical examinations had been made on school children from 154 towns in the State of São Paulo, and the incidence of goiter was measured. Tests in 394 school children, revealed that the radioiodine uptake values and PBI estimates were within normal limits.

A study was made of the effect of iodine prophylaxis on the incidence of goiter which was determined at an earlier time in some of these districts. It was found that the amount of iodine in the iodinated salt was less than that established by law, presumably because of sublimation and, therefore, the results of iodide

prophylaxis were not so good as might have been anticipated. At the present time Dr. Gandra is preparing his material for formal publication.

In Rio de Janeiro, Dr. L. C. Lobo's group has received a substantial grant from the National Institutes of Health / USPHS - for a biochemical, epidemiological, and genetic study of certain communities in the State of Mato Grosso, where goiter incidence is high. This joint operation with the University of São Paulo is well underway at the present time. A complete medical and anthropological inventory is being made on the population of a whole community to include intensive studies of thyroid physiology with particular emphasis on cretinism. A large amount of data has already been accumulated for analysis, but specific results are not yet available. It is of interest that in at least one of these areas the incidence of consanguine marriages is more than 50%.

Dr. Lobo will attend the Fifth International Thyroid Conference in Rome in May, 1965 and present some of his data.

1.4 Colombia

Dr. Eduardo Gaitán's group in Cali has continued their studies in the Cauca River Valley of Colombia, where the incidence of goiter is higher than 50% and where in certain districts 65% of the cattle also have goiter. Iodized salt has been in general use in this area since 1952. Their findings reveal that I^{131} uptake and PBI¹²⁷ and PBI¹³¹ are normal, the urinary excretion of iodine is high, and the glands respond normally to TSH and thyroxine.

A high incidence of thyroid neoplasia, particularly of the anaplastic type, is seen in goitrous patients in this area. Dr. Gaitán's results are not entirely consistent with iodine deficiency as the principal cause of the endemic goiter in this area of Colombia. It will be of the greatest interest to discuss these data during the second meeting of the PAHO Scientific Group on Research in Endemic Goiter in Mexico, 5-9 October, 1965.

1.5 Venezuela

No field studies have been done during the past year by the group under Drs. Marcel Roche and Karl Gaede at the Instituto Venezolano de Investigaciones Cientificas (IVIC). It is the intention of Drs. Roche and Gaede to pursue at the earliest possible time their observations on the Indian isolates of Venezuela, who in many instances have a high uptake of radioiodine without goiter. In the meanwhile, laboratory studies on thyroid physiology continue at I.V.I.C. to include glycolysis and the purification of glycolytic enzymes from the thyroid. A few measurements for iodine content have been made on water from foci of endemic goiter in that country.

1.6 Mexico

The principal activity of the group under Dr. Jorge Maisterrena continues to be the relationship between dietary factors and endemic goiter. It has made surveys of a large number of communities within 100 kilometers of Mexico City, examined the inhabitants and determined the iodine content of their water supply.

In addition they have done detailed metabolic and physiologic studies on patients with endemic goiter, particularly in the Tepetlixpa area. This is the first study ever done on detailed iodine balance in the field. The research is supported by the NIH, U.S. Public Health Service. Several papers from Dr. Maisterrena and his group have been published recently, and others will appear shortly in local and in international journals.

1.7 Second Meeting of the PAHO Scientific Group on Research in Endemic Goiter

Final plans are now being made for the second meeting of this group in Cuernavaca, Mexico, 5-9 October, 1965, immediately preceding the Seventh Meeting of the Pan American Endocrine Society to be held in Mexico City. Each of the laboratories in Brazil, Chile, Colombia, Ecuador, Mexico and Venezuela mentioned above will participate. In addition consultants from Belgium and Holland, the scientific coordinators of the PAHO Research Program in Endemic Goiter (Drs. Stanbury and DeGroot) and PAHO staff will attend. The purpose of the meeting is to summarize results obtained thus far by the participating laboratories and to plan further investigations.

1.8 Salt Iodization and Prophylaxis

A Seminar on Salt Iodization for Endemic Goiter Prophylaxis will be held in Salta, Argentina on 21-25 June 1965. The meeting will be attended by officials of ministries of health and of other

government agencies responsible for iodization programs, representing 15 Latin American countries where goiter is a prominent public health problem, and by PAHO nutrition advisers and consultants.

Its purpose is to bring together for an exchange of views between the technical authorities on endemic goiter in the health agency and those authorities in other agencies responsible for the implementation of the salt iodization program.

It is hoped that these discussions will accelerate the resolution of the problems preventing effective salt iodization in those countries.

2. Nutritional Anemias*

2.1 At the PAHO/ACMR meeting in June, 1964, a report was submitted which summarized aspects of the WHO program on nutritional anemias and emphasized in particular the developments in Latin America under PAHO/WHO sponsorship. It was concluded that in Latin America

- a) information available on the nutritional anemias is inadequate;
- b) anemia is a significant problem;
- c) the establishment of a reference laboratory in the nutritional anemias is essential.

In January, 1965, financial support for establishing the Reference Laboratory became available from the Williams-Waterman Fund, Research Corporation. This was in the form of a grant for \$41,280 to be administered by PAHO in support of the research and training activities of the PAHO Reference Laboratory and Training Center in Nutritional Anemias at the IVIC in Caracas, Venezuela, for a period of three years. The laboratory is now operational. The Scientific Advisory Group for this program includes Drs. Victor Herbert, William Crosby, Maxwell M. Wintrobe and Clement A. Finch.

During the latter part of 1964, pilot collaborative studies between Dr. J. Chopra in Trinidad and Dr. Miguel Layrisse in Caracas were undertaken to work out logistic problems of sample transport between the investigator and the reference laboratory. With the activation of the study in 1965 the collaborators

* Based upon a report by Dr. C. A. Finch, Department of Medicine, University of Washington, Seattle, Washington.

who have been formally taken into the study include Dr. Layrisse in Caracas, Dr. Chopra in Trinidad, Dr. L. Sanchez Medal in Mexico City, Dr. Fernando Viteri in Guatemala, and Dr. Herman Vélez in Medellin, Colombia. These individuals all plan to participate in the studies developed by WHO on tissue iron stores and on the incidence of iron deficiency anemia in pregnancy and infancy. Arrangements are also being made for each of the investigators and a designated laboratory technician to visit the Center in Caracas during this year for the purpose of training in the standardized methodology for plasma iron and iron binding capacity, B₁₂ and folate microbiologic assays.

2.1.1 The following are the recommendations concerning this program:

a) That PAHO take responsibility of appointing the collaborators to the study who have been recommended by the Scientific Advisory Group and who have shown willingness and ability to participate in the anemia studies referred to above. Each participant should also be encouraged to carry out independent studies of other aspects of nutritional anemia of particular interest to him.

b) During the coming year it would be desirable to enlarge the group of participants to represent other geographic areas of Latin America. Such investigators are best identified by direct contact. It would seem important to find collaborators with adequate facilities, permanent positions, backgrounds in hematology, and a high degree of research competence.

c) Plans should be made in one or two years for a meeting of the Participating laboratories and of the Scientific Advisory Group to review the work in progress and plan further research activities.

d) Additional steps should be taken to finance this program. While current funds are adequate for starting the project, stability would be provided if support could be extended over a period of six or more years. Attention needs to be given to finding financial support for the participants' individual research projects in nutritional anemias.

2.2 Report of Consultant Visit by Dr. C. A. Finch to Latin America, January 16 - 25 1965

The purpose of the visit was to discuss with four possible collaborators in Mexico, Guatemala, Colombia and Peru the PAHO/WHO program on nutritional anemias. Through direct discussions, it was hoped that their interest in the program, their particular needs, and the research potential of their laboratories could be accurately characterized.

2.2.1 Dr. Luis Sanchez Medal at the Instituto Nacional de la Nutricion, Mexico City. Dr. Sanchez Medal is prepared to enter into the PAHO/WHO program in collaboration with the PAHO Reference Laboratory in Caracas. He has designated Dr. Pizzuto to personally carry out this program. Dr. Pizzuto is now with Dr. M. M. Wintrobe and will complete his training there in October.

It would seem advantageous that Dr. Pizzuto visit Dr. Victor Herbert's laboratory and possibly Dr. Finch's before returning to Mexico City. Dr. Pizzuto would then be prepared to go to the Reference Laboratory before heading the nutritional anemias program in Dr. Sanchez Medal's laboratory. His salary will be paid by the Hospital de Enfermedades de la Nutricion in Mexico at least for several years. This is an ideal situation for survey studies throughout Mexico because the Hospital already has a program of such studies which are being carried out in the country.

The immediate need in undertaking this program is for funds for a technician, for general expenses, and for some minor equipment needed for the special procedures to be carried out. It is hoped that these funds might be obtained from the Government of Mexico at a later date although this is by no means definite. In evaluating these financial needs, it appears that \$7,000 for the first year and \$5,000 a year for the next two to four years would place the program on a very solid foundation. The general facilities of Dr. Sanchez Medal's laboratories will be greatly improved within the next year by a new building to be constructed with funds donated by the Wenner Gren Foundation of Sweden.

