



PAHO Research Activities 1961-1966

A Summary

PAN AMERICAN HEALTH ORGANIZATION

Pan American Sanitary Bureau, Regional Office of the

WORLD HEALTH ORGANIZATION

PAHO

RESEARCH ACTIVITIES

1961-1966



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PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
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ADVISORY COMMITTEE ON MEDICAL RESEARCH

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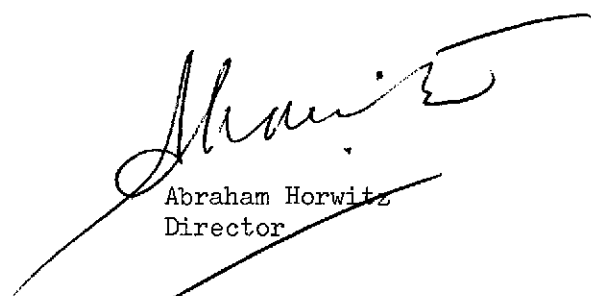
(Note: The document (RES 5/8) pertaining to this agenda item has been distributed separately in book form with the above title)

INTRODUCTION

The past five years mark a period of expansion and intensification of the biomedical research program of the Pan American Health Organization which began with the establishment, toward the end of 1961, of the Office of Research Coordination. The role of this Office, supported at first by a planning grant from the National Institutes of Health of the United States Public Health Service, was recognized as fundamental to the promotion of research and provisions for assuring its continuity through regular PAHO funds were quickly made.

The program broadly encompasses the stimulation of those fields of biomedical research and research training that are related to the objectives of PAHO. Within the guidelines provided on a periodic basis by the PAHO Advisory Committee on Medical Research and the recommendations of consultants, the Organization implements its research program by identifying problems and opportunities, with emphasis placed on those that are amenable to being studied by multicountry collaborative efforts, and by exploring the possibilities of obtaining support for research projects that meet the standards of excellence required by granting agencies.

The following pages present in summary form, by project, the PAHO-sponsored research activities for the period 1961-1966. Included are 90 research projects whose inception, operation or completion fell within the five-year period under review.



Abraham Horwitz
Director

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NUTRITION SURVEYS IN CENTRAL AMERICA AND PANAMA

PROBLEM

At the present time, only qualitative information about the nutritional problems of Central America and Panama is available. The purpose of the surveys is to gather quantitative information from a representative sample of the population of each country on a number of factors that may affect its nutritional status.

METHODS

A trained survey team - consisting of personnel from the host country, the next country, INCAP and the Office of International Research, NIH/USPHS - collects all needed data during a two-to-three month intensive survey in each country. The members of this team typify a wide variety of disciplines represented by physicians, dentists, nutritionists, anthropologists, biochemists, statisticians, food technologists, economists, nurses, social workers, and laboratory technicians.

Aspects covered by the survey include clinical nutritional examinations, anthropometric measurements, bone age and density, oral examinations, dietary surveys, biochemical determinations on blood and urine, intestinal parasitology, immunology, sociocultural interviews, food production and technology, and agricultural economy.

A prevalence survey of diabetes and cardiovascular diseases is also being made.

RESULTS TO DATE

Some reports on specific aspects of the surveys are available, and preliminary summaries of completed surveys are in preparation. The data are being processed for the final reports.

SIGNIFICANCE

Quantification of the nutritional problems of Central America and Panama will provide bases for long-term planning and for the evaluation of future applied nutrition programs. In addition, special problems encountered will be studied in greater detail whenever advisable at a later stage. Pilot programs in areas of special need or interest will point to regional or national programs for improving the nutritional status of the Central American population.

The surveys also serve to train local personnel and to stimulate government interest in applied programs.

OTHER DATA

Grantee: Drs. Guillermo Arroyave and Werner Ascoli,
Institute of Nutrition of Central America and Panama,
Guatemala City, Guatemala.

Funded by: Office of International Research, and the Inter-
departmental Committee on Nutrition for National
Development, National Institutes of Health, U. S.
Public Health Service.

| | |
|---------------------------------|-------------------|
| Timetable: February-April, 1965 | Guatemala |
| August, 1965 | City of Guatemala |
| September-October, 1965 | El Salvador |
| January-March 1966 | Nicaragua |
| March-June 1966 | Costa Rica |
| September-November, 1966 | Honduras |
| January-March 1967 | Panama |

THE EFFECT ON PREGNANT WOMEN OF DIETS LOW IN ANIMAL PROTEIN AND OTHER NUTRIENTS

PROBLEM

The general objective of the research was to study the effect on the individual of restricted nutrient intakes. Major emphasis was given to a longitudinal investigation of pregnant women of low socioeconomic levels, to determine to what extent clinical, physiological, and biochemical variables were affected by a relatively low intake of several nutrients.

METHODS

Soon after volunteer women realized they were pregnant, the following observations were made and repeated at monthly or bimonthly intervals, depending on the type of measurement: a clinical and hematological examination, determination of proteins, vitamin A, carotene and amino acids in blood plasma, of riboflavin in red blood cells, and of urinary urea, creatinine, and riboflavin. The pregnant women were followed until after delivery, at which time cord blood was also taken for study.

A control group was studied, consisting of pregnant women of upper socioeconomic levels, who consumed adequate amounts of all nutrients, and who were under the care of private obstetricians.

RESULTS TO DATE

Compared with the control group, the women of low socioeconomic levels showed a lesser weight gain during pregnancy, with no obvious clinical signs of poor health due to the inadequate diet. Newborns were apparently normal, although slightly smaller. The levels of plasma vitamin A and of red blood cell riboflavin in these mothers were somewhat lower, the ratio of essential to non-essential amino acids was altered, and urea was excreted in lesser amounts. Cord blood analysis in the newborn revealed a low essential to non-essential plasma amino acid ratio, but the nutritional significance of this finding has not been determined.

SIGNIFICANCE

The study served to evaluate the nutritional problems of pregnant women in a rural community, as a model for similar longitudinal studies.

OTHER DATA

Grantee: Dr. Guillermo Arroyave, Institute of Nutrition of Central
America and Panama, Guatemala City, Guatemala.

Funded by: Nutrition Foundation, Inc.

Timetable: 1962-1964.

ANEMIA IN PROTEIN-CALORIE MALNUTRITION

PROBLEM

Almost all reports on protein-calorie malnutrition list as a consistent characteristic the presence of a moderate to severe anemia. The role that protein deficiency plays in the development of anemia is, however, unknown, particularly if considered in the light of other complicating deficiencies and infectious states. The purpose of the research is to define and characterize that role and that of various hematopoietic substances during protein depletion.

METHODS

The degree of protein deficiency has been quantified through the creatinine-height index. Erythropoiesis and the physiological implications of anemia in severe protein-calorie malnutrition have been explored by studies on total circulating hemoglobin, total red cell mass, serum iron and iron binding capacity, sideroblast counts in bone marrow, bone marrow stainable iron, folate, vitamin B₁₂ and vitamin E levels in serum, red cell survival, glucose-6-phosphate dehydrogenase and glutathione levels in red cells, and several red cell fragility tests. Erythropoietin production is also being determined.

Six children have been studied longitudinally from the time of admission to the time of complete protein repletion, in the presence or absence of infection. Records have been kept of the hematopoietic response to various regimens, which include low and high protein intake and supplementation with folic acid, vitamin B₁₂, vitamin E and iron.

RESULTS TO DATE

All children studied show extremely low total circulating hemoglobin and red cell mass, even when, by the usual criteria of hemoglobin concentration and packed cell volume, five out of 13 children would be considered normal. The return of total circulating hemoglobin to normal levels apparently takes as long as the time required for complete protein repletion evaluated by the creatinine-height index.

Serum vitamin B₁₂ levels are normal to high on admission and remain normal during treatment. Six children out of 13 had serum folate levels below 7 nanograms per ml of serum. All levels tended to fall with recovery unless supplemented by folic acid. Serum iron and iron binding

capacity were low with a high iron saturation of transferrin and normal to high sideroblasts in the marrow. Under high protein diets, transferrin levels increased and became markedly unsaturated; bone marrow sideroblasts dropped concomitantly. Several children responded with reticulocytosis to a protein-rich diet.

SIGNIFICANCE

Protein deficiency appears to play a direct role in the production of a markedly diminished total circulating hemoglobin. This may not necessarily be considered an anemic state if viewed as an adaptive mechanism to decreased oxygen transport demands by a concomitantly markedly decreased active tissue mass.

It appears that the response to any hematopoietic stimulus is impaired by a small active tissue mass consequent to protein depletion. A restitution of the total active tissue mass seems necessary for malnourished subjects to reach normal hematological values. It also appears that protein depletion produces a series of abnormalities that affect the normal metabolic pathways for the utilization of certain hematopoietic substances.

OTHER DATA

Grantee: Drs. Fernando E. Viteri and Raymond P. Wood, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1964-1966 (with probable extension).

STUDIES OF ANEMIA IN TRINIDAD

PROBLEM

The clinical impression has long been held that anemia figures prominently as a cause or contributory factor in the admission of patients to hospitals and other treatment centers in Trinidad and Tobago. Evidence was supplied by the Interdepartmental Committee on Nutrition for National Development (ICNND) nutrition survey of the West Indies in 1961, which found a high incidence of anemia in Trinidad and Tobago. Very low hemoglobin levels were encountered among children and adult females. The findings suggested the presence predominantly of iron deficiency anemia, but the survey team recommended studies to establish more precisely the type of anemia, so that appropriate therapeutic measures could be taken.

METHODS

It was proposed to examine 100 subjects in each of the following groups: pregnant women, nonpregnant nonlactating women, adult men, children aged 5 to 9 years, children aged 9 to 13 years, and adolescents aged 13 to 17 years. In addition, 50 lactating women and 50 children aged 0 to 5 years would also be examined. After a full medical history and clinical examination of all subjects, the following constituents would be measured in the laboratory: blood hemoglobin, red cell count, white cell count, differential count, hematocrit, stool parasites or ova, urine glucose, protein and urobilinogen, and serum iron, iron binding capacity, albumin, total protein, bilirubin, folic acid, and vitamin B₁₂.

RESULTS TO DATE

In the study on pregnant and lactating women, anemia was found to be predominantly of the iron deficiency type. The serum folic acid values were somewhat higher than expected and are being repeated on about 50 more subjects. The study on adult men and children aged 5 to 13 is nearing completion. In general, it was found that hemoglobin levels among adult men were quite high, the small percentage of anemia cases being associated with hookworm infestation and sickle type hemoglobin. Hemoglobin levels among children were much lower, and there was a correspondingly higher incidence of hookworm and whipworm infestation. A few cases of hemoglobinopathy were also brought to light in this group. The number of nonpregnant nonlactating women, children less than 5 years of age, and adolescents has so far fallen short of the target. However, the number so far examined has been sufficient to establish a high prevalence of anemia among women of child-bearing age. Serum iron levels have tended to be low.

SIGNIFICANCE

The ICNND survey established that a considerable part of the population subsists on a diet low in protein, particularly animal protein. This would lead one to expect vitamin B₁₂ deficiency to be a significant factor in the causation of anemia. This study may help to establish whether this is so. If iron deficiency should prove to be the main factor involved, the study may indicate whether chronic loss through parasitism, or relative unavailability of iron in local foods is the main factor. In any case, the way might be pointed to appropriate prophylactic measures, e.g., a parasite eradication campaign or food enrichment with ferrous iron.

OTHER DATA

Grantee: Dr. J. G. Chopra, Pan American Health Organization, Port-of-Spain, Trinidad.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1964-1966.

INTERRELATIONS BETWEEN DIARRHEA AND MALNUTRITION

PROBLEM

It is generally recognized that, in the developing countries, malnutrition and common infections act synergistically and that, consequently, the two conditions have a greater impact on the population than would be expected from the simple combination of their effects alone. The present study attempts to investigate and quantify, through field experiments and observation, the patterns of interaction of nutrition and infection.

METHODS

Direct and continued observation has made available the longitudinal records of all children under 6 years of age living in three similar villages in the Guatemalan highlands. Included in the data are clinical examinations; dietary intakes of the family and the individual child; bacteriological and parasite examinations; anthropometrical measurements (height, weight, and mid-tricipital skinfold); hand and wrist x-rays; and type and duration of bouts of illnesses.

The design of the trials made it possible to collect data under modified environments in two of the villages, and under the natural environment in the third village. The modification in one village consisted of a high-quality protein supplement offered to pregnant women, lactating mothers, and children under 6 years of age, whereas in another village, medical attention and specific therapy for infections were introduced. In the latter village, environmental sanitation measures were also attempted.

RESULTS TO DATE

Acute diarrheal disease was shown to be a major health hazard in small children. While its etiology was not completely explained on the basis of enteropathogenic microorganisms, dietary factors were seen to be of importance. The concept of weanling diarrhea and of its epidemiology was established. The prevalence and severity of the disease was greater in malnourished children.

Children not receiving the food supplement tend to be more severely affected and to have more complications - such as acute diarrheal diseases - during measles and chickenpox. Weight loss in the supplemented children during measles is less pronounced than in other children.

Ossification in children of the supplemented village is better than that in children of the other two villages. Variability in the height and weight growth patterns of children in the supplemented village is less than that in the other two. Weight and height are gained at a consistently higher rate in the supplemented village.

SIGNIFICANCE

The studies have helped to demonstrate the importance of the synergism between nutrition and infection in the developing countries, indicating that conventional public health measures are likely to have little or no effect in malnourished population groups when nutritional improvement is not part of the program.

The results have contributed to an understanding of the fundamental nature of acute diarrheal disease in less developed areas of the world, suggesting practical means for its prevention and control, based on a study of its epidemiologic patterns.

OTHER DATA

Grantee: Dr. Werner Ascoli, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

Funded by: National Institutes of Health/U.S. Public Health Service

Timetable: 1960-1966.

INTERRELATION OF VIRUSES, DIARRHEA, AND NUTRITION

PROBLEM

The purpose of the study was to investigate the role of enteroviruses, adenoviruses, enteropathogenic bacteria, yeasts, and intestinal parasites in the etiology of diarrheal disease. An attempt was made to find a relationship between diarrheal disease, microbial and parasitic colonization, and diet on the growth and development of children.

METHODS

A cohort of children, representing approximately 50% of all live births from a typical Mayan village, were followed since birth with anthropometric measurements, pediatric examinations, dietary studies, and weekly examinations for parasites, bacteria, and viruses.

Two types of primary cell cultures and one human cell line were used for routine isolation of entero- and adenoviruses. Specimens from disease episodes and from corresponding matched controls were also processed in suckling mice. *Shigella*, enteropathogenic *Escherichia coli* and *Salmonella* were isolated in selective and enrichment media and investigated by biochemical and serological techniques.