2.2.1.1 The program as visualized by Dr. Sanchez Medal can be summarized under four main headings;

a) The work on the pregnancy and childhood protocols as outlined by the WHO Scientific Group on Nutritional Anemias can be started in the very near future. The assistance of a technician,

while not essential for this, would be of help in insuring the collection of reliable and accurate data. At the moment, such a program would depend on the transport of samples from Mexico City to Caracas for B₁₂ and folate analysis.

b) Liver specimens can be collected and a start will be made in the very near future to carry out the protocol as outlined by the WHO Scientific Group on Nutritional Anemias.

c) A special study will be undertaken on the occurrence of iron deficiency anemia in males. It has been the impression of Dr. Sanchez Medal that this occurs without any obvious explanation. Surveys would be carried out in the vicinity of Mexico City to identify such individuals, and to attempt to determine the cause of their deficiency, including studies of iron loss and iron absorption. Another approach would be to select groups of subjects for evaluating iron balance. These individuals could be bled the equivalent of one, two or three micrograms of iron per day over a prolonged period and their ability to withstand these varying degrees of negative iron balance would be assayed.

d) Since the Hospital with which Dr. Sanchez Medal is associated is intimately concerned with nutritional states, there are opportunities to evaluate various aspects of malnutrition. One of these would be a study of the aplastic state where various factors such as protein depletion, riboflavin deficiency, and others might be evaluated. Transferrin changes could also be examined in malnutrition states and during recovery.

It is the consultant's opinion that this is an ideal situation for collaborative studies in nutritional anemias and that every effort should be made to bring Dr. Sanchez Medal and his group into active collaboration with the Reference Laboratory at the earliest possible moment. There is no question that the work would be of a fine caliber. The opportunities in this particular Hospital for studying nutritional problems throughout Mexico would seem to be ideal. The program as outlined by Dr. Sanchez Medal is consistent with the objectives of the PAHO/WHO program and, furthermore, promises more specific studies in this general area of interest. It would seem important to seek sufficient funds so that the success of these studies could be assured.

2.2.2 Dr. Fernando Viteri at the Instituto de Nutricion de Centro America y Panama, in Guatemala City. Dr. Viteri is prepared to enter into the PAHO/WHO program in collaboration with the PAHO Reference Laboratory in Caracas. Both he and Dr. Ray Wood, who has been assigned for the next year and a half to work with Dr. Viteri through ICNND, would go to Caracas along with a laboratory technician. The exact time is not clear since the ICNND survey starts on February 15th and will run for one or two months. However, after this time they would like to visit the Center. Dr. Viteri's laboratory at the present time is adequately financed so that additional funds would not be required other than those for mailing of samples.

2.2.2.1 The program as visualized by Dr. Viteri can be summarized under the following headings:

a) The pregnancy and childhood protocols as outlined by the WHO Scientific Group on Nutritional Anemias can be started as soon as the ICNND survey is over. In respect to pregnancy, it will be necessary to study the women immediately before delivery since they are not available before that time.

b) Liver specimens will be collected according to the protocol in collaboration with the Department of Pathology (Dr. Tejada and his associates).

c) A special survey to determine the hematologic status of the population will be conducted as part of the ICNND study. The total investigation involves a sample of 25,000 people. Blood samples will be taken from 5,000 of these for hemoglobin, hematocrit and smear.* Five hundred individuals will be selected for detailed hematologic studies involving plasma iron, iron binding capacity, B₁₂ and folate levels along with the other hematologic information.

d) Special studies are planned concerning the anemia of malnutrition. These will include blood volume measurements, erythropoietin assays (in collaboration with C. A. Finch), and various special tests relating to iron, B₁₂ and folate metabolism.

* In view of the time delay between sampling and running the tests, it seemed as if a blood count done by the Coulter counter would be more satisfactory than hematocrit. This would provide an MCH which is probably the most valuable single determination in detecting abnormalities of red cell hemoglobin content.

RES 4/2B

A protocol for the study has been set up to evaluate the effect in malnourished children of a low protein diet with vitamin supplement on the one hand, and a high protein diet without vitamin supplement on the other. The first group would be given a high protein diet after a two week period. These studies should lead to a better definition of protein malnutrition and permit evaluation of the various factors of importance in its etiology.

Since the consultant's visit two years ago to Dr. Viteri's laboratory, notable progress in starting a hematology laboratory and a research program was observed. During the next few years, his laboratory will have an opportunity to demonstrate its ability to focus on specific projects and to carry them through to a successful conclusion. Dr. Viteri is interested in the PAHO/WHO program and in carrying out related studies. It seems most appropriate that he should be one of the original collaborators in the PAHO/WHO program.

2.2.3 Dr. Hernan Vélez, Facultad de Medicina, Universidad de Antioquia, Medellin, Colombia. Dr. Vélez and his associate, Dr. Alberto Restrepo, are interested in joining the PAHO/WHO research program in nutritional anemias. One of the two will visit Caracas for training. Both are prepared to carry out the following studies:

a) Pregnancy and infancy protocols as outlined by the WHO Scientific Group in Nutritional Anemias.

b) Tissue from studies for iron stores as outlined in the protocol by the above Group.

c) Special studies concerning folate metabolism in iron deficiency anemia.

There is an excellent metabolic ward under the supervision of Dr. Vélez where special studies may be carried out. Interesting observations have already been made in the inter-relationships between iron and folate deficiencies and with respect to abnormalities of the small bowel mucosa in patients with anemia. These investigators, with their background in nutritional problems and in hematology, are ready to carry out the protocol of studies and also to make valuable contributions to the study of the problem of nutritional anemias. It seems highly desirable that they be included among the initial collaborators in the PAHO/WHO program.

2.2.4 Dr. Cesar Reynafarje, Hospital Loayza in Lima, Peru. Dr. Reynafarje's interests center around erythropoiesis, erythropoietin, and altitude effects on the blood. He is a hematologist and a clinical investigator of demonstrated competence. The laboratory possesses adequate facilities, although funds for research are limited. Dr. Reynafarje's work is closely related to that of Dr. Emilio Picon of the Nutrition Unit who has recently carried out studies on anemia in villages at the head waters of the Amazon River. Dr. Reynafarje is a highly qualified person for participation in the PAHO/WHO study, and he has expressed interest in the program. It would be highly desirable to further acquaint Dr. Reynafarje with the program by sending him the WHO protocols for studies on tissue iron stores and for studies on the anemia of pregnancy and

RES 4/2B

childhood. Since he is planning to be in the United States this year, it would be desirable that he stop at PAHO Headquarters to discuss the protocols in more detail, and visit with Drs. Crosby and Herbert primarily, and, if possible, Drs. Wintrobe and Finch, so that he could get a better background on laboratory aspects of studies relating to B₁₂, folate and iron. It is hoped that a plan might be developed for bringing him into the collaborative study at a later date.

3. Malaria

3.1 Pilot study of integrated attack measures. A modest start was made late in 1964 to apply to the malaria eradication problem area in Mexico the results of the careful malariological field studies of the past few years. The details of the pilot study consist of a more intense and sophisticated application of the standard malaria eradication operational procedures: DDT-spraying, malaria case finding, and treatment of all diagnosed cases of malaria. Supplementary measures will be used only where absolutely necessary. The progress made with the basic weapons maximally applied in an area where DDT resistance is not a factor will be measured.

This type of developmental research is needed more and more to solve the combined biological and administrative problems that are known now to exist in the Americas and in other malarious areas of the world.

3.2 Mass Drug Treatment. The combination of chloroquine-primaquine has maintained its full therapeutic and prophylactic efficacy in the five Central American countries where it has been used. No resistance has appeared as yet. Two-week cycles remain the preferred schedule of administration and have been used for periods varying from 6 months to 20 months, depending on the epidemiological results. These in turn depend upon the balance between transmission potential and the percentage of persons treated, as well as upon the amount of re-seeding by imported cases. Good long-term programs manage to treat 85-90% of the population in each cycle, and make great efforts to treat the individuals who have missed previous doses. Poor programs may reach only 60 or even 50% of

the people, and these programs usually have to be supplemented or abandoned.

In two concentrated foci in El Salvador and Honduras the same combination, chloroquine-primaquine, has proved very effective in weekly cycles. In Haiti, where the cases are 98% P. falciparum and the people nearly 100% negro, the combination of chloroquine-pyrimethamine has two theoretical advantages namely, that pyrimethamine unlike primaquine is causally prophylactic against P. falciparum and is not hemolytic in the negro, who is known to be more frequently hemolysis-sensitive to certain drugs.

The combination was given a pilot trial on 3 week cycles in 45,000 persons starting in October 1964. The first results were so promising that the schedule will be applied in 1965 to all 570,000 persons in the problem areas in Haiti.

Because of the objections of many people to the discomforts following chloroquine, the combination pyrimethamine-primaquine should achieve better acceptance. Since this combination showed itself so effective in Panama, according to a Gorgas Memorial Laboratory study, it was given a high priority for field testing in 1965 at 2 week cycles, instead of the 1 week periodicity used in Panama.

A small comparative field trial of several methods of mass drug treatment was carried on by the National Malaria Eradication Service of Brazil on the island of São Francisco do Sul, and the evaluation was assisted by a PASB epidemiologist. Three kinds of medicated salt were compared with chloroquine-primaquine tablets for acceptance, and all 4 with a control area for malariological effect:

- A = Amodiaquine in salt
- B = Chloroquine naphthoate in salt
- C = Comosal in salt (chloroquine pre-mixed with salt)
- D = Chloroquine-primaquine tablets
- E = Starch in salt (control)

Both A and B were accepted readily, but C was refused by 30% and D was missed or avoided by 30% for all doses and 43% for all or part of the doses. Only A succeeded in reducing the indigenous malaria to zero. The crude rates, unadjusted for imported cases, showed the following decrease in incidence during 1 year of observation: A = 94%, B = 50%, C = 74% and D = 37%. The control area showed an increase in incidence of 46%.

A detailed manual of operations for mass drug treatment programs has been completed. This manual will be of great importance in the Central American problem areas, where the surest and most widely applicable way of interrupting the transmission of malaria is to treat with antimalaria drugs once a fortnight the entire rural population. The method requires well trained and dedicated personnel, taut supervision for success, and improvement of administrative systems (supervision and reporting).

3.3 A field trial of Cycloguanil-pamoate (CI-501), to begin in the first half of 1965, was planned for 1000 subjects in Colombia. Before the site could be visited by a consultant, it was learned that the standard dose of 5 mg/Kg was not protecting small children for 6 months as it did adults in an African study. The difference was thought to be due to the more rapid excretion of the drug in children. Larger doses will be necessary, but planning was suspended until a revised dosage for children is worked out and tested for safety.

3.4 The Screening Center for Drug-Resistant Malaria Parasites was very productive in 1964 and up to March 31, 1965 when it was closed. Twelve different strains of suspected resistant P. falciparum from different parts of Brazil, Colombia and Venezuela have been studied in 112 subjects who received 232 treatments. The final subjects will continue under observation until 31 May, as all are followed about 4 months. All strains but one failed to respond with 1.5 gms. and/or larger doses of chloroquine, some of them persisting after the administration of more than 5 gms. in 2 days, with blood levels of over 600 µg/liter, the highest level ever seen in the Center. On the other hand, some cleared with doses of 2.4 to 3.6 gms. The only single drug that cured all strains was quinine but 10 days of treatment were required, while 7 days were not sufficient. Some strains were pyrimethamine-sensitive, but even the pyrimethamine-resistant ones regularly responded to a combination of pyrimethamine + sulfonamide. An attempt was made to develop the minimal curative dose of this combination, and a 4-day schedule was shown to be 100% curative (16 cases). However, 5 out of 16 cases developed varying manifestations of bone marrow depression, a toxic reaction caused by both pyrimethamine and sulfonamides and one more likely to occur in folic acid deficiency states. The Center was closed before a shorter schedule could be tested.

3.5 The WHO/PAHO/ME Epidemiology Field Study Team in El Salvador was disbanded at the end of 1963, following the untimely and lamented death of Dr. René G. Rachou. In mid-1964 the Team was reactivated for duty in Mexico, and assigned to the Pilot Study mentioned above.

A paper by Rachou et al. entitled "Synoptic epidemiological studies of malaria in El Salvador" appeared in the American Journal of

Tropical Medicine and Hygiene 14: 1-62, 1965. The article is an example of the type of malariological studies that are needed as bases for the sophisticated malaria eradication operations in problem areas.

3.6 The WHO/PAHO/ME Insecticide Testing Team in El Salvador continued its studies of thirteen residual insecticides selected by WHO as candidates for use against both DDT and dieldrin-resistant anophelines. Two major techniques were used: bioassays of insecticide residues on various types of wall surfaces; and the Excito-Repellency Test Box - PASB Model.

In the bioassay studies no feasible procedure was found for the treatment of highly sorptive mud walls to make them less sorptive. The predominant types of mud walls in El Salvador are exceedingly sorptive to all the new residual insecticides, reducing them to ineffective residues in 2 weeks or less, regardless of the use of new or old whitewash or any of 3 other possible wall-sealing materials. On wooden surfaces, however, one of the new insecticides was found to be highly active for over a year.

In the Excito-Repellency Tests, with DDT-susceptible and DDT-resistant Anopheles albimanus, it was found that 4 out of the 10 candidate insecticides at doses of 0.1 gm/m² "neutralized" the excito-repellent effect of DDT within the limits of the experiment. These are only screening tests, because the insecticides cannot be used in human habitations until their toxicity for man has been determined, but it is valuable to know in advance whether or not they are themselves irritating to a degree that would reduce their operational effectiveness, and whether the DDT already on most walls would interfere with their action.

The Insecticide Testing Team also improved the Excito-Repellency Test box, making it smaller, more easily demountable and providing a

larger opening so that the mosquitoes can more easily escape upon irritation.

The Insecticide Testing Team is being supported by WHO and henceforth will provide a testing unit in the WHO master plan of 7-stage testing of insecticides, capable of performing stages III through VI and participating in stage VII. Hut and village-scale trials of OMS-33, the most promising candidate insecticide now available, are planned for 1965.

A new disc flow regulator proved in field trials to be far more efficient than the unregulated discharge system used in the past and therefore was recommended for use. Studies continue in the search for a more efficient nozzle tip to be used with the new regulator. Preliminary work is being done in the Savannah Laboratory of the Communicable Disease Center, USPHS.

3.7 Defects in the suspensibility of DDT water-wettable powder were discovered early in 1964 and soon were demonstrated in many lots in several countries, although all had met specifications at the time of purchase. Study of this condition revealed the presence of a "latent defect" of unknown origin which produced a progressive deterioration in the suspensibility of the powder. Studies to determine the cause of this defect continue. The Communicable Disease Center, USPHS, is actively engaged in this study.

4. Mental Health Information Center on Latin America

The efforts of the Mental Health Information Center on Latin America were concentrated on annotating a compiled bibliography on mental health in Latin America, and on collecting mental health information, mostly through questionnaires.

By June 1965, the terminal date of the project, the following will have been completed: 1) Annotations of approximately 89 books and over 1,050 periodical articles on mental health, published in Latin America during 1960-1962; 2) a Directory of Psychiatrists in Latin America, containing the following information: name and address, date and place of birth, degrees, schools attended, professional positions, present occupation, and research interests; 3) a Directory of Mental Health Facilities and Institutions in Latin America and 4) a mailing list of key mental health personnel in Latin America.

The Center has also been instrumental in furnishing publications and bibliographies on various mental health subjects requested by individuals in Latin America and in the United States.

If this project is reactivated, its scope would be broadened to include surveys on mental health legislation and on education and training programs in psychiatry.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is essential for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent data collection procedures and the use of advanced analytical techniques to derive meaningful insights from the data.

3. The third part of the document focuses on the role of technology in data management and analysis. It discusses how modern software solutions can streamline data collection, storage, and processing, thereby improving efficiency and accuracy.

4. The fourth part of the document addresses the challenges associated with data management, such as data quality, security, and privacy. It provides strategies to mitigate these risks and ensure that the data remains reliable and secure.

5. The fifth part of the document concludes by summarizing the key findings and recommendations. It stresses the importance of ongoing monitoring and evaluation to ensure that the data management processes remain effective and up-to-date.



5. Population Dynamics*

The PAHO/WHO Conference on Population Dynamics**endorsed the proposals of the Pan American Health Organization and indicated several fields of action which were clearly responsibilities of PAHO. A short progress report of the steps taken to implement the recommendations follows.

5.1. A collaborative project, Epidemiological Study of Natality, was proposed for the understanding of population dynamics and the epidemiology of human reproduction in areas with widely differing environmental and social conditions in the Americas. The approach would be similar to that followed by Gordon and associates of the Harvard School of Public Health in their studies in India (Khanna Study) "to provide long-term information on a prospective basis which would avoid the shortcomings in short cross-section surveys."

In order to carry out a pilot project in this field, the development of an epidemiological study was explored with the Minister and with the Director of Health of Peru in March 1965. A draft research proposal for the Epidemiological Study of Population Dynamics in Peru has been designed. Since the Center of Studies of Population was established in Peru there is special interest in carrying on research for knowledge of population problems. The Dean of the Harvard School of Public Health has expressed his willingness to assist in the development of the project in Peru.

The Organization recommends similar studies in four or five additional

* Based on a report from Dr. R.R. Puffer, Health Statistics Branch, PAHO.

**See RES 4/9 for the conference report.

countries including those with demographic research training programs so that the studies can be used in the training program in medical demography. Efforts are being made to obtain financial support for this program.

5.2. The Conference also recommended a project termed Operational Demographic Research in Areas with Limited Facilities. New methods of collection of records of births and deaths in areas lacking physicians and hospitals would be explored. As part of the research projects (see 5.1 above) some exploration in this field would be possible. In addition efforts are underway to obtain funding for projects in this field.

5.3. The project, Demographic Research on Pregnancy, Natality and Mortality, included investigations of deaths in infancy and childhood and also investigations of all births and fetal wastage (including abortions) in several urban centers.