Intestinal parasites were studied in concentrated material and in fixed and stained smears.

Correlations were established between viral, bacterial, and parasitic colonization, and illnesses and growth rate of children.

RESULTS TO DATE

On the average, children were born one pound deficient in weight. The growth rate was good up to the age of 5 months. The weight then became deficient, and at one year most children were undernourished with first and second degree malnutrition.

A great variation in the excretion of viruses was noted among the children. A few have shown as many as 25 weeks of virus excretion per year, while others have excreted viruses only occasionally.

Entamoeba histolytica, *Shigella*, and enteroviruses (particularly coxsackie and excepting polio) were found more frequently in cases of diarrheal disease than in controls matched case by case, according to age, season and locality.

A correlation was shown between total number of days of diarrheal disease, calorie intake, and growth curve, i. e., children with lower weight increments had more days of illness and lower dietary calorie intake.

SIGNIFICANCE

Diarrheal disease is one of the leading causes of death in under-privileged countries of the world. The problem is more important where malnutrition is prevalent, because of interactions resulting in a greater damage to the host. An understanding of the etiology and epidemiology of diarrheal disease is necessary to design methods of control.

OTHER DATA

Grantee: Dr. Leonardo J. Mata, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1962-1965

INFANTILE DIARRHEA AND MALNUTRITION IN PERU

PROBLEM

Malnutrition in early and late infancy accounts for most of the exceedingly high infant mortality rate in Peru and in many other Latin American countries. The mortality is intimately associated with a high incidence of diarrheal disease, most of which is the result of malnutrition rather than its cause. The purpose of this study is to identify the type of diarrhea resulting from improper and inadequate feeding and responding only to dietary measures, to study the effect of superimposed acute diarrheal disease on malnutrition, to characterize the changes in body composition that result from the above, to measure in infancy the effect of malnutrition on growth and development, and to consider measures for the prevention of malnutrition.

METHODS

A twelve-bed metabolic unit and laboratory was established for the admission and treatment of severely malnourished infants with acute and/or chronic diarrhea, and also a 28-bed convalescent unit for studying growth during recovery and for the long-term evaluation of new sources of dietary protein. A field station in a remote rural area was established for the ambulatory treatment of diarrheal disease under primitive conditions.

RESULTS TO DATE

Over 100 severely malnourished infants with diarrhea were admitted, treated, and studied in the metabolic unit. Mortality was 13%, with more than half of the deaths due to acute metabolic derangements and occurring in the first two days after admission; the remaining deaths were caused by severe infection and usually occurred later. If severe dehydration and acidosis were present, hydration by the intravenous route was found necessary. Feeding was initiated much sooner after admission than is usual in the well-nourished; otherwise, it would have been impossible to correct the altered body composition. Severe systemic infection was frequently present, although specific enteric infection was seldom found. The unusually high caloric requirements of these infants have been related to the degree of undernutrition. Over 500 cases of diarrhea were treated in the field unit, most of them by the oral route, but those with obvious acidosis received short, intensive intravenous hydration.

SIGNIFICANCE

The study reconfirms that most diarrheal disease in impoverished infants is due to malnutrition and can only be controlled by the availability of adequate amounts of human breast milk or a reasonable substitute, preferably cow's milk. An understanding of the relationships between malnutrition and diarrhea makes possible successful treatment of most cases of diarrhea in primitive environments.

OTHER DATA

Grantee: Dr. George G. Graham, Department of Research,
British American Hospital, Lima, Peru.

Funded by: National Institutes of Health/U.S. Public Health Service

Timetable: 1960-1965.

NUTRITION, PHYSICAL GROWTH AND MENTAL DEVELOPMENT

PROBLEM

Human growth in general must be envisioned both as an increase in size and as a change in function until maturation is completed. Hereditary factors determine to a large extent the characteristics of growth. Growth, however, may be modified or adversely affected in one or more ways by disease, or by nutrition as the main resultant of the interaction of environmental factors.

The hypothesis of this study is: In preschool children lack of nutrients at various levels will produce modifications in physical growth and mental development. Therefore, working with an undernourished population of preschool children, the following specific aims will be studied:

1. To explore the physical growth parameters of each child, from birth to school age in order to determine the state of nutrition, and to study the interrelationship of nutrition to infection and mental maturation.
2. To determine at what age adequate nutritional supplements promote favorable changes in physical growth and mental maturation.
3. To study the cultural and socioeconomic factors that influence physical growth and mental maturation at various ages.

METHODS

The experimental design calls for comparing two groups of children 6 years of age and under, and newborn infants entering each group, on a longitudinal basis over a six-year period. Both groups will be drawn from relatively isolated communities. One (experimental) group will be provided on a daily basis with a nutritionally adequate diet. The second (control) group, drawn from other communities, will receive no nutritional supplement and subsist on its "traditional" dietary regimen. These children and all women of child-bearing age will be followed cross-sectionally and longitudinally with:

- Demographic studies
- Dietary surveys
- Cliniconutritional examinations
- Neurological testing
- Anthropometric measurements
- Psychological testing
- Biochemical determinations

RESULTS TO DATE

Surveillance of forty rural Guatemalan villages has been completed in search of a proper research site. In ten of these villages that appeared to have most of the basic requirements for the study, a cross-sectional anthropometric survey of parents and preschool children has been obtained. Work has begun in one of these villages using pre-coded IBM forms devised expressly for this study. Methodology is being perfected, and a cross-sectional study of the population is being completed that can be used as baseline data in the future. Included are: (a) a map of the community with pertinent physical description; (b) complete census by age, sex, and ethnic groups, as well as general sanitary conditions; (c) preliminary family dietary surveys, in order to define the basic family diet; (d) cross-sectional psychological testing of preschool children. The baseline cliniconutritional and neurological testing will be completed in the near future.

During this exploratory phase of the investigation, the methodology will be tested and perfected and the team standardized in its use. This will be followed first by a pilot and then by the definitive study.

SIGNIFICANCE

All available data show that great numbers of children from rapidly developing countries are subject to different degrees of malnutrition. This is the consequence of protein-calorie deficiencies, as well as of the high rate of infectious diseases, mainly of the respiratory and digestive tracts. These two environmental factors - nutrition and infection - are overwhelmingly present in these populations during most of the preschool age when children's growth rates are very marked. As a consequence of this interaction, many investigators have shown that children's physical growth, mental development and maturation are being seriously impaired at this age for an unknown period of time and is likely to be permanently arrested. This proposed study will attempt to determine and quantify the relative importance of nutrition and infection on the genetic growth potentials of an individual, as well as the relationship between permanent sequela and the age of onset of undernutrition. If the hypothesis is correct, it is even more mandatory to apply immediate measures to prevent protein-calorie malnutrition as well as infectious diseases in the preschool age, in view of present difficulties in obtaining proper amounts of good quality animal proteins for all people in the developing nations.

The results obtained in this study will emphasize the importance of developing inexpensive protein in sufficient amounts to meet the physical growth needs of preschool children and to prevent the onset of mental retardation of the millions of children now being exposed to the dangers of undernutrition.

OTHER DATA

Grantee: Dr. Cipriano A. Canosa, Institute of Nutrition of Central
America and Panama, Guatemala City, Guatemala.

Funded by: National Institutes of Health/U.S. Public Health Service

Timetable: 1964-1966

THE INFLUENCE OF NUTRITIONAL STATUS ON PHYSICAL WORKING CAPACITY

PROBLEM

There are no data with which to test the widely accepted belief that the capacity of chronically malnourished adults for physical work is impaired.

The purpose of the research is to define the nutritional status of malnourished adults by means of body composition studies and to correlate this with several physiologic measurements of physical working capacity. Another object of the study is to determine the metabolic response of chronically malnourished adults to a carefully measured stress produced by physical activity.

METHODS

Body composition is being estimated by basal oxygen consumption, total, extracellular, and intracellular water, 24 hours urinary creatinine excretion, and body density, as determined by underwater weighing. The physical activity of the subjects is quantified by means of graded exercise in a treadmill, bicycle ergometer, and Harvard step-test. Cardiorespiratory response to exercise is measured, as well as oxygen debt, pyruvate and lactate production, nitrogen balance, free fatty acid mobilization, and steroid excretion.

RESULTS TO DATE

Body composition studies indicate that adult males, from populations that by dietary intake and clinical-nutritional criteria are malnourished, have an adequate lean body mass. Their body fat is markedly reduced.

Comparing Mayan Indian population to populations of predominantly Spanish descent having the same socioeconomic and dietary intake characteristics, it has been found that the Indian has smaller limbs, lower lean body mass, and a greater amount of fat than the adult of Spanish descent.

The performance of both groups in the Harvard step-test is excellent, the average score being 83 - 21 for civilian and 83 - 13 for military groups (average for U. S. soldiers after three weeks of intense physical training is 69). Less than 10% of the adults studied had a Harvard step-test score that could be considered unsatisfactory.

SIGNIFICANCE

If this preliminary information is representative of the results of more detailed studies to be made in the near future, it would seem that adults, classified as malnourished on the basis of dietary survey and clinical-nutritional examinations, have an excellent working capacity. The common finding of severely malnourished adults in our hospitals would, however, indicate that these individuals live on the verge of physiological failure due to adaptation to very limited food intakes and which can easily be precipitated by moderate stresses not affecting well-nourished individuals.

OTHER DATA

Grantee: Dr. Fernando E. Viteri, Institute of Nutrition of
Central America and Panama, Guatemala City, Guatemala.

Funded by: United States Army Medical Research and Development
Command.

Timetable: 1965-1967

BIOCHEMICAL EVALUATION OF NUTRITIONAL STATUS

PROBLEM

The basis for undertaking this study is the recognition that there is a lack of simple biochemical methods to assess the status of nutrition, particularly in relation to proteins. Many of the biochemical changes in malnutrition appear only when the deficiency, or deficiencies, have become so severe as to be clinically evident. Such is the case with the concentration of blood serum proteins and several enzymes. The detection of malnutrition at an early stage would, therefore, have prognostic value.

METHODS

The turnover rate of body proteins is decreased when there is restriction of protein intake, resulting in a reduction in the excretion of basal urinary nitrogen. Similarly, it would be expected that the amino acid pool of the body fluids and the blood plasma would be affected by an insufficient supply of amino acids, either from the diet or from the catabolism of tissue proteins. Methods were, therefore, developed to investigate several of the dynamic aspects of protein metabolism. Among the indices investigated more extensively were the urea nitrogen:creatinine ratio in urine; the ratios of several amino acids in blood plasma such as valine:glycine or cystine:glycine; and the essential:non-essential amino acid ratio. As an index of muscle mass, creatinine excretion per given time period (usually 24 hours) was also studied.

Experimental designs included: (a) the estimation of the parameters under investigation in groups of subjects known to differ in adequacy of nutrient intake; (b) the study of the changes in the indices resulting from the administration of controlled levels of protein intake varying from 0.5 g. to 2.5 g. of protein per kg. of body weight.

RESULTS TO DATE

A. The urea nitrogen:creatinine ratio was found to be a very useful and sensitive index for distinguishing population groups according to their protein nutrition. An interesting effect of the amount of water ingested on the excretion of urea was observed. It consists of an increase in the excretion of urea with increased water intake, only noticeable when the protein intake is low. In practical terms, it means that the urea nitrogen:creatinine ratio should be measured under conditions of minimum urinary flow rate.

B. Even at a relatively early stage, protein-calorie malnutrition produces marked decreases in the essential amino acids of the plasma, mainly valine, leucine and isoleucine. Cystine also decreases. In general, the ratio of essential to nonessential amino acids appears to decrease early under conditions of inadequate protein intake. A simple paper chromatography technique can be used for its measurement.

C. Marked differences in the amount of creatinine excreted in 24 hours has been found between well-nourished and poorly-nourished population groups. The index applied in the present study is the excretion of creatinine per 24 hours per centimeter of body height. Retardation in muscle mass development corresponding to 2-3 years of chronological age is found in chronically undernourished rural Central American children of preschool age.

SIGNIFICANCE

Methods for detecting characteristic changes in chronic malnutrition at a relatively early stage are of great value in the evaluation of nutritional status of populations. These methods ought to be simple, relatively inexpensive, and sensitive, as is true of the ones proposed in this study. In our experience, a study can be made of the urea nitrogen:creatinine ratio of children as young as 2 1/2 years, since there is no need for a timed urine specimen. In addition, it is possible to apply the test to between 20 and 30 children at a time without much difficulty. The plasma amino acid ratio can be measured in a 200 microliter fingertip blood sample.

The method would seem particularly useful in following the course of recovery of children recuperating in hospitals, health centers, and nutritional rehabilitation centers.

OTHER DATA

Grantee: Dr. Guillermo Arroyave, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1961-1965.

METABOLIC FACTORS IN PROTEIN MALNUTRITION

PROBLEM

Protein-calorie malnutrition is the most prevalent nutritional disease among preschool and early school-age children in the developing areas of the world. The magnitude of the problem among adults is unknown, although preliminary observations have disclosed many cases of adult protein-calorie malnutrition.

The purpose of the investigation is to study metabolic changes that lead to protein-calorie malnutrition and that are present in malnourished individuals. The role of protein-calorie malnutrition in metabolic abnormalities must then be determined to gain a better understanding of the importance of protein nutrition in the maintenance of normal body functions.

METHODS

Quantification of the degree of protein-calorie malnutrition has been accomplished by measuring serum proteins, plasma amino acid levels, amino acid load tests, and urinary nitrogen compounds. The best index of protein depletion is the creatinine-height index, which consists of the urinary creatinine excretion in 24 hours by a malnourished subject divided by the urinary creatinine excretion in 24 hours of a normal subject of the same height.

Special emphasis has been given to the study of intestinal absorption, where protein, fat, labelled fatty acid and triglyceride, D-glucose and D-xylose have been explored. The pancreatic exogenous secretion and intestinal disaccharidase content and physiological competence are also being explored. Adrenal function studies have included 17-hydroxysteroid excretion in the basal state, and after Metopyron blockage or ACTH stimulation, as well as by means of ^{14}C -Cortisol turnover and Cortisol secretion rates.

The sample consists predominantly of children and of some malnourished adults.

RESULTS TO DATE

The average creatinine-height index of malnourished children upon admission has been 0.55 and, upon protein repletion, it approaches a normal index of 1.0. The time and characteristics of recovery are variable, but in general, the process is much slower than indicated by the usual clinical and biomedical criteria of protein repletion.

Upon admission, all intestinal absorptive functions are impaired to a variable degree. There is a striking relationship between protein repletion as measured by the creatinine-height index and the return to normal of the absorption of substances that require active transport or specialized mechanisms. Impaired pancreatic function does not appear to be physiologically important.

Adrenal activity appears to be high in marasmus and low in kwashiorkor, although overlap between the two groups is very marked, based on their ability to respond to Metopyron and ACTH administration. Preliminary information on Cortisol turnover indicates decreased turnover in kwashiorkor.

Adult malnourished subjects appear to be metabolically identical to malnourished children.

SIGNIFICANCE

Protein-calorie malnutrition is being viewed in terms of specific functional impairments, so as to obtain a clear understanding of the metabolic consequences of different degrees of deficiency.

OTHER DATA

Grantee: Dr. Moisés Béhar, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1960-1967.

AMINO ACID METABOLISM IN CHILDREN

PROBLEM

A major concern confronting present and future populations of the world is that of a protein supplies. Protein malnutrition is a health problem of great importance today in children of developing countries. There is an urgent need to learn how available foods can be improved nutritionally without other deficiencies being induced and how reference patterns of use in formulating new foods or improving others can be developed. More needs to be known about the nutritional significance of deficiencies, excesses, and balance of essential and non-essential amino acids.

METHODS

Nitrogen balance has been determined in children who have recovered from protein-calorie malnutrition in order to ascertain the effect of amino acid supplementation of staple foods, of lowering the level of the protein intake and of altering the ratio of essential to non-essential amino acids in specific foods.

RESULTS TO DATE

Amino acid supplementation of rice: These studies indicate that the protein value of rice protein can be improved only when supplemented with lysine, methionine and tryptophan. These findings are contrary to studies in rats in which rice protein has been found deficient in lysine and threonine.

Effect of feeding decreasing levels of protein foods: Protein levels ranging from high (3.5 g protein per Kg/day) to zero have been fed to a total of 33 children using skim milk, egg protein, mixtures 9, 14 and 15 and a soybean textured food. The results indicate that the decreasing order of protein quality is: egg protein, skim milk, textured soybean protein and the vegetable protein mixtures. Endogenous fecal nitrogen averaged 24 and endogenous urinary nitrogen averaged 57 mg. Best protein intake for maximum difference in protein quality between proteins was found to vary between 150-230 mg N/Kg/day.

SIGNIFICANCE

The final objective in research programs of this nature is to obtain information to be applied in the establishment of protein requirements, reference amino acid patterns, development of better quality protein foods,

better utilization and improvement of available protein foods as well as to obtain basic information on protein and amino acid metabolism by human population groups of the age used in these studies. The information obtained can serve as the basis for programs designed to provide people with more and better quality protein foods.

OTHER DATA

Grantee: Dr. Ricardo Bressani, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: National Institutes of Health, U.S. Public Health Service.

Timetable: 1964-1967

RESEARCH IN AGRICULTURE AND FOOD SCIENCE

PROBLEM

The need for research in the fields of agriculture and of foods in developing countries is becoming better recognized and appreciated. Efforts so far are minor when compared with the ensuing benefits. A strong correlation exists between malnutrition and poor agricultural productivity and utilization. Research on the many and difficult problems facing agricultural productivity and the proper utilization and processing of foods would undoubtedly be of benefit to other health programs.

The purpose of this study is to contribute to the solution of problems in food processing, animal nutrition, utilization of by-products for human and animal feeding, and the effects of fertilizers on nutritive value of food crops.

METHODS

Because of the breadth of the project, no specific methodology can be described other than that indicated immediately below.

RESULTS TO DATE

A study whose objectives are to determine the kinds and extent of fungi infection in cereal grains grown and stored in Central America indicates that many samples are highly infected with fusarium, penicillium, Aspergillus niger and A. flavus, diplodia, alternaria and others. Chickens fed corn experimentally infected with these fungi showed significantly less growth. This is particularly true of those fed on corn infected with A. flavus.

Leaves of high protein content are being studied as raw materials for the preparation of protein isolates. Using appropriate methods, 75 to 85% of the leaf proteins can be extracted. Their nutritive value and utilization are being studied.

Studies are under way on:

The utilization of the by-products of cereal grain industries as food for man and animals.

The utilization in ruminant feeds of agricultural by-products such as coffee pulp, cottonseed husks, corn stalks, leaves and cobs, citronella and lemon grass, bagasse with cottonseed flour and sugar cane molasses.

The effect of different fertilizer treatments and of levels of nitrogen on the protein quality of beans.

The nutritive value and utilization of dehydrated fish.

Studies on opaque-2 corn have begun. The gene in Guatemalan corn is being sought with the aim of introducing it into common Guatemalan corn varieties.

SIGNIFICANCE

The results of these studies are providing valuable information on problems raised by nutritionists, stock raisers, feed millers, and farmers, and are helping to produce more and better quality food per unit area. Information on the fungi present in corn that produce mycotoxins is of importance to health institutions and industrial concerns and enables preventive measures to be taken and better systems of storing agricultural products to be developed.

OTHER DATA

Grantee: Dr. Ricardo Bressani, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: W. K. Kellogg Foundation.

Timetable: 1964-1967

THE NUTRITIVE VALUE OF TEXTURED FOODS MADE FROM VEGETABLE PROTEIN ISOLATES

PROBLEM

Edible protein isolates from various oleaginous seeds and other raw materials have become available during the last ten years. These products are characterized by a content of not less than 95% protein and by many desirable physical properties of practical use and interest to the food industry, such as their dough and film-forming, moisture binding, emulsifying, stabilizing and thickening properties. They are being used as protein supplements to common food products, in the preparation of protein-rich foods and, through appropriate technology, into food products similar to those of animal origin.

Information on the nutritional value of these processed foods is not readily available. The purpose of this study is to evaluate the quality of the protein in the meat-like products made from protein isolates, using feeding tests in experimental animals and man.

METHODS

Standard chemical procedures are used for ascertaining the moisture, fat, crude fiber, ash and protein content of the products. Determination of amino acid content is by ion-exchange chromatography after acid hydrolysis.

Protein efficiency ratio (PER) and net protein utilization (NPU) in young growing rats are obtained using standardized techniques and controls. Limiting amino acids are determined by growth and PER studies in rats. Growth and nitrogen balance is studied in young growing dogs using natural beef as a control.

Nitrogen balance is also studied in normal children who have recovered from protein-calorie malnutrition.

RESULTS TO DATE

Several studies with young growing rats indicated that the protein value of simulated ground beef is equivalent to about 92% of the nutritive value of casein. Textured food gave essentially the same weight gain, NPU, PER and plasma protein picture as casein. Its palatability was extremely good as judged by the amounts of food consumed. Studies in dogs indicated that the protein value of textured food is equal to that of fresh beef and that feeding for 5 months did not cause adverse effects. Other studies with

rats fed the textured food indicated lysine and methionine to be limiting when levels of dietary protein are low.

Nitrogen balance studies were made of eight children whose intake of textured protein ranged from 300 mg of N/kg body weight per day to zero. At high intake levels, the nutritive value of textured food was similar to that of milk. At intakes below 1 g of protein/Kg/day, nitrogen values of textured food were lower than those of skim milk protein.

SIGNIFICANCE

It is of importance to the manufacturer to know the nutritive value of the protein in meat-like and other products so as to evaluate its processing, and also to nutritionists and dietitians to enable them to use such products in feeding programs and as substitutes for unavailable foods. Knowledge of the protein quality of these products will permit more efficient use to be made of them as supplements to diets low in both quality and quantity of protein or as foods.

OTHER DATA

Grantee: Dr. Ricardo Bressani, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

Funded by: General Mills, Inc.

Timetable: 1965-1966

ENRICHMENT OF CORN, WHEAT, AND RICE FLOURS WITH PROTEIN CONCENTRATES OF ANIMAL AND VEGETABLE ORIGIN

PROBLEM

Cereal grains are widely consumed foodstuffs but they contain little protein and, what there is, is of poor quality. This poor quality is due to the deficiencies of certain essential amino acids. They may be added to cereal grain flour in the form of small amounts of protein concentrates.

The purpose is, therefore, to find the minimal amounts of protein concentrates necessary to give the maximum increase in protein value.

METHODS

Experimental animals are fed a basal cereal grain diet supplemented by graded levels of given protein concentrates. Weight gain, feed utilization, and such other parameters as blood proteins, carcass, and liver composition are measured. Among the concentrates used are skim milk, casein, fish protein concentrate, soybean, and cottonseed flour.

RESULTS TO DATE

In all studies, total protein and protein quality can be significantly improved by single additions of several protein concentrates varying in amounts from 3 to 12% to corn, rice, and wheat flours (whole or white). Better results are obtained by supplementation with animal protein concentrates than with vegetable protein. Although the levels of vegetable proteins needed are slightly higher, the enriched product retains essentially the same organoleptic and texture characteristics.

SIGNIFICANCE

Gruels made of cereal grains and of mixtures of cereal grains are popular and consumed in great quantities in Central America. If they could be prepared with enriched flours, more and better quality protein would be eaten. Higher nutritive values would also be available in tortillas, bread and similar formulations.

OTHER DATA

Grantee: Dr. Ricardo Bressani, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: W. K. Kellogg Foundation.

Timetable: 1964-1967

USE OF WO-100 PROTEIN IN VEGETABLE MIXTURES FOR HUMAN FEEDING

PROBLEM

In view of the protein-calorie malnutrition problem of many developing countries, extensive research has been carried out to develop protein-rich foods which could be used as the common animal source to supply the protein in need. Examples of such protein-rich foods are INCAP Vegetable Mixture 9, 14 and 15. These mixtures, which contain 3% torula yeast, provide some protein and relatively good amounts of B-complex vitamins. Other microbial proteins could be used to replace yeast or they could be used as the main ingredient in similar protein-rich foods. However, two of the conditions which new sources must meet are that they be free of any toxic or deleterious influence on the animal or human health and that have a relatively high protein quality.

It is the general purpose of this investigation to study the supplementary protein value of bacterial protein and to develop vegetable protein mixtures with it, as well as to learn more about its limiting amino acids, its biological value and true protein digestibility, and its effects on the chemical composition and general health of experimental animals as prerequisites to nutrition tests in man.

METHODS

Standardized methods have been used for the determination of proximate chemical composition. Amino acid content is ascertained by ion-exchange chromatography.

Biological methods include:

PER assays for protein quality in young growing rats,

Determination of limiting amino acids by growth and PER experiments in rats,

Determination of biological value and true protein digestibility by nitrogen balance methods in rats,

Nutritional significance of ash content of bacterial protein,

Long-term feeding tests and gross pathological and biochemical studies,

Supplementary value tests of cereal proteins to other vegetable proteins and mixtures,

Use of bacterial protein in the formulation of vegetable protein mixtures.

RESULTS TO DATE

Only some preliminary results are available from two feeding tests. In one case the PER value of the bacterial protein was 1.07 as compared with 2.09 for cotton-seed flour using 10% protein diets. A higher quality product tested in a second study gave a PER value of 1.90 as compared with 2.12 for cottonseed flour. It was also found that the bacterial protein under study did not complement the proteins from cottonseed. Additional samples received recently will be tested for their protein value and, if possible, production and processing conditions will be adjusted to give products of high quality.

SIGNIFICANCE

Efforts are constantly being made to produce sources of proteins for the feeding of large population groups. Bacterial proteins offer good possibilities because they are relatively easy to produce at low cost. They can provide good quality protein as well as other essential nutrients. If the protein quality of bacterial protein were high, it could find many uses in the formulation of foods, and it would decrease the scarcity of available protein in the world.

OTHER DATA:

Grantee: Dr. Ricardo Bressani, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: Medical Research Division, Esso Research & Engineering Company.

Timetable: 1965-1966

UTILIZATION OF LEGUMINOUS AND OTHER INDIGENOUS SEEDS OF CENTRAL AMERICA AS FEEDSTUFFS IN ANIMAL PRODUCTION AND AS PROTEIN SOURCES IN HUMAN DIETS

PROBLEM

The Central American area has a wide variety of oleaginous plants, seeds and legumes which could become common sources of food for man and animal and serve as raw materials for the agricultural industries, if chemical and nutritional knowledge were available. The purpose of this investigation is to conduct a chemical and nutritional study of such seeds and plants.

METHODS

The seeds and plants to be studied are selected on the basis of general availability, possibilities of increased production, and reported use. Several different samples will be analyzed for their chemical and amino acid composition. If the oil content is high, a sufficient amount of it will be prepared for specific analysis. The general nutritional quality of the samples will then be determined by feeding tests in rats and chicks. Toxicity, protein quality, and other characteristics will also be ascertained.

RESULTS TO DATE

So far, the chemical composition and essential amino acid content of the gandul seed (Cajanus indicus) and of the conacaste seed (Enterolobium) have been studied. The protein content of both seeds was similar to that found in other leguminous seeds. As for essential amino acid composition, both seeds were deficient in methionine and the gandul seed was also deficient in tryptophan.

Raw seeds did not support good growth in rats or chicks, but those cooked in the autoclave showed increased animal growth, protein efficiency, and feed conversion values. Supplementation with 0.1% tryptophan and 0.3% methionine improved the growth and the protein efficiency ratio of the animals fed on the gandul seed, while the same effect was observed when the conacaste protein was supplemented with methionine. Similar studies are being carried out with other native seeds.

SIGNIFICANCE

The information is essential for increasing the availability of such materials for the feed industry and also as human foods. If these seeds contain organic components of industrial importance, such as oils and vegetable gums, they could be used as raw materials for new industries.

Programs of agricultural, industrial and economic development can benefit from these data.

OTHER DATA

Grantee: Dr. Ricardo Bressani, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: W. K. Kellogg Foundation.

Timetable: 1964-1967

THE METABOLIC FATE OF GOSSYPOL AFTER INGESTION OF COTTONSEED PRODUCTS BY ANIMALS

PROBLEM

During the last few years, particularly since the time INCAP Vegetable Mixture 9 was commercially produced and distributed as human food, cottonseed protein concentrate has been receiving increasing attention. Mixture 9 contains 38% cottonseed flour and its processing is designed to remove gossypol while keeping protein quality high. Relatively small amounts of gossypol, however, remain although intensive investigation has shown no toxicity or adverse physiological effects. It is, nonetheless, of interest to study the fate of the small amounts of ingested gossypol to learn how it is inactivated and what is its mechanism of action.

METHODS

The initial studies used dogs and swine and, the later one, children receiving INCAP Vegetable Mixture 9. The amount of ingested and excreted free and total gossypol is measured in dogs and children.

After 48 hours of inanition, swine are fed a cottonseed flour meal of 300 g to be consumed in half an hour. Animals are sacrificed every hour up to 9 hours after ingestion of the food. Intestinal contents are analyzed for free and total gossypol.