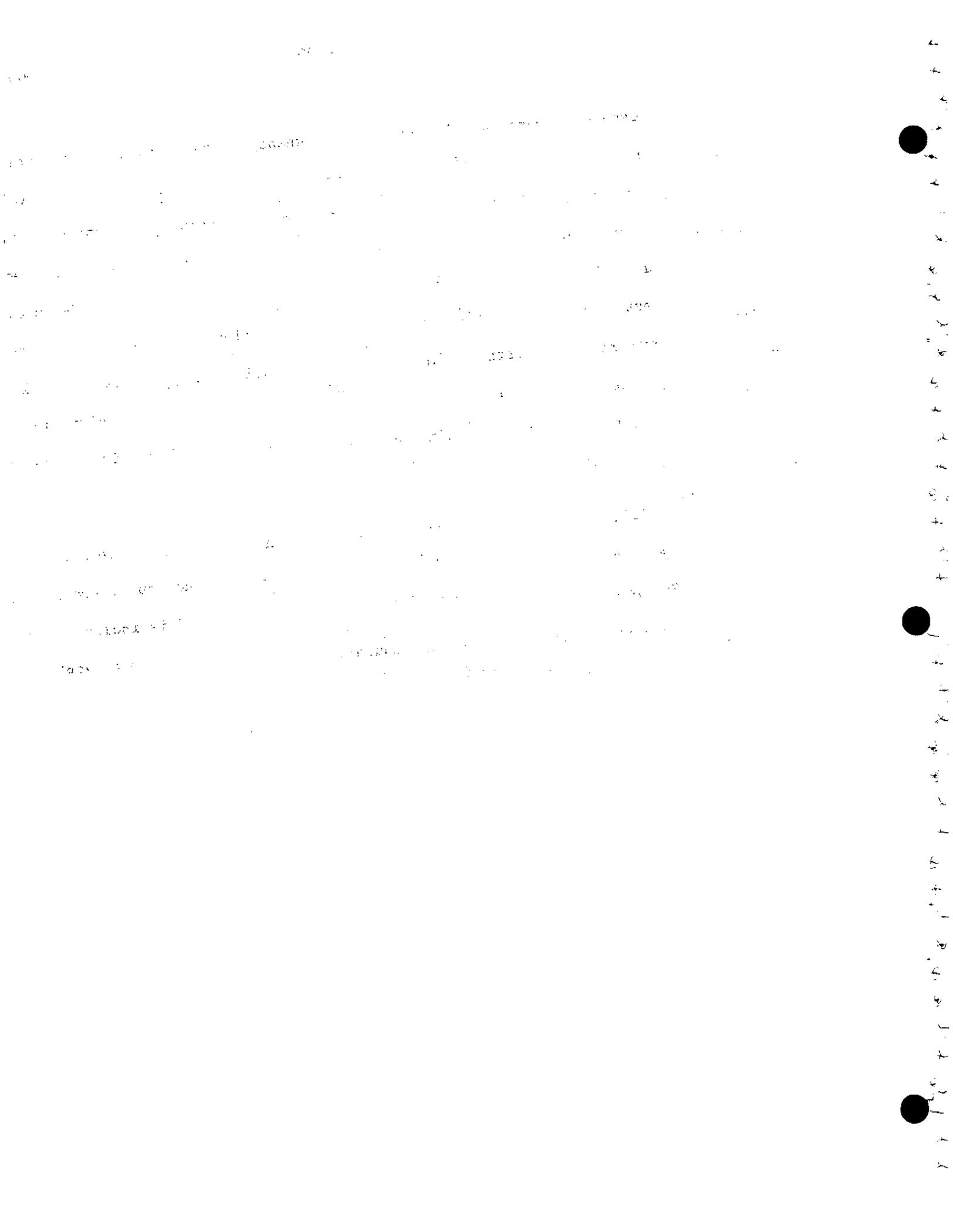
At the present time, a proposal for development of an Inter-American Investigation of Mortality in Infancy and Childhood is being discussed. This Investigation would be similar to the Inter-American Investigation of Mortality which has been carried out successfully in the years 1961-1965 with the collection of data for over 40,000 deaths of adults 15-74 years of age.* However, the objective would be broader: "to develop accurate and comparable death rates in infancy and early childhood considering the nutritional, sociological and environmental factors responsible for excessive mortality." Also it is anticipated that the Investigation would be carried out in a few rural areas in addition to large cities giving 10-14 areas in all.

In order to understand the problems of abortions and other fetal wastage, and for knowledge of live births and the condition of infants at birth,

* See RES 4/2A, P. 1-26.

research regarding all births and pregnancy wastage would be desirable in the large cities in which the Investigation is carried on. An analysis of such material on all births and fetal deaths (including abortions) would reveal important health problems which are not sufficiently recognized at present. This type of research is proposed for pilot work in Caracas, Venezuela, and for later extension to other cities. In this city a high proportion of births occurs in one large hospital and in this hospital 17.6 per cent of the admissions in 1964 were due to abortions. Initiation of research on births is being proposed for one city with possible extension to other cities.

5.4. In accordance with another recommendation, plans were made for two research training centers in medical demography in São Paulo, Brazil, and Santiago, Chile. A report of activities in this field is included under Research Training and Medical Education. (See Section 6, RES 4/2B).



6. Research Training and Medical Education*

The Pan American Health Organization has continued working on its program for the establishment of faculty and research training centers in Latin America.

The general objectives of the program is to accelerate the growing capability of selected Latin American university medical centers to provide teaching and research training opportunities for nationals of other countries, to qualify them for full-time positions in their home institutions, with special reference to those schools which lack adequate specialized manpower resources in the medical sciences. It is noted that of the 108 medical schools in Latin America, 30 have come into being since 1955.

Plans have materialized in two specific areas:

6.1 Faculty and Research Training in Microbiology ✓

The specific objective of this project is to take advantage of the training resources of the Institute of Microbiology of the University of Brazil (and affiliating institutions) for the training of teachers and investigators in microbiology.

The Institute, under the leadership of its Director, Dr. Paulo de Góes, has a well-organized high quality graduate training program in the field of microbiology for physicians from Brazilian medical schools, which has been in operation for several years. Site visits by PAHO personnel have confirmed the fact that the Institute resources will accommodate additional trainees from other countries, and Dr. de Góes is eager to assume the added responsibility this would entail.

* Prepared by Dr. R. Villareal, Medical Education and Research Training Unit, PAHO.

Through a joint effort of the PAHO and the Rockefeller Foundation, a program for faculty and research training in microbiology has been formalized; and the Institute is ready to start accepting international trainees under this project.

6.2 Faculty and Research Training in Medical Demography

The Pan American Health Organization has continued its studies to establish two centers for research training in medical demography.

The purpose of the training centers is to prepare faculty members of medical schools and schools of public health so as to bring into their teaching programs concepts of medical demography (population structure, methods of collection and use of natality, morbidity and mortality statistics) and to develop community-centered research in these fields.

PAHO invited faculty members of several medical and public health schools to an informal working group on Research Training Centers in Medical Demography held in the Faculty of Hygiene and Public Health of the University of São Paulo, on 18-19 March 1965. The aim of the meeting was to design a program whereby faculty members and scientists in schools of medicine and public health in Latin America could obtain greater understanding of population dynamics and its interrelationship to health. They would also be better equipped as investigators to study population changes affecting health and prevention of diseases. Full agreement was reached on: a) the need for the program; b) the nature and content of the curriculum; c) dates of the course; d) the number of students to be recruited; and e) the budgetary cost figures.

It is hoped that this project may be jointly financed by PAHO, the

Agency for International Development of the United States of America (AID) and other interested agencies. If funds become available, the research training program may start recruiting trainees early in 1966 for São Paulo, Brazil, and Santiago, Chile and in 1967 for Venezuela.

6.3 Health Manpower Study in Colombia

The Health Manpower Study in Colombia attempts to examine the teaching programs and patterns for medical education, and in general for education in the health professions, in the light of: a) the health needs of all of the people, both those who receive medical care and those who do not, b) the demands for health services, and the physical, social, economic factors that influence those demands, c) the availability of health services in Colombia, d) the present and potential supply of health workers, e) the present and potential responsibilities and activities of those health workers, f) social and economic factors which affect the availability of health services.

The plan of operations for the study has been worked out and includes the following study areas, in most of which work is already underway:

Physician supply

Nurse and nurse auxiliary supply

Medical education

Nurse education

National health survey

The study will lead to the development of a method that can be used in other Latin American countries to obtain data for a more rational

planning of health personnel education and training. It serves as an educational experience for faculty and students of the medical schools of Colombia that will give them a better knowledge of their country's health conditions and needs. It will also develop a closer understanding - for a more effective joint action - between health authorities and those responsible for medical education to attain a common goal of rising health standards in Colombia.

7. Dental Public Health*

7.1 Salt Fluoridation in Colombia

The analysis of the clinical nutrition and dental data collected prior to fluoridation of salt has been completed and will be published in the Boletín de la Oficina Sanitaria Panamericana.

The laboratories for the analysis of fluoride have been set up and tests for fluoride content in table salt have begun prior to its being distributed to the populations under study.