RESULTS TO DATE

Gossypol balances in dogs have shown that 85-100% of the total gossypol ingested is found in feces. Free gossypol content in feces was, however, 2-3 times higher than intakes, suggesting hydrolysis of bound gossypol in the gastrointestinal tract. No other explanation is available yet.

Results in children are similar to those in dogs. Recoveries of gossypol are, however, much more variable.

Free and total gossypol analysis in swine again indicated an increase in free gossypol in the material present in the large intestine as compared with the intake. Total gossypol values were, however, equal to intake values. More work is needed to interpret these results and those indicated above for dogs and children.

SIGNIFICANCE

The mode of absorption and mechanism of action of gossypol is not known although the toxic effects of this compound when fed in high amounts have been described.

Studies on the metabolic fate of gossypol are of both academic and practical interest, since they may make it possible to eliminate it or inactivate its effects, and to increase the utilization of cottonseed protein in human and monogastric animal feeding.

OTHER DATA

Grantee: Dr. Ricardo Bressani, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: UNICEF
W. K. Kellogg Foundation.

Timetable: UNICEF 1964-1965
W. K. Kellogg Foundation 1964-1967

THE ROLE OF CALCIUM AND IRON ON GOSSYPOL TOXICITY IN SWINE AND POULTRY FED ON COTTONSEED MEAL RATIONS

PROBLEM

The dearth of animal protein in this area of the Western Hemisphere can be traced to the lack of appropriate protein and calorie concentrates for the formulation of economical and highly nutritive animal rations.

Since cottonseed is available in Central America, research on an increased, safer, and more effective use of cottonseed protein for swine has been carried out for the last 4 years. The utilization of cottonseed meal for swine and poultry is limited by the pigment gossypol. Toxic to both animals when ingested in large amounts, it so darkens the eggs of hens consuming it that they become unacceptable for human use.

The problem is to find ways of destroying, decreasing, or inactivating gossypol so that more cottonseed meal can be safely used in swine and poultry feeding.

METHODS

Simple feeding tests are done in swine fed on rations containing relatively large amounts of cottonseed meal. Compounds found in vitro to decrease or inactivate gossypol are then added to the basal ration, alone or in combination. The response is measured by growth, feed utilization, and analysis of samples of blood and tissues. Blood and levels of certain enzymes and proteins in plasma are studied.

Hens are fed rations containing cottonseed meal and their eggs are collected and graded.

RESULTS TO DATE

Several studies have shown that swine fed on rations containing 42% cottonseed meal either die from toxic effects or become marasmic. The addition of calcium hydroxide or ferrous sulfate alone protects the animals to a limited extent. Complete protection and good performance are obtained only when calcium and iron are given together. Without these minerals the pigs become anemic and have high glutamic-oxalacetic transaminase levels in the blood. The hair becomes highly depigmented.

The addition of calcium hydroxide and ferrous sulfate to rations containing 42% cottonseed meal allows hens to produce normal eggs.

SIGNIFICANCE

These studies are providing information on the mechanism of gossypol toxicity in swine. They will also help to develop more efficient and safer ways of utilizing larger amounts of cottonseed meal protein in the animal industry and to increase the production of animal products at lower costs.

OTHER DATA

Grantee: Dr. Ricardo Bressani, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: W. K. Kellogg Foundation.

Timetable: 1964-1967

ENDEMIC GOITER AND CRETINISM IN WESTERN BRAZIL

PROBLEM

Both endemic goiter and cretinism are common in the States of Goiás and Mato Grosso. Studies of etiological factors in endemic goiter or of cretins have not been made in this region. The purpose of the research is to determine the extent of endemic goiter in Goiás and in Mato Grosso, to look for possible etiological factors, including their genetic component, and to make a detailed study of cretins transferred from this area to the laboratory in the Institute of Biophysics in Rio de Janeiro.

METHODS

Teams have made surveys in three towns in the Mato Grosso area. Full familial data have been collected. A study of bone, nervous and sensory systems, and iodine metabolism has been made on typical cretins from the Goiás region. Samples of serum from goiter patients in Goiás were obtained for thyroid antibody determinations and complement fixation tests for Chagas' disease.

RESULTS TO DATE

Data showed little, if any, relationship of endemic goiter to genetic inbreeding with no indication of a relationship to any other endemic factor.

Radioiodine uptakes and PBI values were low in cretins from Mato Grosso, but normal in cretins from Goiás. All cretins responded to thyrotropin and had normal karyotypes. Spasticity is the typical neurological finding in these cretins. Typical brain wave patterns were obtained. Pneumoencephalograms revealed no noteworthy abnormalities.

SIGNIFICANCE

The studies point to the need for further research on the etiology of endemic goiter in western Brazil. Although the neurological state of the patients has been defined, the relationship of these findings to embryonal development as governed by the availability of thyroid hormone is not clear and needs further investigation.

OTHER DATA

Grantee: Dr. Luis Carlos Lobo, Institute of Biophysics, University
of Brazil, Rio de Janeiro, Brazil.

Funded by: National Institutes of Health/U.S. Public Health Ser-
vice.

Timetable: 1962-1968

EPIDEMIOLOGICAL STUDIES OF ENDEMIC GOITER IN THE STATE OF SÃO PAULO AND THE EFFECT OF IODIDE SUPPLEMENTS

PROBLEM

Although endemic goiter is recognized as a problem in Brazil, and especially in the state of São Paulo, up to now its magnitude has not been measured. The role of genetic and environmental factors in the etiology of the disease has never been fully studied.

METHODS

Endemic goiter surveys have been conducted over the past several years on thousands of elementary school children in the state of São Paulo. Detailed maps have been drawn of the disease distribution. Radioiodine uptakes have been obtained on selected children to measure the effects of graded doses of iodide under various dosage schedules. Protein-bound iodine and antibody determinations have been made in blood.

RESULTS TO DATE

In many of the rural and semirural areas of the state of São Paulo, the incidence of goiter in school children is about 15 to 20 per cent. Large or nodular goiters are seldom encountered. Accordingly, the endemic is not a severe one. No etiological factor, apart from iodine deficiency, has been found. There is no excess of thyroid antibody in these patients. The customary elevated uptake of radiiodine slowly returns to normal levels when supplements of iodide are given. This swing is measured in terms of many weeks rather than days. The uptake is depressed much more rapidly by administration of thyroid. In spite of long-continued, seemingly adequate iodide prophylaxis, there is a small residue of goiter patients in the younger age group in which etiological factors other than iodine deficiency evidently cause the goiter to persist. These factors remain to be determined. The iodide prophylaxis program acts as a natural screening for those patients who must have etiological factors other than iodine deficiency per se.

SIGNIFICANCE

This investigation serves to point out the prevalence of endemic goiter in the state of São Paulo, and its good but incomplete response to administration of prophylactic iodide. It also indicates that a residual group of patients failed to respond to this program and must have etiological factors other than iodine deficiency. The results also indicate and

measure the slow fall in iodine avidity occasioned by administration of iodide, and the fact that in this region endemic goiter does not seem to be related to autoimmunological factors.

OTHER DATA

Grantee: Dr. Yaro R. Gandra, School of Public Health, University of São Paulo, São Paulo, Brazil.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1962-1967

ENDEMIC GOITER IN A GENETIC ISOLATE IN CHILE

PROBLEM

Adequate data are not available on the question of the relationship of endemic goiter to genetic factors. A genetic isolate exists in the Pedregoso district of central Chile, where endemic goiter is present and intermarriage is frequent. A unique opportunity is presented for a study of the epidemiology and physiology of endemic goiter and its possible relationship to genetic factors.

METHODS

A full clinical examination of all inhabitants in the district has been made. Radioactive iodine uptake, turnover, and excretion have been among the determinations made. Some pathological specimens have been obtained following surgery of the thyroid. Items in the diet of the inhabitants of the district have been tested for goitrogenic activity.

RESULTS TO DATE

Findings on the Indians of the Pedregoso region are in accord with the general concept that iodine deficiency is the principal cause of goiter. No evidence for a contributing genetic factor has been found, except in three brothers in whom there appears to be some impairment in dehalogenase activity. Preliminary studies in rats have indicated that the seed of the Araucaria tree, a principal dietary item of the inhabitants of Pedregoso, is goitrogenic.

SIGNIFICANCE

No evidence has been adduced to support the hypothesis that genetic factors have contributed importantly to the pathogenesis of endemic goiter in the Pedregoso region. This being the case, iodine deficiency appears to be the only definable cause.

OTHER DATA

Grantee: Dr. José Barzelatto, Hospital del Salvador, University of Chile, Santiago, Chile.

Funded by: National Institutes of Health/U.S. Public Health Service and the International Atomic Energy Agency.

Timetable: 1961-1969

ENDEMIC GOITER IN THE CAUCA VALLEY OF COLOMBIA

PROBLEM

Although severe endemic goiter has been known in the Andean region of Colombia since the time of von Humboldt and Boussingault, the endemic goiter now available for study in the Cauca Valley is mild in degree. Iodide prophylaxis has been in effect since about 1953, but thyroid abnormalities can still be found in preadolescents of this district, and new goiters are appearing in spite of normal iodine content in urine. To date, there seems to be no increased iodine avidity by the thyroids of these subjects. A study of the etiology of the disease was therefore proposed.

METHODS

The town of Candelaria - about 20 miles from Cali - has been chosen for study. All school children there have been surveyed repeatedly over the past several years, and a careful study has been made of the relationship of endemic goiter in these children to water supply and food sources. Measurements of iodine metabolism are being made, and the effect of various food and water samples is being tested.

RESULTS TO DATE

Surveys to date have disclosed the presence of a mild endemic without associated cretinism or other elements of retardation. There seems to be some relationship to water supply and to changes in water supply. One area of the town supplied by one water source seems to have far more thyroid abnormalities than another. There is no increased ^{131}I uptake in this group.

SIGNIFICANCE

The principal significance to be derived from these studies is that obviously mild endemic goiter may exist without evidence of a growth stimulus to the gland, as indicated by increased ^{131}I uptake. The possibility of a positive goitrogenic effect with compensatory hyperplasia exists. Thus, conditions are favorable for demonstrating with certainty for the first time a positive goitrogenic factor in the pathogenesis of endemic goiter.

OTHER DATA

Grantee: Dr. Eduardo Gaitán, Facultad de Medicina, Cali, Colombia.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1964-1968

THE PATHOPHYSIOLOGY AND PROPHYLAXIS OF ENDEMIC GOITER AND OF CRETINISM IN ECUADOR

PROBLEM

The purpose is to study the incidence of goiter and related disturbances, such as cretinism, short stature, and mental retardation, with respect to their genetic and environmental factors and with major emphasis on pathophysiological changes in endemic cretinism. To test the beneficial effects of iodinated oil on endemic goiter and cretinism, a prophylaxis program is contemplated to assess its effects on physical and mental deficiency states that coexist with endemic goiter, but whose relationship to it is undetermined.

METHODS

A complete inventory of several villages in Ecuador has been made. Data on many aspects of the health of each individual in these communities and on their family background have been tabulated. A number of classical cretins have been brought to Quito to study their bone, cardiopulmonary function, iodine metabolism, sensory perception, and psychological attainments. A large number of selected preadolescents in each of three villages chosen for the iodinated oil prophylaxis program have had bone age determinations made by X-ray. The inhabitants of one of these villages have been injected with ethiodol, an iodinated oil. Careful follow-up of newborns in these groups is in progress.

RESULTS TO DATE

Data obtained to date regarding the goiter endemic in the highlands of Ecuador are consistent with the theory that severe deprivation of iodine is the responsible factor in this disease. Goiter, however, was inexplicably found in some areas where the daily iodine urinary excretion seemed normal. Deafmutism is found only in villages where cretinism is present. Retarded bone age and EEG abnormalities were demonstrated in cretins. It may be concluded that cretinism in a severely endemic area is in no sense an all-or-none phenomenon, but rather, that all grades of retardation are found in the iodine deficiency syndrome spectrum. The iodinated oil prophylaxis program was instituted early in 1966, and evaluation will not be forthcoming for at least a year.

SIGNIFICANCE

These studies make possible a more comprehensive view of the nature and impact of endemic goiter than has been available up to now. An appreciation of the nature of cretinism and of the impact of severe iodine deficiency in a population is emerging. The ethiodol prophylaxis program should provide information on the impact of severe iodine deficiency. For the first time, data will be available on the effect of iodine deficiency and of endemic goiter on postnatal and infant physical development.

OTHER DATA

Grantee: Dr. Rodrigo Fierro, Escuela Politécnica, Quito, Ecuador.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1962-1969

IODINATED OIL IN THE PREVENTION OF ENDEMIC GOITER AND CRETINISM IN ECUADOR

PROBLEM

In 1965, the PAHO Scientific Group on Research in Endemic Goiter recommended that, in areas of Latin America where iodization of salt proved impractical, alternative methods be explored for the prevention of endemic goiter and cretinism. One of the methods suggested was iodinated oil by intramuscular injection, such as has been used in New Guinea (McCullagh 1959 and Hennessy 1964). These studies indicated it to be a practical and effective method for the prevention of endemic goiter in young children.

The objectives of the study in Ecuador were to establish the prophylactic effectiveness of intramuscular iodinated oil and to determine its feasibility in public health practice in rural areas of developing countries.

METHODS

Two isolated rural communities in Ecuador (La Esperanza and Tocachi) of similar size, composition, and physical, biological and sociocultural environment were selected. Both towns have approximately 1,000 inhabitants and migration is low (less than 3%). Food consumed by these populations is grown locally, and there is practically no trade in food with the rest of the country.

A complete census of each town was made, identifying each person by house, name, age, sex, and an index number. Community leaders prepared the population for the study, and full cooperation in both towns was obtained. In March 1966, anthropological, clinical, biochemical, and dietary surveys were begun. In Tocachi, the inhabitants were injected with iodinated oil. La Esperanza served as the control town. In both populations, clinical assessment was made of goiter size, and obvious physical or mental defects related to endemic goiter were identified. Children underwent a detailed pediatric examination including: (a) height and weight data; (b) assessment of psychomotor and mental development; (c) clinical examination, including the size of the thyroid gland; (d) an X-ray examination of the hand and, in some cases, of the head and foot; and (e) a brief dental examination, stressing tooth development.

A twenty-four-hour recall study of dietary intake was carried out in a subsample of 25% of the families in each town. Special attention was given to any possible iodine-rich elements in the diet.

All clinicians involved in the study participated in an initial standardizing procedure for classification of clinical enlargement of the thyroid gland.

In both towns, more than 80% of the inhabitants received a clinical examination of the routine and detailed variety, and in Tocachi, the population examined received an injection. In adults, this was given in the deltoid or triceps areas, and in children, in the buttocks, according to a dosage schedule that varied with age. It is planned to complete the survey and injection of the residual population at the earliest opportunity. The preparation used was a 37% solution of iodine by weight (475 mg per ml) in poppyseed oil.

Samples of blood and urine previously had been taken in subsamples of each population for determination of protein bound iodine (PBI) and for ^{127}I in random samples of urine. In both populations, levels of urine iodine were very low.

All cases of obvious mental deficiency (deaf and dumbness and cretinism) were recorded and received treatment similar to the rest of the population.

Future plans call for a physician to be stationed in the town of Tocachi to observe the effects of iodine injection and to inject all children born in the area. Follow-up studies, similar to the baseline study described, will be carried out at 12-month intervals.

RESULTS TO DATE

Earliest results will be available after a year.

SIGNIFICANCE

After 25 years of intensive efforts to introduce iodinated salt for the prevention of endemic goiter in the Americas, only 3 countries out of 20 now have effective programs. Endemic goiter and cretinism continue to represent a widespread public health problem in the highland areas of Latin America, affecting over five million people.

In view of the administrative and commercial problems involved in salt iodization, the use of intramuscular iodinated oil can serve two important purposes: (1) to provide protection against endemic goiter and cretinism until such time as iodized salt is effectively introduced in a given area; and (2) to provide protection to isolated population groups living in mountainous areas outside of normal commercial channels, e.g., isolated Indian communities.

The cost of this procedure is approximately 36 US cents per adult and 18 cents per child and will probably give adequate protection for at least 2 years. Because of the convenience of this method, the injection can be given in the same way as smallpox vaccination, diphtheria and tetanus inoculation, etc. This can be conducted through local health services under medical supervision.

OTHER DATA

Grantee: Dr. Rodrigo Fierro, Escuela Politecnica Nacional, Quito, Ecuador.

Funded by: Pan American Health Organization/World Health Organization.

Timetable: 1966-1970

FACTORS GOVERNING IODINE BALANCE IN ENDEMIC GOITER IN RURAL MEXICO

PROBLEM

Iodine deficiency is regarded as the principal, if not the sole, cause of endemic goiter in Latin America. Although the intake of iodine presumably varies daily and seasonally, the effect of these fluctuations on iodine metabolism and balance and on the development of endemic goiter has not hitherto been observed because of difficult field conditions and the impossibility of making meaningful interpretations of data when acute changes in iodine intake take place. No measurements have been made, either of the effects of specific regional diets on iodine balance over protracted periods of time, or of the acute effect of sudden change in iodine balance. The purpose of the research, therefore, is to obtain useful information on these problems.

METHODS

A small field laboratory and metabolic unit was established in a village in rural Mexico where endemic goiter was already recognized and its extent and severity measured. Patients were kept in the metabolic unit or fed from the metabolic unit on standard diets. Various manipulations were made in these diets. Measurements of iodine and other nutrients were made in food, iodine excretion in urine and feces was determined, and the effects of added iodide and of other changes in the diet were appraised.

RESULTS TO DATE

Long seasonal swings in iodine balance have been observed. In some seasons of the year, when exogenous foods are primarily consumed by the native group, the patients subsist in strongly positive iodine balance, whereas in other seasons the balance is negative. Addition of iodide supplements to the diet results in a slow, even fall in radioiodine avidity to a new steady state. These fluctuations are measured in terms of weeks. No effect of any dietary item has been observed on iodine balance, and this has been taken to indicate the absence of significant goitrogenic factors in the local diet. It was found that radioiodine uptake can be maintained at normal levels by the administration of 5 to 10 mg. of iodide once per week. In the iodine deficiency state, obligatory fecal loss of iodine became relatively large, and evidently contributed to the severity of the iodine deficiency.

SIGNIFICANCE

The studies indicate that the thyroid responds slowly to fluctuations in available iodine. By dietary supplements at weekly intervals, the activity of the gland can be maintained at a level that does not produce thyroid disease. Administration of large doses of iodide can quickly reduce the retention of radioiodine to negligible levels, and this may be of significance in the event of nuclear accidents with radionuclide fallout. In an iodine deficiency state, fecal loss may be of importance. The results suggest, but not prove, that in the endemic under study, dietary goitrogens are not significant factors.

OTHER DATA

Grantee: Dr. Jorge Maisterrena, Hospital de Enfermedades de la Nutrición, Mexico, D.F., Mexico.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1962-1968

THYROID FUNCTION IN VENEZUELAN INDIANS

PROBLEM

Earlier studies have shown that thyroids of many Indian groups in Venezuela have high avidity for iodine, without increasing in size. The aim of the research is to investigate the nature of the change without compensatory thyroid growth as opportunities for observations on these remote and primitive groups become available.

METHODS

Expeditions to the various Indian tribes have been made. Measurements include uptake and turnover of radioiodine and analysis of blood samples. Samples of urine and feces are difficult to obtain because of tribal prejudices.

RESULTS TO DATE

The thyroids of aboriginal tribes have been shown to exhibit the curious anomaly reported before of high avidity for iodine in the absence of goiter.

SIGNIFICANCE

The anomaly of thyroid function exhibited by native groups in Venezuela suggests the possibility of additional growth factors, apart from TSH, in the development of goiter resulting from iodine deficiency. Further studies of this phenomenon are indicated because of its bearing on the general problem of endemic goiter.

OTHER DATA

Grantee: Dr. Karl Gaede, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

Funded by: Instituto Venezolano de Investigaciones Científicas.

Timetable: 1962-1968

ATHEROSCLEROSIS IN LATIN AMERICA
(INTERNATIONAL ATHEROSCLEROSIS PROJECT)

PROBLEM

The immediate aim of the study is to determine quantitative differences in the severity and extent of atherosclerosis among several population groups in the Western Hemisphere with differing social, economic, and environmental conditions, as well as genetic characteristics.

Two general questions are raised: 1. What is the history of atherosclerotic lesions in qualitative and quantitative terms for population groups of different environmental and racial backgrounds? 2. Are there any significant differences in the development of these lesions that can be related to differing environmental conditions?

METHODS

Aortas, coronaries, and basic information from more than 20,000 cases were forwarded periodically to the central laboratory at INCAP by 20 collaborating laboratories in the following countries: Brazil, Chile, Colombia, Costa Rica, Guatemala, Jamaica, Mexico, United States, Venezuela, West Indies, India, Italy, Japan, Korea, Norway, Philippines, and South Africa.

All the specimens were grossly stained, bagged in plastic envelopes and filed for later examination. A protocol was prepared in order to standardize the sample. A group of trained pathologists, all of them senior investigators, met periodically to estimate visually the extent (in percentage) and quality of the lesions observed (fatty streaks, fibrous plaques, complicated and calcified lesions).

All the information collected was transferred to punch cards for statistical analysis. The visual evaluation meetings were held periodically, either at INCAP or at the School of Medicine of Louisiana State University. The final statistical analysis and tabulation is being done in the latter institution.

RESULTS TO DATE

Conclusions will be reached upon completion of final statistical analyses. Trends indicated by preliminary analysis are summarized as follows:

1. There is a complete spectrum of lesions from such areas as South Africa and Guatemala to the United States of America and Norway.

2. In all populations so far studied, fatty streaks are practically universal beyond 10 years of age.

3. The greatest difference between groups is mainly in fibrous plaques and more advanced lesions.

4. Population differences are greater in coronary arteries than in the aorta.

5. On a group basis, aortic lesions are positively correlated with coronary artery lesions.

6. Each of the three coronary branches behave similarly.

7. Fibrous plaques, complications, and calcification parallel the general accepted prevalence of coronary heart disease in each group.

8. Patients with history of diabetes and hypertension show more extensive lesions.

SIGNIFICANCE

Basic information on the distribution of preclinical atherosclerotic lesions will be available as differentiated from that obtained through the usual epidemiological methods for studying atherosclerosis and its clinical complications.

Since atherosclerotic lesions begin many years before the clinical disease, any preventive measures must take into account the long natural history of the disease upon which this study is providing data.

That part of the study being undertaken in populations from developing countries will permit the conduct of future surveys to determine the nature and extent of changes associated with economic, social, and nutritional differences.

These data will test present hypotheses covering the pathogenesis of this disease and will permit the development of additional hypotheses to be tested by other investigations.

OTHER DATA

Grantee: Dr. Carlos Tejada, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala, and Dr. Henry C. McGill, Louisiana State University, School of Medicine, New Orleans, Louisiana

Funded by: National Institutes of Health/ U.S. Public Health Service

Timetable: 1960-1965

RELATION OF ATHEROSCLEROSIS TO ENVIRONMENTAL FACTORS

PROBLEM

The specific aims of this project were as follows:

To contribute to the determination of the true incidence of atherosclerosis and related complications in Guatemala, as part of the coordinated study established under the International Atherosclerosis Project.

To continue observations of the variations of serum lipids among Central American population groups, in an effort to determine more precisely the relative influence of various environmental factors, including specific dietary components, in bringing about the lower serum cholesterol levels observed among the lower socioeconomic groups in Central America.

METHODS

To fulfill the first objective, approximately 2,000 aortas and coronary arteries from persons necropsied in charity hospitals and the medicolegal service in Guatemala City were collected. Furthermore, in approximately 500 of those cases, the vertebral, common, and internal carotid arteries, as well as the arteries from the base of the brain, were also collected. All this material was processed according to the operating protocol of the International Atherosclerosis Project.

To accomplish the second objective, several studies were performed. Each has its own methodology according to its purpose. In general, the following techniques were used:

Clinical, anthropological, and dietary surveys;

Collection of blood samples for blood viscosity and several biochemical determinations (cholesterol, proteins, electrophoresis, gas chromatography, etc.);

Supplementation of the diet with different foods and substances (such as pure cholesterol);

Experimental studies, using animals, in order to reproduce dietary patterns and nutritional conditions similar to those observed in the human population of Central America.

RESULTS TO DATE:

Based on data from sudden accidental deaths in Guatemala, no correlation exists between aortic atherosclerotic lesions and serum cholesterol or lipid phosphorus levels.

Crystalline cholesterol added to the diet of Guatemalan rural school children failed to raise serum cholesterol and other lipid fractions. When the diet included two boiled eggs daily, the serum cholesterol increased significantly.

Surveys among population groups in Central America indicate that the differences observed in serum cholesterol are due primarily to dietary factors.

Young males in the United States of America have higher levels of total cholesterol, phospholipids, and neutral lipids than rural Guatemalan. Arachidonic acid in cholesterol esters and phospholipids, as well as in total serum, are also higher in the United States of America.

Blood viscosity is apparently independent of the total serum cholesterol and of fat intake. Fasting and postabsorptive lipemia show no correlation with blood viscosity.

Essential hypertension is practically absent among low-income agricultural Indian workers. Blood pressure in high-income and Indian industrial workers is more elevated than in agricultural Indian groups.

SIGNIFICANCE

While recognized as one of the most important causes of death in population groups from highly developed countries, atherosclerosis is seldom seen in underdeveloped countries and has a very low mortality risk. It is important to establish the differences observed in these two population groups, so as to study the pathogenesis and etiology of this disease.

OTHER DATA

Grantee: Drs. José Méndez de la Vega and Carlos Tejada,
Institute of Nutrition of Central America and Panama,
Guatemala City, Guatemala.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1960-1966

EPIDEMIOLOGICAL RESEARCH ON CANCER IN LATIN AMERICA: A PLANNING CONFERENCE

PROBLEM

By 1960, cancer ranked as one of the first five principal causes of death in 11 of 20 Latin American republics. Almost one-sixth of the deaths among the population 15 years of age and over is due to cancer. In the age groups in which the incidence of malignant neoplasms is high, the risk for most Latin American populations appears to be as great as for those in the United States, Canada, or countries of Western Europe. In many countries there is a growing recognition that cancer control must be widened to include prevention and early diagnosis. Epidemiological study of cancer is a direct approach to this goal.

Marked differences in the incidence of various forms of cancer have been noted between Latin American countries and also between countries in this and in other areas of the world. Variations in environmental conditions in the Americas appear to offer a fertile area for epidemiological research on the occurrence and etiology of cancers of different anatomical sites.

METHODS

To establish a sound and coordinated approach, the Pan American Health Organization called a Planning Conference on Epidemiological Research on Cancer in Latin America, held in Lima, Peru, at the Instituto Nacional de Enfermedades Neoplásicas, from February 25 to March 1, 1963. Participants were from countries of the Western Hemisphere and represented such disciplines as epidemiology, pathology, surgery, statistics, and administration.

The conference agenda centered on three broad fields in cancer epidemiology and cancer control. The first pertained to methodology and criteria for obtaining reliable information on cancer incidence and cancer mortality; the second related to the specific fields in which epidemiological research should preferably be carried out because of unusual occurrence of the disease or special population characteristics; the third considered the education and training of personnel required for epidemiological studies on cancer.

RESULTS TO DATE

The Planning Conference made the following recommendations to the Pan American Health Organization for developing cancer control activities and epidemiological research on cancer in Latin America:

That the Organization begin to provide consultation services in the field of epidemiology and cancer control.

That the Organization assist in the identification and assessment of suitable experimental or demonstration areas for study of special problems in cancer.

The criteria suggested at the Conference for improving mortality statistics and for developing morbidity statistics should be used as guidelines by the Pan American Health Organization.

Periodically, the Organization should obtain comparable information on morbidity and mortality from cancer and should circulate reports of special interest.

In developing epidemiological investigations, the Organization should help to establish definitions, standards of clinical and pathological diagnosis, and epidemiological methodology to produce comparability of data among the study areas.

Collaborative epidemiological investigations into the incidence and causes of cancer of four sites with high morbidity or mortality should be designed and initiated in several areas in Latin America. The four sites recommended for study are: the stomach, cervix uteri, skin, and lung.

In selected areas investigations should be planned on cancers of five sites of unusual interest or unusual geographical distribution: esophagus, liver, gall bladder and biliary tract, urinary bladder, and oral cavity.

An inter-American reference center for pathology should be established in connection with the center for research training in pathology now being proposed by the Organization. Such a reference center would assist epidemiological investigators by setting up standards and assessing the comparability of pathological material.

A meeting of medical school faculties should be convened to plan the integration of instruction on cancer throughout the medical school curriculum, stressing epidemiological approaches.

The Organization should encourage schools of public health to give more emphasis to cancer and chronic diseases as community responsibilities.

Research training centers for epidemiology and biostatistics should be established in close association with schools of public health and health services.

Plans of the Organization for training medical record librarians should be expedited to improve the quality of hospital morbidity data as rapidly as possible.