The fluoridation of the water supply serving one of the populations acting as control has been started. The University of Antioquia has taken the necessary administrative steps and prepared the communities for the distribution and acceptance of the fluoridated salt. The initial stages have met with no unfavorable reactions on the part of the population.

The analysis of urinary excretion of fluoride as a means of adjusting the fluoride intake to optimal levels has begun.

7.2 Center for Dental Epidemiological Training and Research for Latin America

With the participation of the University of São Paulo, the Division of Dental Public Health and Research/USPHS, the W.K. Kellogg Foundation and PAHO, the Center for Dental Epidemiological Training and Research for Latin America started to function in February 1965.

As a first step, a manual for epidemiological studies on dental caries is being prepared jointly by the University of São Paulo and the Dental Health Center/USPHS in San Francisco.

The training in epidemiology and statistics of two staff members of the Faculty of Public Health and Hygiene in São Paulo is planned for 1965.

* Based on a report from Dr. D. Restrepo, Health Promotion Branch, PAHO.

The training in dental public health epidemiology will begin in 1966.

Research activities are scheduled for the same year.

Mark

8. Radiation and Isotopes*

8.1 Studies on the Effects of Radiation

- 8.1.1 Studies of possible biological effects on human populations of increased background radiation due to abnormally high levels of radioactive elements in the ground.

For this project, the PAHO has provided a consultant during the past three years whose function has been to advise and technically coordinate. The consultant, Professor M. Eisenbud of the Institute of Environmental Medicine of New York University has provided a summary of the situation relative to the high background radiation areas of Brazil which is attached as Appendix I.

The program continues to remain a cooperative effort of the Institute of Physics of the Catholic University of Rio de Janeiro and the Institute of Biophysics of Brazil (each receiving grants for this project from the U.S. Atomic Energy Commission) as well as the Institute of Environmental Medicine of New York University.

The PAHO provided two more fellowships for this project during the past year - one in radiochemistry and the other in cytogenetics.

The persons in the inhabited areas under investigation are being exposed to external levels of radiation that vary from 3 to 100 times or more the normal background radiation. Interestingly enough, there would appear to be minimal biological intake of the kind of heavy radionuclides present locally in the soil of Guarapari and Maipé - probably due to the limited quantities of food grown there. However, in the Araxá region, a

* Based on a report from Dr. I.M. Lourie, Health Promotion Branch, PAHO.

greater amount of produce is grown for local consumption and the radioactive minerals contained in the soil of the farm land is providing opportunities for studying the effects of elevated levels of radium in food.

Certain difficulties have been encountered in following through on the cytogenetics work reported upon last year. While the early leukocyte cultures went well, later cultures were technical failures. A separate room has now been set aside at the Biophysics Institute for the cytogenetics studies and the head of that laboratory has received a travel grant from PAHO to work with Dr. Michael Bender (who has been the adviser for this phase of the work) at the Oak Ridge National Laboratory in the U.S.A. - in the hope of isolating the particular problem involved. There is no question, however, that chromosomal aberrations have been found in persons from the high background areas of Brazil in excess of what one would expect to find under ordinary conditions. While the culture problems have presented difficulties (which we hope to resolve by the travel grant previously referred to) we can happily report that the material from the successful cultures has been scored, the karyotypes worked out and good data recording and filing methods developed - as well as photographic techniques for making permanent records of the karyotypes.

8.1.2 Studies of the mechanisms involved in the production of aplastic anemia and the CNS syndrome following irradiation - using large animals.

As indicated in last year's report, modifications in the research protocol became necessary in order to include a very important neurological phase involving the study of radiation-induced C.N.S. syndrome in the burro. Consequently, at the suggestion of the U.S. Atomic Energy

Commission that a total proposal be presented - even though they might find difficulty giving complete support to the entire project at one time - a full scale meeting, arranged for by PAHO, was held in New York late in November 1964. Present were representatives from among the proposed Northamerican and Peruvian investigators as well as representatives of the U.S. AEC and PAHO to elucidate the various limits of the total proposal. A further working session was held in Peru in February 1965 between the Peruvian investigators and a PAHO representative to clarify further the problems which would have to be handled in Peru - particularly by the Faculty of Veterinary Medicine of San Marcos University as well as the Veterinary Institute for Tropical & High Altitude Research. The latter will be providing both space and facilities for the various hematological, pathological, biochemical and other studies to be carried out in the high Andes utilizing both burros and llamas. At the Veterinary School in Lima most of the virological studies will be performed as well as some of the pathology studies. The Medical School of San Marcos University will also be conducting much of the hematological study.

As now being proposed in final form for partial or total financial support by the U.S. AEC the study to be carried out in the high Andes of Peru as well as at sea level will have a two-fold purpose:

- a. To determine whether hypoxia exerts a protective influence by reducing the number of aplastic anemia cases which can be anticipated following the irradiation of burros with an LD 50/30 dose of gamma radiation. In this context the mechanisms concerned with the development of aplastic anemia will also be studied and related if possible to leukemia development in humans.
- b. A second aspect is to determine whether the burro does develop a CNS syndrome following an LD 50 or smaller dose of radiation - and if so, to attempt to study, the actual mechanism which makes the burro this sensitive - and the subsequent pathological effects.

There is some thought, held by a few veterinarians, that the CNS effects may be caused not by the radiation but rather by latent equine viruses which may be activated as a result of irradiation. This aspect is to be investigated likewise through the virological facilities available at the Faculty of Veterinary Medicine in Lima.

8.2 Studies on the utilization of radiation or radioisotope tracers to elicit various biological, physiological or pathological mechanisms.

8.2.1 Manganese Poisoning.

This project is now in its second year of operation under an N.I.H. grant, with a PAHO staff member acting as project coordinator.

The intent of this project is to elucidate the mechanisms by which chronic industrial inhalation of manganese ores induces a schizophrenia-like syndrome followed by either Parkinsonism or a "Wilson's disease-like syndrome". It is expected that the distortion of the homeostasis of manganese might reflect itself in distortion of cholesterol and lipid metabolism.

During the second year, work continued on defining the aberrations of manganese metabolism. Attention continued to be given to the: (a) turn-over of manganese in normal subjects and manganese poisoned individuals (which involved radioisotopic studies concerned with tissue up-take of carrier-free manganese and turn-over studies for determination of life spans of erythrocytes); and (b) biochemical studies of plasma protein transport of manganese in both normal and manganese intoxicated persons.

To carry out these studies, patients were transported from the mine district - approximately 400 miles - to the Catholic University Hospital in Santiago, Chile, where they were hospitalized. In addition to the

extensive routine chemical and laboratory work-up which was carried out, detailed neurologic and psychiatric evaluations were continued on these patients.

Furthermore, a considerable expansion has occurred during the second year of the investigative step of neutron activation analysis for natural manganese in body fluids and cell samples sent from Chile to the Brookhaven National Laboratory in Upton, New York, Dr. G.C. Cotzias of the latter has sent a most informative summary of this work which is attached as Appendix II.

8.2.2 Copper Metabolism

Following the visit of the PAHO advisor to Chile last year for the purpose of determining the feasibility of conducting a research program on copper metabolism among persons exposed occupationally to high levels of soluble copper, blood samples obtained from Chilean miners were submitted to analysis for ceruloplasmin and total copper at the Albert Einstein College of Medicine in New York. Dr. Herbert Scheinberg, our advisor at Albert Einstein, reported these analyses to be generally within normal ranges.

However, the more important examination, i.e., of liver biopsy samples to determine total copper content, has not been carried out as the promised liver samples from two mines in Chile have not been forwarded to the Albert Einstein Medical College. Negotiations are still being carried out between Dr. Scheinberg, the Chilean investigators and the mine physicians to obtain these samples. Only after an analysis of a representative number of such samples can it be determined whether it is worth proposing a research program

on copper metabolism among persons occupationally exposed to high levels of soluble copper.

8.2.3 Biodynamics of vitamin D and certain trace metals in osteomalacia.

In late 1964 a PAHO advisor, Dr. John J. Canary, from the Georgetown University School of Medicine in Washington, D.C. was sent to Chile to cooperate with a Chilean counterpart, Dr. Jorge Litvak, to determine the feasibility of establishing a project of this nature. The report subsequently submitted by Dr. Canary indicated that there were a number of unknowns which could be more readily investigated and hopefully resolved in Chile than in the U.S.A. A grant request will be submitted to the N.I.H.

Dr. J. Canary's outline of the proposed study is attached as Appendix III.

8.2.4 Investigation of the Biology and Ecology of Rhodnius prolixus.

As indicated in the report to the PAHO/ACMR in 1964, this research proposal for studies to be carried out by the Instituto Venezolano de Investigaciones Científicas (IVIC), and the Atomic Energy of Canada, Ltd. had been approved by the NIH/USPHS. The funds for the first year were later provided by the NIH and the project was accepted for a three-year period. The first year of investigation under the grant is now coming to an end (work had actually started in Venezuela before the grant was issued). A brief progress report on the first year's activity is provided as Appendix IV by Eng. J. Gómez Núñez.