SIGNIFICANCE

The recommendations of the Planning Conference serve as guidelines for the countries and the Organization to develop research on the epidemiology of cancer and its control.

OTHER DATA

Grantee: Miss Mary H. Burke, Pan American Health Organization,
Washington, D.C.

Fundedby: National Institutes of Health/U.S. Public Health Service.

Timetable: 1963

SALT FLUORIDATION STUDIES IN FOUR COLOMBIAN COMMUNITIES

PROBLEM

Dental caries is regarded as the principal problem in dental health. The long-term objective of this study is the development of salt fluoridation as a practical mass method of caries prevention.

There is enough knowledge now available concerning the effectiveness of small amounts of fluoride in the diet during the years of teeth formation to increase the resistance of teeth to caries attack. There is also considerable evidence to make the assumption that fluoride in the proper dosage will be effective with different vehicles.

A study in Switzerland indicates the effectiveness of salt fluoridation and calls for further studies, not only to confirm its efficacy, but also to develop a methodology for the establishment of safe optimum doses of fluoride to be used under those conditions.

The specific aims of this project are: 1. to study the effectiveness of common salt as a vehicle in fluoridation programs for caries prevention; 2. to compare the effectiveness of sodium and calcium fluoride as salt additives in caries prevention; and 3. to establish optimum levels of fluorides in salt, for general, safe application.

METHODS

Four communities in Colombia have been selected for the study. They are basically similar in composition, population, geographic location, climate, social development, and health status (including dental health). One community is receiving its normal supply of common table salt as used in Colombia (control population). Two of the other communities respectively are receiving on a continuous basis common salt with added sodium fluoride tricalcium phosphate mixture and common salt with added calcium fluoride. The remaining community is being provided with water fluoridation, so as to obtain comparable data on caries prevention and urinary fluoride excretion.

A basic dietary survey (with special attention to the consumption of salt in the younger ages) was planned on a 10 per cent randomly selected sample of each community, using a seven day observation weighing technique. Clinical nutrition studies are also to be conducted, and x-ray examinations to be made of hand and wrist bones to determine bone density and development. A survey of caries experience is planned using the American DMF index, with tooth as the unit of measurement. Urinary fluoride excretion studies in the four communities are to be used as a means of adjusting the fluoride intake to optimal levels.

RESULTS TO DATE

A detailed census was taken in 1963 of the four communities in both urban and rural areas. Each inhabitant was identified by name, age, sex, residence, and socioeconomic status. Also, a determination was made of hematocrit, hemoglobin, total proteins, electrophoresis of protein fractions, vitamin A, carotene and riboflavin. X-ray examination of the wrist bones was made to observe bone density and development. The distribution of fluoridated salt started in June 1965 as planned: one community receives a mixture of sodium fluoride (1%) and calcium pyrophosphate (99%); another receives calcium fluoride (0.93%), and tricalcium phosphate (99.07%); and a third community receives fluoridated water. The results of this experiment are quite satisfactory. They indicate that the salt is uniformly mixed with the phosphate fluoride and that the distribution to the communities is being extremely well accepted by the people, who are aware that they are receiving fluoridated salt. The average daily consumption of table salt per capita (10.63 gms) serves for the dosage of fluoride. Pooled samples of urine for all ages (12 to 14 years old) is used for dosage adjustments of fluorides in the community fluoridated water and in the experimental communities.

The next step, a second dental survey to be conducted by the middle of 1966, will show if the salt fluoridation measure is useful in caries prevention.

SIGNIFICANCE

At present, the only effective mass method of caries prevention is by fluoridation of water, which usually implies the processing of the community water supplies. It must, however, be borne in mind that about a hundred million inhabitants of Latin America are without an adequate water supply and, consequently, are physically unable to avail themselves of water treatment procedures. Even when water supplies in Latin America are improved, a great percentage of rural dwellers (nearly 60 per cent in some countries) will still be without normal and minimum treatment procedures, since they will remain dependent on small, local water supplies, not very adaptable to fluoridation. It is therefore urgent to seek some other vehicle of universal need and use, such as salt, so that these populations can benefit from fluoridation programs. In short, salt fluoridation in rural areas could be a good supplementary measure to water fluoridation.

OTHER DATA

Grantee: Dr. Hernán Vélez, University of Antioquia, Medellín, Colombia.

Funded by: National Institutes of Health, U.S. Public Health Service.

Timetable: 1963-1968

MANGANESE POISONING: A METABOLIC DISORDER

PROBLEM

It is planned to elucidate the mechanisms whereby chronic industrial inhalation of manganese ores induces a schizophrenia-like syndrome, followed by either Parkinsonism or a Wilson's disease-like syndrome. It was expected that the derangement in manganese homeostasis might reflect itself in distortion of cholesterol and lipid metabolism.

METHODS

The methods used were: (a) turnover of manganese in normal subjects and manganese-poisoned individuals, through radioisotopic studies concerned with tissue uptake of carrier-free manganese, and turnover studies for determination of life span of erythrocytes; and (b) biochemical studies of plasma protein transport of manganese in both normal and manganese-intoxicated persons.

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, detailed neurologic and psychiatric evaluations were continued on these patients. Attention was given to neutron activation analysis for natural manganese in body fluids and cell samples sent from Chile to the Brookhaven National Laboratory, U.S.A.

RESULTS TO DATE

The methodology of neutron activation analysis for manganese was re-investigated and improved. This was necessary because values obtained have been lower than those of other laboratories. Manganese-poor human samples (i.e., blood serum, spinal fluid) were always yielding markedly lower values than those of others. Reinvestigation has shown the following: (1) Contamination is the most serious of the hazards of ultramicroanalysis. (2) Contamination was occurring under the laboratory conditions of this investigation. Its control has lowered serum values for manganese even further, from about 1 to 2 parts per billion to 0.6 to 1.5 parts per billion. (3) Undesirable nuclear reactions were shown to be highly improbable under the conditions of this study. (4) This method was shown to agree with other methods, provided that the materials were handled similarly. (5) Normal values were obtained and the stability of the metal's concentration in serum and whole blood was investigated under several metabolic conditions (Cotzias, G.C., Miller, S.T. and Edwards, J. Neutron Activation Analysis: The Stability of Manganese Concentrations in Human Blood and

and Serum. Journal of Laboratory and Clinical Medicine, May 1966, in press).

Data from the study of healthy Chilean miners were compared to those from patients with chronic manganese poisoning and a normal control group. The healthy miners showed a surprising incidence of the cog-wheel phenomenon as an isolated finding and a markedly accelerated total body turnover of ^{54}Mn . The clinical findings pertaining to the sufferers with chronic manganese poisoning were tabulated. The disease emerged mainly as an extrapyramidal one, but damage to parts of the brain other than the extrapyramidal system was also elicited. These patients had normal total body turnovers of ^{54}Mn and displayed no hematological or metabolic differences from normal controls within the parameters tested.

SIGNIFICANCE

Viewed together with animal data, the present findings indicate: (1) normal, working miners must be living with a significant tissue burden of manganese; (2) the patients with chronic manganese poisoning lost their manganese burden after removal from the mines, without losing the symptoms and signs of chronic manganese poisoning; (3) chelation therapy is not indicated in such cases.

Data from neutron activation analysis of tissues confirm the impression already presented that healthy miners have high tissue burdens of manganese. Several subtle physiological points have become apparent, the most significant of which is this: high tissue burdens with manganese do not cause manganese poisoning, and the question arises whether burdening patients with Mn^{++} protects them against Mn^{+++} and Mn^{++++} .

Data from plasma clearance and tissue distribution studies of ^{54}Mn are being tabulated. At the moment, it appears that besides tissue analysis for manganese, there exists at least one nondestructive method for detecting high manganese tissue burdens. This method is the total body turnover measurement of ^{54}Mn .

OTHER DATA

Grantee: Pan American Health Organization: Project Coordinator -
Dr. Irvin M. Lourie.

Principal Investigators: Dr. George C. Cotzias, Brookhaven
National Laboratory, Upton, New York, and Dr. Ismael Mena,
Pontificia Universidad Católica de Chile, Santiago, Chile.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1963-1970

STUDIES OF HIGH BACKGROUND RADIATION IN BRAZIL

PROBLEM

Increasing use of nuclear energy for civilian purposes will expose a growing number of people to levels of ionizing radiation over and above those normally encountered. The biological effects of ionizing radiation are delayed and, in the case of the genetic ones, may not become manifest for many generations. Since man has had only about one quarter of a century of experience with nuclear energy, every opportunity must be taken to study the biological effects of chronic exposure to ionizing radiation at dose rates above those normally encountered from natural sources.

There are three distinct areas in Brazil in which the levels of ionizing radiation exposure are known to be abnormally high because of radioactive minerals in the soil. The purpose of this study is to define the extent to which abnormally high radiation levels exist and to determine whether biological effects can be identified in the indigenous population.

METHODS

The radiation levels to which the population is exposed are determined by field surveys, by dosimeters worn by samples of the native population, and by analysis of food, water, excreta, and human tissues for the specific radioactive substances known to be present in the area. With the cooperation of local health agencies, demographic data are being obtained to determine how many are living in the area, the sources of their food, and the duration of residence.

The frequency with which somatic mutations are occurring is being studied by chromosomal analyses of peripheral blood lymphocytes. The extent to which genetic mutations are accumulating will be studied by chromosomal analyses of cells obtained from newborn babies. Epidemiological studies on the incidence of leukemia, bone cancer, and other known delayed radiation effects may be undertaken if justified by the levels of exposure and the size of the population. The extent of radiation effects on the native flora and fauna are also being studied by biologists working in the field to catalog the native species and to select certain ones for special studies.

RESULTS TO DATE

It has been determined that in Guarapari, a coastal community where population is exposed to radiation from monazite sands, chromosomal aberrations are found in peripheral blood at a frequency greatly in excess of the control population. The types of aberrations noted are those normally

associated with internal emitters, and measurements have therefore been made of the thoron content of the expired breath. It has been shown that the amounts of thoron in the breath of individuals with abnormal frequencies of chromosomal aberrations are higher than normal. The significance of this observation is that thoron is a radioactive descendant of ^{228}Ra , and it is, therefore, presumed that this nuclide is present in the bodies of Guarapari residents. Some of the Guarapari homes use radioactive beach sand as a building material and the thoron concentrations within the homes are currently under study as a possible secondary source of internal exposure. External radiation exposure at Guarapari has been measured in samples of the population who have cooperated by wearing solid state dosimeters for periods of several months.

At Araxá, in the State of Minas Gerais, the principal source of exposure is the radium in food. Many of the foods range from 20 to 100 times normal in their radium content. Studies are proceeding to obtain data on the sources of food in Araxá, to estimate the daily intake of radium. It appears that there are about 100 people whose radium intake is more than 20 times normal.

It is difficult to obtain surgical or autopsy material for radiochemical analysis. To date, there has been no direct determination of the amount of ^{228}Ra in human tissues. Teeth have been obtained, but they do not provide large enough samples for such analysis. However, it has been determined that placentas contain about 30 grams of ash, and arrangements are being made with midwives to obtain placentas from representative mothers. These will be analyzed for the various heavy radionuclides.

The third radioactive anomaly is at Poços de Caldas where the Morro do Ferro has external radiation levels that range up to 300 times normal. Although there are no human inhabitants on the Morro do Ferro, studies of the flora and fauna provide a unique opportunity to study the long-range effects on subhuman populations. It has been determined that the lung of the rat receives a very high radiation dose, in the range of 3,000 to 30,000 rem per year, and that one plant, of the genus *Melastomataceae*, concentrates radium in far greater amounts than other plants. Studies to estimate the dose received by the various tissues of *Melastomataceae* are under way.

SIGNIFICANCE

These radioactive anomalies are probably the most radioactive places in the Western Hemisphere. Physical and biological studies can go a long way towards providing information that cannot be obtained in the laboratory.

OTHER DATA

Grantee: Dr. Eduardo Penna Franca, Instituto de Biofísica, Universidade do Brasil, Rio de Janeiro, Brasil and Father Francis X. Roser, Instituto de Física, Universidade Católica, Rio de Janeiro, Brasil.

Funded by: U.S. Atomic Energy Commission and the Pan American Health Organization.

Timetable: 1963-1969

MULTIDISCIPLINARY STUDIES ON PRIMITIVE POPULATIONS IN LATIN AMERICA

PROBLEM

The primary purpose of these studies is to ascertain the population structure of primitive man, as exemplified by the American Indian, and to define the selective pressures and the milieu within which that structure functioned. Secondary objectives are 1) to collect data on gene frequencies which, properly programmed for large-capacity computers, will provide new insights into the taxonomic relationships of various Indian groups, and 2) to study changing patterns of disease, especially those with a genetic component, as the Indian makes the transition from the Stone Age to the Atomic Age.

METHODS

A multidisciplinary team, which will always have been preceded by an anthropologist thoroughly familiar with the group under study, will obtain genealogies; perform medical, dental, and anthropometric examinations; and collect blood, saliva, urine and stool specimens. Each specimen of blood, saliva, and urine will be typed in the laboratory with reference to a variety of genetic marker systems and studies will be made of the antibodies, biochemical constituents, and parasites involved.

RESULTS TO DATE

An extended account of the first period of field work with the Xavantes Indians in Mato Grosso, Brazil, has been published and other reports are in preparation (Neel, Salzano, Junqueira, Keiter, & Maybury-Lewis, *Am. J. Human Genet.* 16: 52, 1964). In brief, data concerning the vital statistics, inbreeding levels, polygamy structure, etc., of several relatively undisturbed groups have been collected and will help build a population model. Other data on the gene frequencies, the state of health and the disease pressures of this group have also been compiled.

SIGNIFICANCE

Primitive man is rapidly disappearing. These studies are designed to provide additional insights into the circumstances under which human evolution occurred. They may help explain some of the biomedical aspects of modern man, who, in the genetic sense, has only very recently left the primitive state.

OTHER DATA

Grantee: Dr. James V. Neel, University of Michigan Medical School,
Ann Arbor, Michigan.

Funded by: World Health Organization / Pan American Health Organization,
National Institutes of Health, U.S. Public Health Service,
U.S. Atomic Energy Commission and the National Research
Council of Brazil.

Timetable: 1962

EPIDEMIOLOGICAL STUDY OF POPULATION DYNAMICS IN PERU

PROBLEM

To gain full understanding of cultural, economic, medical and biologic factors influencing the growth or decline of a population, the Ministry of Health of Peru, through the School of Public Health and with the assistance of the Pan American Health Organization, plans to carry out an epidemiological study in population dynamics in three communities.

Retrospective studies and surveys made in the past are usually deficient in records of early fetal losses. The prospective findings in the Khanna study provide a significant five-fold increase in the frequency of abortions in comparison with the findings by the retrospective method. The Inter-American Investigation of Mortality indicates excessive mortality of women from complications of early pregnancy and abortions. As in the Khanna field investigations, the study of abortions as part of a broad study of population dynamics would be in accordance with the principle that the population must be observed, in order to trace the course of events as they happen.

METHODS

It is planned to select three communities in Peru, each with a population of from 2,500 to 3,000, located in the three regions of the country. Two of these communities would be near Lima, one on the coast and one in the altiplano. The third community would be in the jungle area. After reasonable experience has been gained in the first town on the applicability of the method, it will be extended to the other communities.

A record would be obtained of each family and on each member. At the first interview, retrospective data would be collected on all women of child-bearing age (15 to 44 years). As part of the local health program, all but single women would be followed at monthly or bimonthly intervals through household visits by trained local interviewers, who would be supervised by local health personnel. Interviewers would record pregnancies, menstrual periods, dates and termination of pregnancy, breast feeding, fetal losses, live births, and deaths in each family. Observations on each woman would be continued for at least three complete years. Time would also be allowed to complete observations on deliveries of women becoming pregnant before the end of the third year.

A census of these communities would be taken at the initiation of the program. A recording of additions, removals, births, and deaths would be made at one year intervals. Each birth and death would be investigated by

the health officer of the area, using special forms and procedures developed to ensure complete and uniform information (in accordance with internationally recommended definitions). Such data would be used to promote adequate registration procedures, as well as for this study of population dynamics.

The staff would include specialists in epidemiology, statistics, obstetrics, sociology, and anthropology. Faculty members of the School of Public Health, medical schools, and other university departments could contribute their services to this project. A public health nurse would be in charge of and supervise the staff trained to serve as local interviewers. Officers of the health centers would be prepared to participate in this program and to supervise it.

RESULTS TO DATE

Initial planning and development of records and procedures are under way.

SIGNIFICANCE

Observational (or longitudinal) data on women of child-bearing age would provide a complete record of pregnancies that occur and their outcome, intervals between pregnancies and their effects on early fetal, late fetal and infant mortality. Such observational data are considered preferable to retrospective data for complete recording. This is especially true for populations with many provoked abortions. The research program would provide valuable data for understanding the level of fetal and infant mortality, the condition of infants at birth, and the underlying causal factors and sequence of events leading to deaths (fetal, infant, and maternal).

The problem of provoked abortions and its relation to subsequent fetal and infant deaths and maternal health is studied best by the longitudinal method. Knowledge of the health implications of provoked abortions would result from this research.

OTHER DATA

Grantee: Dr. Mario León, School of Public Health, Lima, Peru.

Funded by: World Health Organization.

Timetable: 1966-1969

MALARIA ERADICATION EPIDEMIOLOGY TEAM

PROBLEM

The most serious problem area in the Americas for malaria eradication lies in the Pacific coastal plain of Central America. There seem to be a number of reasons for this. The purpose of the study was to collect and analyze data that might shed more light on the factors causing persistence of transmission in the face of DDT spraying and to develop study methods and techniques of epidemiological analysis that might be useful in the investigation of other problem areas. DDT- and dieldrin-resistance were known to be operating. Irritability to DDT is known to be present in the vector. Other elements are suspected and are under study.

METHODS

Recognized methods of susceptibility testing, bioassays of residual deposits on various surfaces, measurements of irritability, anopheline density, longevity, and sporozoite indices, plus studies of breeding places, climate, and the use of new entomological techniques were combined with continuous monitoring of malaria activity by epidemiological methods, regular mass surveys, search for fever cases, case follow-ups, etc. The behavior of the population, its housing, and changes in housing, movements, and occupation, were studied, together with agricultural practices, irrigation, insecticide spraying of cotton, and cattle raising.

All these factors were analyzed and evaluated.

RESULTS TO DATE

Many contributions were made to a better understanding of the causes of failure to halt malaria transmission in problem areas. Less known elements were brought out, such as irritability, and poor housing, and outdoor habits of the people. Better methods of study were devised or perfected, such as the demountable experimental huts with large cone-less window traps; the excito-repellency test box, which is considered to be a major contribution toward accurate and replicable measurements of the lethal effects of wall deposits, and from which the mosquito is free to leave any time it becomes irritated. The importance of irritability was shown in many situations, and seasonal differences in the behavior of the one responsible vector were described.

A synoptic method was developed for periodic study of all the important epidemiological elements of a problem locality.

SIGNIFICANCE

The problem area of Central America is now much better understood. The causes and dynamics of the persistence of malaria transmission in the area are much clearer. New techniques for studying other problem areas were developed. A highly valuable device for measuring the lethal effects of insecticide in the form of wall deposits, in the face of resistance and/or irritability of the vector, was invented and its use perfected. Its usefulness in predicting the effectiveness of new insecticides, either singly or when placed over irritating deposits of DDT, has been demonstrated.

OTHER DATA

Grantee: Dr. René G. Rachou, Pan American Health Organization.

Funded by: Pan American Health Organization/World Health Organization.

Timetable: Costa Rica, May 1960-January 1961
El Salvador, January 1961-December 1963.

OPERATIONAL RESEARCH IN MALARIA ERADICATION:
I. MASS DRUG DISTRIBUTION BY MEDICATORS

PROBLEM

When residual house spraying fails to halt transmission of malaria, due to technical factors involving the vector or to outdoor exposure of humans to the vector, alternative or supplementary methods of attack must be sought.

In the Pacific coastal region of Mexico and all of Central America, irritability and/or resistance of the vector, frequently coupled with appreciable outdoor biting, poor or altered housing, sorption of insecticide by mud walls, migration, etc., have prevented the interruption of transmission. One of the potentially effective supplementary attack methods is the mass administration of anti-malarial drugs. Since P. vivax is the pre-dominant species in the entire region, a drug combination is desired that, besides suppressing parasitemia (to prevent infection of mosquitoes), would at the same time produce radical cures of P. vivax, i.e., it would attack the exo-erythrocytic cycle and cut short the potential of late relapses in this species.

In about 1961, Alving's work showed that chloroquine plus primaquine in weekly doses would do both these things. The larger the dose of primaquine, up to 60 mg., the fewer the number of weekly doses needed to obtain radical cures equal to the 14-day standard dose of primaquine. Doses of 45 mg. once a week were as well tolerated as 15 mg. once daily.

It was decided that chloroquine could be given in doses that would suppress parasitemia for 14 to 21 days; so, from this standpoint, two-week cycles could be used. Theoretically, the size and the number of doses of primaquine were the important elements; the interval between doses should have no bearing since weekly doses were effective. For the sake of economy, two-week cycles were, therefore, selected for field trials in severe problem areas. Monthly cycles had already shown promise in Trinidad where the persistence problem was a very mild one.

METHODS

Combined tablets of chloroquine 150 mg. of base and primaquine 15 mg. of base were made up for convenience in obtaining gradations of dosage for children. Adults would receive 3 tablets, children 9 to 12 years, 2 tablets, and 4 to 8 years, 1 tablet. A tablet of half size, 75 mg. of chloroquine and 7.5 mg. of primaquine, was made up for children 6 months through 3 years. Medication was given by employees of the service to everyone who could be found at his home or place of work. This was done in El Salvador, especially.

Blood samples were taken initially and routinely from all fever cases and from persons who had missed prior treatments. A 3 to 6% sample of the population was obtained each month. The census was brought up to date during each visit, and arrivals and departures were recorded. Health education methods were developed and evaluated. Side effects of the drugs were watched for and tabulated. Forms were developed and modified as they were used to keep track of every aspect of performance, efficiency of coverage, effects on malaria incidence, quantity and type of supervision, etc.

Relapse rates after the end of various treatment cycles were analyzed. In two instances, weekly cycles were used in small foci of intense transmission over a short period (11 to 12 weeks).

RESULTS TO DATE

The demonstrations showed that the mass administration of combined chloroquine and primaquine was effective if well carried out, but ineffective otherwise. The potential of producing radical cure of P. vivax by 8 or more doses of 45 mg. of primaquine (to adults) even at two-week intervals was confirmed.

Operational norms were worked out: Depending on population density, up to 1,100 or 1,200 persons could be treated in two weeks by one medicator. In sparse areas, where access was difficult, this figure could be as low as 600. The costs of mass drug administration are considerably higher than those of house spraying, but if the job is well done, the duration of attack can be much shorter. Health education is an absolute essential before and during the program. Strict supervision and intelligent, personable medicators are necessary. Poor supervision ranks high as the cause of failure. The second most important cause is that treated or cleared areas are continually reinfested by other problem areas not yet under control.

The dosage for children had to be increased in the upper segment of each age bracket. Reactions are only those due to chloroquine: nausea, dizziness, vomiting, diarrhea, and a host of minor symptoms, mostly irritation of the G.I. tract. About 25% of people had some side effects, but with proper health education, most of these continued to take the drug.

In Haiti, where virtually all the population is negro and 98% of the malaria is P. falciparum, the combination of chloroquine and pyrimethamine is being used in three-week cycles. High acceptance is achieved. The medication is technically effective, but very high mobility of the population results in continual reinfestation from untreated areas unless the entire problem area is under treatment at the same time. As of December 31, 1965, 1,350,000 persons were under treatment. It had, however, been started too recently for the success of such a sizeable operation to be determined.

SIGNIFICANCE

These studies have shown the potential success and the limitations of mass drug administration by paid medicators, which is one of the potent supplementary attack methods for use when spraying alone fails. The most effective and economical methods of assuring a high percentage of regular participation on the part of all the population have been developed and evaluated, and are now ready for wider application. The safety of the method has been demonstrated.

OTHER DATA

Grantee: Investigators in the Malaria Eradication Services of El Salvador, Guatemala, Nicaragua, Mexico, Costa Rica, Honduras, and Haiti. Principal Investigators: PAHO Malaria Advisors in each country. Coordinated by Malaria Eradication Branch, Pan American Health Organization.

Funded by: Pan American Health Organization.

Timetable: El Salvador, June 1961-March 1964
Guatemala, August 1962-November 1963
Nicaragua, August 1962-December 1963
Mexico, August 1962-April 1963
Costa Rica, September 1962-November 1964
Honduras, May 1963-December 1963
Haiti, October 1964 to present.

OPERATIONAL RESEARCH IN MALARIA ERADICATION II. MASS DRUG DISTRIBUTION BY MEDICATED SALT

PROBLEM

In the coastal plain of British Guiana and Surinam, malaria has been eradicated by residual house spraying. In the interior of British Guiana, house spraying was not undertaken because of the high cost of covering a small population. But there was the constant threat that malaria from the interior might be reintroduced into already clear areas. The distribution of chloroquine by adding it to table salt might prove to be an effective and economical way to obtain full coverage, because all the salt for the interior came through wholesale dealers in the capital city. To determine the effectiveness of the method, a field trial was proposed.

In the interior of Surinam, spraying of houses has been carried on for 7 years but has never been well accepted by the majority of the Bush negro population. In 1965, two small pilot trials were begun, using table salt treated either with amodiaquine or with chloroquine silicate, to determine acceptability by the people and feasibility of distribution by local missionary doctors. In view of favorable results, a larger scale epidemiological study is about to begin.

The purpose of the study in Brazil was to run a very small controlled trial for a period of one year, comparing 3 medicated salt mixtures against chloroquine-primaquine combined tablets and against a control.

METHODS

The British Guiana project depended on a law requiring all dealers shipping salt to the interior to first send it to the government processing plant for the addition of medicated salt. The plant added 0.4% chloroquine (as base) to make an estimated average adult dose of 280 mg. per week. Inspectors regularly checked all salt dealers in the treated area to see if their supplies of salt contained chloroquine. They also checked a representative sample of salt in various private houses. Both an active and passive search for cases was continued assiduously throughout the period of treatment. After 4 years, with only 3 known cases, treatment was stopped in two areas, but it was continued 6 months longer in the third, where there had been importation of untreated salt and of resistant P.falciparum from Brazil.

For the small scale Surinam project, medicated salt was prepared with 0.45% and 0.50% amodiaquine or chloroquine silicate in the British Guiana mixing plant. It was offered to all the villagers attending a missionary clinic on the upper Surinam river. Within the time expected, almost all of the families were back requesting more salt.

A small-scale field trial was carried out in Brazil on the island of São Francisco do Sul, comparing 3 kinds of medicated salt with combined chloroquine-primaquine tablets for acceptability and effectiveness.

RESULTS TO DATE

In British Guiana, the medicated salt was completely effective in two districts where there was no source of salt except the medicated salt. Among 28,000 persons, malaria cases dropped to zero in 3 months and remained there, except for 3 sporadic cases whose source was never found. After 4 full years, supplying of medicated salt was stopped. About 100 cases of solar dermatitis were seen, a lichen planus type of reaction considered to be probably due to chloroquine. The reaction was unpleasant for 2 to 5 months and self-limiting whether or not medicated salt was suspended. In the third district, among 8,000 persons, after a sharp drop in the first year, cases began to rise with a very high preponderance due to P. falciparum. Non-medicated salt from Brazil was widely used, and it was known that resistant strains of P. falciparum were crossing the border from Brazil. Medicated salt was continued because of its highly beneficial effects against P. vivax, and because incidence of P. falciparum also appeared to be lower in persons using medicated salt. Transmission was halted by careful house spraying in 1963-1965, and no cases have been found since July 1965.

Preliminary trials in Surinam indicated that medicated salt was accepted without complaint by Bush negroes, when amodiaquine or chloroquine silicate were used. Both are practically tasteless. The trial is about to be extended to the entire upper Surinam river, the area of highest incidence in the country.

The comparative trials in Brazil showed good acceptance of medicated salt only when amodiaquine and chloroquine silicate were used. The former was the only one of 4 regimens that reduced locally acquired malaria cases to zero.

SIGNIFICANCE

Under certain conditions, antimalarial medication in table salt has been shown to be an effective and very economical method of malaria eradication. Factors influencing its success or failure have been explored.

OTHER DATA

Grantee: Investigators in British Guiana, Surinam and Brazil, and
PAHO Malaria Advisors in these countries.

Funded by: Pan American Health Organization.

Timetable: British Guiana, Jan. 1961-Dec. 1965
Surinam, Apr. 1965-1967
Brazil, Jan. 1963-Apr. 1964.

SCREENING CENTER FOR DRUG RESISTANT MALARIA PARASITES

PROBLEM

In a few instances, strains of Plasmodium falciparum have been found to be resistant to chloroquine. This was proved in Colombia, and strongly substantiated in Venezuela, Brazil, and British Guiana. Many suspected cases of chloroquine resistance and sometimes multiple drug resistance are being reported.