9. Tuberculosis*

9.1 At a meeting in New York on 20 July 1964 with Drs. Walsh McDermott and René Dubos it was agreed that the following projects should be undertaken:

- a) Long-term and short-term comparison of the administration of BCG vaccine by scarification and by the intradermal route and
- b) The effect of nutritional status on the appearance of post-vaccination allergy and, if possible, on the protective power of the allergy itself.

Interest was expressed in undertaking a long-term investigation on:

- a) the relative influence of specific and non-specific factors in the development of tuberculosis infection and the appearance of the active disease, and b) the effect of a 5-year program during which chemotherapy is given to all cases of tuberculosis and chemoprophylaxis to every tuberculin-positive person under 15 years of age in a community in which optimal resources exist for implementing the program. The purpose of the study is to ascertain whether the funds for such a complete program are justified by the results obtained.

9.2 Tuberculosis Research Meeting.

A one-day meeting following the Regional Seminar on Tuberculosis (Maracay, 29 Nov. - 5 Dec. 1964) was convened to examine possible tuberculosis research projects.

9.2.1 BCG vaccination.

Drs. A. Paz de Almeida and M. Fontes Magarão expressed interest

* Based on a report from Dr. Rogelio Valladares, Communicable Diseases Branch, PAHO.

in a comparative study of intradermal vaccination and oral vaccination, both in terms of the allergenic action and of the protection conferred on vaccinated groups and the variations in tuberculin sensitivity with concurrent vaccination. They stated that the National Tuberculosis Service of Brazil would provide the necessary resources for the execution of a project of this type.

Dr. Horacio Rodríguez Castells expressed interest in an experiment aimed at establishing the practicability of BCG vaccination without prior tuberculin testing and, in addition, its acceptability simultaneously with smallpox vaccination. He gave an account of WHO experience in this field and expressed his willingness to work in this project.

Interest was expressed in the proposal of the Will Rogers Foundation, New York, to undertake an experiment with isoniazid-resistant BCG in a high prevalence area. There was, however, no concrete offer on the part of the participants.

9.2.2 Tuberculosis bacteriology.

Study of the prevalence of primary resistance to first line drugs and of the prevalence of atypical bacilli. Interest was expressed in this study by Drs. A. Fontes Magarão, Justo López-Bonilla, and Horacio Rodríguez Castells.

Routine use of the Sula medium for the culture of Koch bacilli - its applicability and yield, as compared with direct microscopy and culture in L-J medium. Dr. José I. Baldó submitted a written outline of a research project on the use of the Sula medium for the culture technique for which the Ministry of Public Health and Social Welfare of Venezuela would provide

the necessary national resources. It was agreed that, if the joint execution of this project (Government-PAHO/WHO) was accepted, the comparative study should include the other two procedures.

9.2.3 Chemotherapy.

Dr. Pedro M. Iturbe expressed the interest of the Latin American Regional Committee of the International Union against Tuberculosis in co-operating in a concrete project in this field. There was general interest in research projects on intermittent drug regimens and the possibility of using thioacetazone as a companion drug; however, there was no concrete proposal by any of the participants. The impression was that some of these projects might be accepted when the respective protocols are submitted to national agencies or institutions.

9.2.4 Epidemiology.

Despite the importance assigned to projects involving the long-term study under varying circumstances of the evolution of tuberculosis, it was considered that the technical and organizational difficulties as well as the financial requirements for carrying out these projects make it advisable, if one is undertaken, to limit it to small areas and then only if there is considerable financial and technical support at the international level.

9.2.5 Mycosis.

Dr. José I. Baldó drew attention to Resolution XXIV of the Directing Council of the Pan American Health Organization (September 1959) on the advisability of carrying out in the countries an epidemiological survey of deep mycosis with pulmonary localization and suggested that this

Resolution should be put into practice and that studies on these lines should be combined with those on tuberculosis. He explained that other more serious problems in the health field had claimed the preferential attention of the Government; but that there was no reason why certain basic research projects should not be included, for example on the prevalence of infection by particular spores in the population studied in a tuberculosis project.

9.3 Activities subsequent to the Caracas Meeting

Dr. William R. Barclay who attended both the Seminar and the Research meeting has expressed his interest in the BCG vaccination projects and the study of the prevalence of atypical or unclassified myco-bacteria, in relation to the prevalence of non-specific tuberculin sensitivity.

Dr. William Lester, who also attended both meetings, has expressed interest in participating in a complete study of atypical mycobacteria in Latin America, starting with a large-scale investigation of primary resistance to antituberculosis drugs. He believes that in order to carry out this project on a large scale, subsidiary projects will be necessary to study the applicability, yield, and effectiveness of bacteriological diagnostic methods applied to large numbers of persons and in places distant from well-equipped laboratories.

Correspondence is being maintained with Dr. Luis Cano Girona, Peru, about the project concerning isoniazid-resistant BCG; with Dr. José Ignacio Baldó, Venezuela, on laboratory methods applicable in semi-urban and rural areas; and with Dr. Fontes Magarão, Brazil, on research on the frequency of primary resistance to major drugs; a "Guide for a Study of the Effectiveness

of a Simple Method for Determining Isoniazid Resistance," distributed by the Tuberculosis Section, WHO, Geneva, has been put in circulation.

Argentina, Colombia, Nicaragua and Honduras have expressed their interest in participating in the last-mentioned project.

BIOLOGICAL EFFECT OF BACKGROUND RADIATION IN MAN

The Pan American Health Organization has assisted the University of Brazil and the Catholic University of Rio de Janeiro in undertaking studies in the areas of high natural radioactivity. Professor Merrill Eisenbud, of New York University, has been serving as a PAHO Consultant in these investigations. PAHO has also awarded three fellowships to Brazilian scientists who have journeyed to the U.S. for special training under Professor Eisenbud's direction at New York University.

There are two distinct types of high natural radioactivity areas in Brazil. In both, the high levels of ambient radiation are due to abnormally high concentrations of thorium and/or uranium in the local soils and rocks. The first is the region of monazite sands along the Atlantic coast where the town of Guarapari and Maipé are located and a population of about 20,000 people is exposed to levels of external radiation that vary from 0.03 to 1 mr/hr. The second type is found in the region of volcanic intrusives in the State of Minas Gerais, of which Morro do Ferro and Araxá are the most interesting.

A coordinated multidisciplinary approach to the subject has been underway for about two years. The cooperative venture combines a wide range of professional techniques in an investigation of three sites, each of which is quite different in character, and each of which offers different research opportunities. The Institute of Environmental Medicine of New York University is collaborating with the two Brazilian institutions.

* Prepared by Prof. M. Eisenbud, Institute of Environmental Medicine, New York University, N. Y.

Guarapari is a city of about 5,000 people on the Atlantic coast in the State of Espirito Santo. Weathering of the mountains that parallel the coast has provided an abundant source of insoluble mineral sands that include monazite, a rare earth phosphate containing thorium and uranium. The monazite, being a relatively heavy mineral, is deposited by an interplay of river and ocean currents in sharply defined areas that elevate the ambient radiation levels. The radiation levels in the streets of Guarapari run from 0.1 to 0.2 mr/hr; in homes up to 0.6 mr/hr; and in selected spots on the beach up to 2 mr/hr. Maipé, a fishermen's village 30 miles south, presents similar conditions in which the radiation levels in inhabited places range to 1.0 mr/hr. Small quantities of foods are grown in the radioactive soils of these areas, but preliminary measurements indicate minimal biological intake of heavy radionuclides in humans. The present work is, therefore, centered on the effects of external radiation.

The second region studied is Morro do Ferro, near the city of Poços de Caldas, an area of alkaline intrusives and intense mineralization. In the center of the Poços de Caldas intrusives, Morro do Ferro appears with two well developed magnetite dikes, having secondary fractures that yield rare earth oxides with thorium and traces of uranium. The radiation levels range up to 3.2 mr/hr. The mountain is not inhabited, though it is used for cattle grazing. The environment is one in which radio-ecological studies should be rewarding, and a broad study of the total radioactive environment is in progress.

Araxá (including a nearby village of Tapira) is the third natural laboratory. The radioactivity of this region also originated from an alkaline intrusive, but here the mineralization consists to a great extent of apatite.

The radioactive minerals occur almost exclusively in the form of pyrochlore, or as a complex niobium-tantalo-titanate compound of rare earths with a high content of thorium and uranium. Over extensive pasture areas, the background is elevated six to eight times the normal level of 0.01 to 0.02 mr/hr. In more localized areas, the levels are up to 0.4 mr/hr. The principal interest in this region arises from the fact that the radioactive minerals are contained in the soils of farm land, where appreciable produce is grown for local consumption. Therefore this region offers the opportunity for studying the effect of elevated levels of radium in food on the local population.

Some of the investigative techniques are standard procedures while others have been developed as necessary. Solvent extraction methods developed by Petrow for the determination of Ra^{228} and Th^{228} in biological materials have been used. Ra^{226} has been analyzed by the emanation technique or by co-precipitation with barium sulphate. At the Pontificia Universidade Católica the physics laboratory is equipped with a 4" x 4" sodium iodide crystal and multichannel analyzer. An alpha spectrometer similar to that designed by Osborne is also in use. Total alpha counting and fast alpha pair coincidence counting are employed at the Instituto de Biofísica. The facilities of the Environmental Radiation Laboratory at New York University have also been employed. Several field techniques such as measurements of radon and thoron in the air and gamma spectra have been developed.

CHRONIC MANGANESE POISONING IN CHILE*

This report covers activities at Brookhaven either initiated or completed over the last year, and which pertain directly to the study of chronic manganese poisoning. In addition to the one technician sponsored by the USPHS, several investigators of varying seniority have participated in these investigations. Individual contributions can be assessed only broadly, while the scientific interdependence of these contributions is obvious. Therefore any of the manuscripts now under preparation which include any contribution by the technician in question will be considered as having been supported in part by the USPHS, although the overwhelming support for this work has come generously from the USAEC.

The subject matters investigated are grouped under the following categories:

- 1) Determination of manganese in biological materials.
- 2) Homeostatic control of manganese in animals.
- 3) Preliminary observations on Chilean manganese miners and cases of chronic manganese poisoning.

1. Determination of manganese in biological materials

1.1 The problem.

It became apparent early that pools accessible to the physician at the bedside contain only scant traces of manganese. Hence the hazard of contamination was great. Contamination can be minimized with analyses based on neutron activation if handling of the samples is postponed until

* Prepared by Dr. G.C. Cotzias, Physiology Division, Brookhaven National Laboratory, Upton, N.Y.

they have exited the reactor. After that point, contamination with manganese does not matter much, because carriers are added anyhow in most radiochemical separations.

It was stated in the first of our publications on this subject (Papavasiliou, P.S. and Cotzias, G.C., J. Biol. Chem. 236:2365, 1961) that our values for solid tissues confirmed the majority of recorded values whereas our values on liquid pools were unprecedentedly low. Later several papers were published, both here and in Western Europe, reporting much higher values than our own. This discrepancy was accentuated by one group which reported values for "nondialysable serum manganese" one order of magnitude higher than our "total serum manganese". Indeed, to date only one group has published results identical to, and one group has obtained results much lower than our published ones.

The persistence of gross discrepancies in the quantitation of this element is indeed indicative of a general state of flux in the field of ultramicroanalysis as applied to medical samples. Therefore, a detailed, systematic study is in the final stages of completion, aimed at controlling each step of our own methodology and at comparing various procedures which have as a common end-point neutron activation analysis.

1.2 Results.

A detailed description of the many experiments is included in a manuscript now being prepared for publication. In summary, the search for loss of either part of the sample or of manganese in the sample was conducted with human serum and by means of measuring either the radioactive Mn^{54} or the natural Mn^{55} . No measurable losses were found. Linearity was

obtained: 1) between manganese added to serum and manganese recovered from serum; 2) between stepwise increments in sample volume and manganese per sample. Replication was satisfactory among samples from the same serum, from day to day in the same patient and from person to person in a group of 10 fasting individuals.

An international biological standard kindly supplied by Dr. H. M. Bowen of Great Britain yielded in our hands the value of $14 \mu\text{g}/\text{gm}$ that had been found spectrometrically in Great Britain. Parallel analyses of microgram quantities of manganese by the colorimetric permanganate procedure and by our method yielded identical results. Analysis of serum and blood ash by means of copper spark spectrometry elsewhere versus our method showed about twice the concentration of manganese than it did by the spectrometric method. Since the radiomanganese excreted in rat bile had been found to fluctuate in a predictable, almost periodic fashion, the readily determinable Mn^{54} of rat bile was measured together with the natural Mn^{55} as quantified by our method. Both Mn^{55} and Mn^{54} fluctuated together in two differing and extensive experiments.

The above checks suffer from the drawback of having been applied to samples containing higher concentrations of manganese than, say, serum. However, we do not know of a control ultramicro method which presents as good a safeguard against contamination as does neutron activation analysis.

As opposed to the negative results of the search for losses, the search for contamination yielded some positive results. Iron was suspected as being an artifactual source of radioactive manganese in activated biological samples, but iron proved to be a negligible source. Samples of iron wire analyzed in parallel by neutron activation and by chemical

means in two separate laboratories here were found to contain the same amounts of manganese (200 $\mu\text{g}/\text{gm}$) regardless of the method used. On the other hand, a careful search for "blank" showed the existence of manganese in the sample holders. Cleaning the sample holder with established techniques increased this blank to around 0.1 millimicrogram, which is a significant contamination if one is analyzing manganese-poor serum, but totally insignificant if one is analyzing manganese-rich solid tissues. Rinses with triple distilled demineralized water of sample holders constructed from spotchecked polyethylene tubing reduced this blank by about two orders of magnitude. Several additional but less significant sources of contamination were found and eliminated. The sum of this experience has resulted in a further diminution of our already "low" values although the work was intended to raise our values to those of the majority.

The comparison of our method to that of the other users of neutron activation analysis is not concluded as yet. Several sources of contamination were found in these other methods. As yet the sum of the contaminants does not fully account for the discrepancy between our results and theirs. The most significant single difference between the respective approaches is that in our case separation of manganese is always begun after radioactivation, whereas in the case of some other laboratories, extensive handling is practiced before the sample has been activated. The manuscript under preparation (Cotzias, Miller, Edwards) will not be published until we can account fully for the contribution of this cardinal difference to the disparity of the respective results.

2. Homeostatic control of manganese in animals

2.1 The problem.

Regardless of whether one believes our own values for serum manganese or the recent much higher values of others, one has to agree that the common feature of all reports is a rather striking stability of these values, albeit at markedly discrepant levels. If this stability is not artifactual it should be reflected also in the easily analysable solid tissues and should persist under conditions in which the supply of this element is varied. Human material is not desirable to start with, because overloads occur accidentally and cannot be quantified or timed. Therefore such work was done on animals and the three manuscripts now under completion are briefly summarized below.

2.2 Results.

In a set of experiments with mice, the animals were injected with Mn^{54} and were followed by means of total body counting until their total body radioactivity reached about 1% of the original burden. These animals had been given milk as their basal diet, and various subgroups received variable amounts of manganous sulfate as a dietary additive. It was found that the total body turnover had reflected the concentration of manganous sulfate in the diet throughout the wide range of concentrations tested. This manifested itself in two ways: a) ranking of the total body curves duplicated the ranking of the diets: more rapid turnover occurred with higher manganese levels in the diet. b) the time necessary for the loss of 98% of the radioactivity was inversely and linearly proportional to the molarity of manganese in the milk.

In these experiments, the manganous sulfate supplements were initiated immediately after the isotope was injected and might reflect

only the behavior of loosely bound manganese. Therefore in other experiments, mice were kept on milk alone for 20 days, injected with radioisotope and kept for two additional weeks. They were then subdivided into groups which were fed a wide range of manganous supplements. The ranking found earlier was found again with the exception of the three lowest levels of manganous additive in which no effect was seen. Sacrifice of these animals was followed by the determination of the radioactivity remaining in their organs. A linearity between the percent of total body radioactivity found in a given organ and the level of manganese supplied held for all parenchymatous organs, but the carcass showed only a slight change and in the opposite direction. These results indicated that increasing amounts of manganese were absorbed as the manganese levels in the diet were increased, resulting in progressive increase in the rate of excretion of the radioisotope from the body. Isotopic exchange must have occurred primarily in the parenchymatous organs rather than in the carcass suggesting that the turnover of manganese in tissues might be kept constant (Britton, A.A., Rozanski, C. and Cotzias, G.C., manuscript under completion).

The suggestion that tissue manganese levels might be kept constant meant that one should look for such constancy. For this, two tissues representing parenchymatous organs and "carcass" respectively (liver and diaphragm) were analyzed for manganese by our method for neutron activation, under a large variety of experimental conditions. It was first found that two strains of mice, from two different medical centers, eating two different commercial diets, showed strikingly similar concentrations of this element in these two tissues. Administration of the gluco-

corticoid hormone prednisone or of ACTH resulted in a marked drop of the manganese concentration in the liver which could not be restituted to normal with extra manganese. Following adrenalectomy, on the other hand, animals comparable to those which had shown the prednisone or ACTH effects did not show any effect from total adrenalectomy. Eventually adrenalectomy did reflect itself, but only in animals consuming high manganese diets. Now the adrenalectomized animals accumulated in both tissues more manganese than did the controls. These results raised the question of what the physiological limits of this element's stability of concentration might be. For this there were supplied diets like those used by Britton, Rozanski and Cotzias discussed before. Indeed analyses of animals eating such diets for two months showed that the concentration of manganese in the liver remained at the level of about 2 $\mu\text{g}/\text{gm}$ dry weight even when the manganese level in the diet was increased about 1000 times over the basal level (from 4×10^1 to 3×10^5 $\mu\text{g}/\text{liter}$ of milk). Similarly the concentration of manganese in the diaphragm remained constant at about 1 $\mu\text{g}/\text{gm}$ over the same range of concentrations. Adrenalectomized animals could not be differentiated from normal ones over this range. The manganese level of 3×10^5 $\mu\text{g}/\text{liter}$ of milk corresponds to a calculated daily manganese intake similar to that provided by Purina pellets. Furthermore the tissue manganese concentrations of animals fed this commercial chow were indistinguishable from the corresponding milk-fed ones. Above that dietary level, the manganese concentration of both tissues increased rather smoothly, but, as expected, more steeply in the adrenalectomized animals than in the controls.

The sum of these experiments proved that there exists a homeostatic

control for manganese, that this control operates primarily on the routes of excretion rather than those of absorption and that the adrenal plays some role, but the precise role remains undefined (Hughes, E.R., Miller, S.T. and Cotzias, G.C., manuscript under completion).

Since the above experiments indicated that the excretory routes seemed to be the controlling ones in the homeostasis of manganese, experiments were conducted in rats to identify these routes. It was found that biliary ligation markedly curtails the rate of loss of Mn^{54} from the body, but that rectal ligation totally abolishes excretion under a variety of conditions. Hence, only gastrointestinal routes and not the kidney are involved in the homeostasis of manganese. Biliary ligation induced a significant rise in the concentration of Mn^{55} within the liver, and within the psoas muscle but not within the other organs tested. In the latter organs there was indeed observed a constant rise with the advent of time which was most notable in the kidney. This rise was identical in bile-ligated and sham-operated animals, suggesting that the stress which follows operation might mediate a prednisone-like effect similar to the one alluded to above. This effect would tend to diminish the rise of the manganese level caused by ligation by accelerating the normal redistribution of the element among various extrahepatic sites, such as the kidney. Indeed, confirmatory evidence for such a mechanism was gathered by means of observing the distribution of Mn^{54} . Nonetheless it was also found that animals with biliary ligation lose the ability to increase their turnover when given small loads of manganese, while operation animals fully retain this ability. With large manganese loads, jaundiced animals do demonstrate a small response of their total body turnover of Mn^{54} . This means that

a gastrointestinal excretory route other than the bile can be brought into action by large loads of manganese. Preliminary experiments show that this route is the mucosa of the jejunum, which excretes manganese at rates which diminish progressively from the cephalad to the caudal segments of the intestine (Papavasiliou, P.S., Miller, S.T. and Cotzias, G.C., manuscript under completion).

3. Preliminary observations on Chilean manganese miners and cases of chronic manganese poisoning.

3.1. The problem.

To determine whether the manganese concentration of selected tissues reflects the state of chronic manganese poisoning.

3.2. Results.

Until the methodological controversy discussed above is settled most of the samples received from Santiago will not be analyzed.

Duplicate analyses were performed on the whole blood of 8 healthy manganese miners and 4 patients suffering from chronic manganese poisoning. The manganese concentration in the first group ranged from 14 to 39 μg of Mn/liter of blood, whereas in the second it ranged from 7.2 to 12.2 μg of Mn/liter of blood. The higher values of the working miners by comparison to the patients suffering from manganese poisoning held also in analyses of plasma, skin and urine. The miners showed a range of 1.37 - 3.22 μg /liter of plasma while the patients showed a range of 0.39 - 1.62 μg /liters of plasma. Skin samples from miners showed a range of 0.6 to 12.5 $\mu\text{g}/\text{gm}$ dry skin whereas skin from patients showed 0.28 to 0.55 $\mu\text{g}/\text{gm}$. Urine concentrations have ranged in miners from .858 $\mu\text{g}/\text{liter}$ to 3.4 $\mu\text{g}/\text{liter}$ whereas in patients

they have ranged from .30 to .97 $\mu\text{g}/\text{liter}$. The above values indicated higher manganese concentrations in samples obtained from the dust-covered miners than in the cleaned, hospitalized patients. Still, this difference in manganese concentration might not be artifactual, since it was not evident when hairs were examined. Indeed manganese concentration in the hair of miners ranged from 5.5 to 348 $\mu\text{g}/\text{gm}$ whereas that of patients ranged from 1.5 to 30.5 $\mu\text{g}/\text{gm}$, but the difference of the means is not statistically significant because it is due to a single very high value in one of the miners. Similarly the spinal fluid manganese of miners varied between .98 and 3.22 $\mu\text{g}/\text{liter}$ and that of the patients between .77 and 1.28 $\mu\text{g}/\text{liter}$.

The sum of such data, if substantiated, might show that the currently advocated chelation therapy for human chronic manganese poisoning is inapplicable to the chronic phases of this disease. Concomitantly it seems that the healthy manganese miners have a large manganese pool, in parallel to the animals which had been given large amounts of manganese in their diets. It is possible that the healthy population of miners might be as worthy of study as the cases of chronic poisoning for which they are presumed to be controls. By the same token a Chilean control group will be sought.

It is gratifying that the changes observed with samples of easily analyzable skin were clearly evident also in our controversial analyses of the liquid pools.

BIODYNAMICS OF THE VITAMINS D AND CERTAIN TRACE METALS IN OSTEOMALACIA.*

1. Purpose

To increase the knowledge of mechanisms of action of the vitamins D in man by the serial application of some recently developed techniques of histo-, radio- and biochemistry to a group of vitamin D deficient patients under controlled conditions and to correlate sequential changes in levels of certain trace metals in bone, blood, stool and urine with the above parameters before and after vitamin D's administration in the same patient group in order to gain further knowledge of the role of these elements in the actions of vitamin D and in the pathogenesis of osteomalacia.

2. Significance

Despite more than fifty years of study, the mechanisms of action of vitamin D remain unknown. Amply confirmed has been the effect of vitamin D to increase absorption of calcium by the intestine. More recently studies have indicated that vitamin D produces an increased rate of re-absorption of bone and even more recently data has been presented from this laboratory, indicating that vitamin D has an effect on the renal tubule, decreasing the tubular reabsorption of filtered calcium in toxic amounts.

The disorders produced by deficiency of or resistance to vitamin D's action continue to produce morbidity in many parts of the world. The unique opportunity provided by the presence of a number of patients with these disorders in an area with adequate facilities and staff to carry out the proposed study is of great significance.

* Prepared by Dr. J.J. Canary, Georgetown University School of Medicine, Washington, D.C.

These controlled studies in vitamin D deficient and in vitamin D resistant man have been stimulated by the recently demonstrated extra intestinal action of vitamin D. The opportunity to perform classical external balance studies in a group of patients with vitamin D deficiency and vitamin D resistant osteomalacia in conjunction with radio, chemical, histological and histochemical studies on bone in addition to the application of some of the newer biochemical techniques in these biologic specimens before and after administration of vitamin D afford us an opportunity to correlate changes in external balance produced by vitamin D with changes sequentially in bone tissue. The application of the newly developed tetracycline labelling technique to these specimens should also afford further insight into the actions of vitamin D on the bone.

We believe these studies should shed new light on the actions of vitamin D in a representative number of patients. Despite the great advances in our ability to study bone metabolism, few of these recently developed techniques have been applied to this old problem and we believe the opportunity afforded us in this instance will be a unique one. The ability to accurately quantitate trace metals, particularly zinc and magnesium in the above fashion, will afford us more information about the role of these substances in the action of this vitamin.

INVESTIGATION OF THE BIOLOGY AND ECOLOGY OF
Rhodnius Prolixus*

Considerable progress was made on the biology and life history of R. prolixus. Some effects of environmental factors were observed. Temperature (within the limits of 16 to 34°C) controlled egg eclosion, duration of stadia, eggs per female and longevity. The highest biotic potential was found at 29°C. Relative humidity affected all these functions only when it dropped below 50%. Studies with light indicated that its presence increased population growth.

In our colonies an abnormal ratio of one male to two females did not affect egg eclosion; higher ratios depressed the production of nymphs. Crowding had an adverse effect on feeding, and incidentally, on oviposition. Crowding becomes a limiting factor when the space per adult insect is less than 97.5 cc. For a population with a normal age group distribution, crowding becomes significant only at about 1 cc per insect. Feeding occurs at intervals varying from 7 to 40 days but at intervals greater than 14 days the animals become sluggish and egg production is impaired. Blood of any vertebrate is acceptable.

Tests at IVIC and at Chalk River have proved that ionizing radiation at exposures from 8,000 to 20,000 r interferes seriously with mating behavior in the male. However, significant lethality occurred in eggs from females mated to males treated at 5,000 r. Also, our results indicated that males

* Prepared by Eng. J. Gómez-Núñez, Department of Ecology, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

exposed to this dose level mated more frequently, lived longer, and for a short period were more active than controls. These phenomena followed treatment with both X and gamma radiation.

Field observations have shown that R. prolixus in Venezuela is found in homes, in palms and in arbored nests of birds. Sylvatic *Rhodnius* are found up to 900 m above sea level, whereas indoors the range increases to approximately 2,000 m. Populations fluctuate seasonally, but from preliminary surveys maximum densities vary from 90 individuals per palm to 500 when the palm is occupied by vertebrates. Indoors, as many as 3,000 may occupy a single dwelling, although the average infestation is usually about 300. Within homes R. prolixus is always found near human sleeping places. A new and efficient method for evaluating house infestation has been developed and details will appear in a future paper. To enlarge on the observations of *Rhodnius* ecology, methods of tagging the insect with radioactive cobalt wire, developed at Chalk River, are being field-tested in Venezuela.