It was necessary, therefore, to have a center where, through human subjects and in a controlled environment, suspected resistant strains could be studied to confirm or refute reports of resistance and where alternative methods of treatment could be investigated when standard treatments failed.

METHODS

The Center organized a field screening method for determining, through the preliminary treatment of patients, that the suspected strain of malaria was not responding well to drugs. In special iced containers, samples of blood were shipped to the Center and inoculated into human subjects. Subjects were followed daily or several times a day by recording the body temperature and examining blood for parasites. During the acute attack, smears were taken 3 times a day and examined at once. Full records of all examinations were charted for each patient. If the planned treatment did not work and the patient was in any danger, he was given quinine either for 2 to 3 days to reduce parasitemia or for 10 days to cure the infection. There was a regular and frequent follow-up with blood smears after each treatment. If recrudescence occurred, retreatment was given, either with a larger dose of chloroquine or with a different drug.

Combinations of drugs- particularly the combination of pyrimethamine and various forms of sulfa drugs - were also tested in the treatment of resistant strains. All cases were followed for a 120-day period of negative blood smears before being considered cured. WBC, RBC, and hemoglobin were done routinely, and chloroquine levels in urine and sometimes blood when indicated. Transfusions and leucovorin were given to a few cases who showed marked leukopenia under the combined drugs, pyrimethamine and sulfamethoxy-pyridazine.

RESULTS TO DATE

In all, 15 strains of malarial parasites were studied, 10 from various areas in Brazil, 4 from Colombia, and 1 from Venezuela. Two more were picked up from persons who came to the Center seeking help for resistant infections.

One-hundred-thirty-one candidates were given 169 inoculations, either original from the field, or sub-inoculations. Inocula were either fresh, chilled, or frozen blood. Only one strain was sensitive to standard doses of chloroquine. Doses up to 5.4 gm. total were given. The larger the dose, the higher the percentage of cures, although some strains resisted even the highest dose. Two thirds of the cases treated were resistant to pyrimethamine alone, but only 6% showed failure, even after smaller doses of pyrimethamine, when this was combined with sulfa drugs. The stage of infection was of importance. There were 83% failures in the first round of chloroquine, but only 50% of treatments of the second or later parasitemias were failures. Three out of 7 strains tested with pyrimethamine were sensitive to this drug.

Quinine was usually effective, but only if a total of 17 to 20 grams was given in 10 days. One case resisted 26.5 grams in 17 days, and is considered solidly quinine-resistant. Three more were not cured by 16.5 to 19.5 grams.

SIGNIFICANCE

The confirmation of resistance to chloroquine and other antimalarial drugs is obviously necessary when many field reports of suspected resistance are not clear or reliable.

Knowledge about effective schedules of combined drug is of the utmost importance. Field trials are now going on to test the value of pyrimethamine with sulforthomidine.

OTHER DATA

Grantee: Dr. A.J. Walker, PAHO Screening Center, Ribeirão Preto, Brazil.

Funded by: Pan American Health Organization/World Health Organization.

Timetable: 1963-1965

INSECTICIDE TESTING TEAM

PROBLEM

The major single factor obstructing malaria eradication in the Americas is resistance of the vector A. albimanus to DDT and to dieldrin. This is found in the Pacific coastal region of Central America. El Salvador is the center of the problem and manifests it in all its varieties and degrees.

The project aimed to study new insecticides and better and more economical ways of using old insecticides to overcome problem areas, i.e., areas that did not respond adequately to residual spraying of houses alone.

The project first undertook evaluation of baytex, malathion, and guthion in 1959. In 1960-1962, more precise studies were made of the activity of DDT on all types of walls, in both El Salvador and Bolivia. In 1962-1963, intensive studies were made of larvicides, including Paris green and baytex. In 1963-1964, basic tests were made of the activity of 18 insecticides, mostly new ones, both alone and in combination with DDT, to evaluate their efficacy against resistant, irritable, and resistant-irritable vectors. Since 1965 a seven-stage scheme for the evaluation of new insecticides has been under way.

METHODS

Initially, the use of new insecticides and of DDT on different types of surface was evaluated mainly using bioassay, with susceptibility tests to measure evolution of resistance in the field and insectary colonies. Methods of testing muds as panels were perfected. Antilarval methods included both hand and aircraft dispersal methods. They required careful and continuous checking of breeding places and adult densities at weekly intervals throughout the year.

Laboratory measurements of resistance and irritability used standard WHO methods. For measuring the impact of irritability on lethality of residues, the mosquitoes had to be given free exit from contact with the insecticide. Refinements of the E-R test box and of techniques for using it were made, and hundreds of tests were performed, using new insecticides in various concentrations, with and without DDT.

Since April 1965, work in the WHO integrated study is concentrated on a new carbamate, OMS-33. It includes Stage IV, Experimental Hut Trials, where 7 huts have been built and have been under test since July; and Stage V, Village Scale Trials, where 3 villages now have received two rounds of spraying. Preparations have been made to start an extended village scale

trial in 3200 houses in April 1966. This will include epidemiological evaluation of effectiveness.

RESULTS TO DATE

In the earliest studies, malathion and baytex were found to be effective against resistant anophelines. This effectiveness, however, was of short duration, averaging only 3 months and much less on some surfaces. DDT was found to be effective against susceptible strains for 6 1/2 months on mud, and much longer on other surfaces. It was partially effective against all resistant forms, according to the bioassay test, but when the mosquitoes were given the option of breaking off contact when irritated (E-R test box), it killed almost none.

Larviciding by Paris green proved disappointing, due to protection afforded larvae by either scum or algae in many breeding places. It proved impractical in rural areas where breeding places were extensive and very changeable. Nevertheless, a great deal was learned about varying ecological factors and seasonal changes in density of A. albimanus.

Many important observations were made in the use of E-R test box for evaluating new insecticides. The probable resultant effect of a new insecticide placed over old residual DDT deposits was measured. Carbamates were especially good at "neutralizing" the irritation caused by DDT.

Panel test showed a tremendous effect of relative humidity on the killing power of OMS-33 before it was tried in huts. Trap huts and village scale trials have shown the limitations of OMS-33 (about 13 weeks, and less in dry air) and the feasibility of spraying it in routine operations (toxicity observations).

SIGNIFICANCE

All these studies are fundamental to finding an effective and economical way to overcome the problem of persistence of malaria, when this is due either to resistance or irritability of the vector, or to both. Resistance and irritability are the principal reasons for failure to halt transmission in the Americas.

OTHER DATA

Grantee: Mr. J.R. Austin, Pan American Health Organization.

Funded by: Pan American Health Organization/World Health Organization.

Timetable: 1959-continuing.

PILOT PLAN OF INTEGRAL ATTACK ON MALARIA TRANSMISSION

PROBLEM

The malaria eradication program of Mexico has progressed well on the Atlantic slope. On the Pacific coast and in the foothills, however, it appears that the level of malaria cannot be further reduced by routine measures of DDT spraying. About 2 million persons live in this so-called problem area, making it the largest in point of population in the Americas, but in degree, the level of residual persistence is only about 2 to 8 cases per 1000 population per year. The worst of the problem area has received a 6 1/2 month course of mass drug treatment, an effective but very expensive method, which, because of migration, cannot be assured of permanent success until the entire problem area is brought under control. This has been followed by attempts to halt transmission by increasing the frequency of spraying, but with only slight benefits.

As an additional method of attack, the present effort is to determine the possibility of success in overcoming residual persistence of transmission by an intensive and complete search for cases, plus radical treatment of all those discovered.

METHODS

DDT will be sprayed inside all houses at the rate of 2 gm./m² three times a year.

Active evaluators are assigned to cover the entire problem area once a month, looking for cases of fever or other illnesses that could be malaria, and taking blood slides from each case found. The slides are examined quickly (within 2 to 3 days) and the information on positives is conveyed to an investigator. In each case, he determines whether it represents current transmission and initiates radical treatment, consisting of five days of primaquine in early stages when cases are numerous, and of 14 days (standard) when they become few.

Mass surveys are made periodically in selected localities to see how effective the screening procedure is in finding cases. Mass radical treatment is tried in a few localities where malaria persists after one year. Intensive entomological studies are made in localities that respond well or poorly, to determine factors operating in each circumstance.

RESULTS TO DATE

Localities known to be positive in 1964 were nearly all negative in 1965. Those on which no information was available in 1964, due to incomplete

coverage with case-finding, turned up a number of cases after full coverage case-finding was initiated. Cases began to rise again in June or July 1965, and were judged to be instances of late relapses or long latent period. From July 1965 onward, the incidence dropped progressively below the seasonal trend and reached an encouragingly low level. Transmission apparently has ended in all but a few localities during the peak of the 1965 season. Spraying will be stopped in many localities and supplementary methods used in the few with obvious persistence.

SIGNIFICANCE

Malaria eradication by use of residual DDT has progressed a long way in Mexico. Some small additional attack measures beyond residual spraying of houses will be required among about two million people. The feasibility and efficacy of a supplementary measure, less expensive than mass drug treatment - namely, complete coverage with active search for cases and radical treatment of all cases found - is thus being explored. This is far more acceptable to the public than mass drug treatment. If the scope of its potential is discovered, it may be used in many localities instead of the more costly mass treatment, thus making it possible to complete this and other programs at a savings of present funds, or within funds that might later be made available.

OTHER DATA

Grantee: Dr. Pérez Yekutiél, Pan American Health Organization, Mexico.

Funded by: World Health Organization.

Timetable: 1964-1967

IRRADIATION EFFECTS ON THE BIOLOGY OF RHODNIUS PROLIXUS

PROBLEM

It was supposed to determine, both under laboratory and field conditions, the effect of ionizing radiation on the biological and ecological factors regulating the dynamics of Rhodnius prolixus, the principal vector of Chagas' disease in Venezuela, with specific reference to the effects of sterilization by irradiation on the biotic potential of the vector.

METHODS

The frequency, duration and reproductive effectiveness of successive copulations, number of eggs and their eclosion was observed under different environmental conditions, varying sex ratios, feeding, frequency and insect ages. Long term genetic alterations, if observable, were to be followed. The ecology of the insect was studied, chiefly by means of radioisotope tagging - with special attention to the distribution, dispersion, movement, longevity, mortality, density, population interactions, and limiting factors.

The effects of ionizing radiation on the biological and ecological factors enumerated was observed in the laboratory - which included a study of the effects of sterilization by irradiation on the biotic potential of Rhodnius prolixus. Depending upon the results of the latter investigations, it was contemplated moving the experiment to semi-natural conditions, and possibly, finally, to natural conditions on a presently uninfested island of Lake Valencia.

RESULTS TO DATE

Studies of the biology and life history of R. prolixus were brought near completion. Feeding frequency and other environmental changes do not affect the survival of the insect as much as they do its reproductive capacity and the intrinsic rate of population increase. Flask population studies were started at different sites to obtain information on population growth potentials in different areas of the country. Preliminary results suggest that areas up to 800 meters of altitude with a mean temperature range of 26° to 29° C, a yearly rainfall of 1200 to 2000 mm and a mean relative humidity from 50 to 80% offer the best environment for R. prolixus.

By the use of ⁶⁰Co tagged individuals, it was also found that the differences between the so-called intradomiciliary and sylvatic strains of R. prolixus are due more to dietary conditions than to genetic mechanisms. Contrary to what was believed before, R. prolixus moves by its

own locomotion between its sylvatic habitats, from these habitats to human dwellings, and between human dwellings. The frequency of dispersion depends on the distance between habitats and the degree of starvation of the insects.

Tests at IVIC and at Chalk River, Canada, have proved that ionizing radiation at exposures from 8,000 to 20,000 R interferes seriously with mating behavior in the male. Significant lethality, however, occurred in eggs from females mated to males treated at 5,000 R. The results also indicated that males 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.

SIGNIFICANCE

Tests with laboratory populations, utilizing R. prolixus males exposed to ionizing radiations (5,000 R gamma), gave results suggesting that, although 25% fertile, these males are capable of reducing normal populations if introduced at a greater ratio than 4 to each normal male of the population. These population studies produced data suitable for biometric analysis of population dynamics and a life table of R. prolixus. Genetic observations were also made of a red-eye mutant which was analyzed and found to be due to a recessive gene that was not sex linked.

A study of certain phytosociological relationships of the palm Attalea humboldtia was accomplished. This palm is one of the two sylvatic habitats where R. prolixus is most frequently found. The results indicate that, within a specific environment, the presence of the palm, although associated with other plants, is not dependent on them. The palm itself supports a complex biocenosis which permits the survival of R. prolixus and the transmission of T. cruzi between vertebrate reservoirs and vectors.

Some progress on the study of sex attractants in R. prolixus has been achieved. It is known now, from laboratory tests at Chalk River, that only virgin females will attract males and that feeding has some effect on increasing this attractiveness.

The work will be augmented during the next year by field tests in Venezuela.

OTHER DATA

Grantee: Eng. Juan C. Gómez Nuñez, Instituto Venezolano de Investigaciones Científicas, (IVIC), Caracas, Venezuela.

Funded by: National Institutes of Health/ United States Public Health Service.

Timetable: 1964-1967

CHEMOTHERAPY OF CHAGAS' DISEASE

PROBLEM

Chagas' disease is a debilitating parasitic infection, often fatal in either its acute or chronic stage. It affects a minimum of 7 million persons in South and Central America and perhaps several times that many. There is as yet no accepted vaccination procedure nor any really satisfactory drug treatment.

The purpose of this study is to encourage and to conduct research leading to the development of a potent and safe therapeutic agent for use in Chagas' disease. Collateral purposes are to consult, advise, exchange information, arrange for clinical trials, and establish criteria that can be used both in the selection of patients for clinical trials and in the evaluation of the results of therapy. To accomplish these objectives, a Chagas' Disease Chemotherapy Research Group was organized in 1963 consisting of six individuals, four from Brazil, one from Chile, and one from the United States, representing both clinical and research interests and coming from different organizations, such as medical schools, national laboratories, and pharmaceutical laboratories. These individuals have been consulted separately by the principal investigator, have met together during the 7th International Congresses of Tropical Medicine and Malaria to exchange ideas and agree on procedures, and have been kept informed of each other's work through correspondence.

METHODS

The discovery of a new chemotherapeutic agent may result from efforts along two major lines, known commonly as the empirical and the basic (or theoretical) approaches. It is considered important to employ both approaches. In the empirical methods, many chemical compounds from both natural and synthetic sources are tested systematically in laboratory animals to discover activity against the causative organisms of the disease. In the basic or theoretical approach, the causative organism of the disease is studied to determine its requirements, physiology, biochemistry, and metabolism, in the hope that knowledge in these areas may point the way to the development of a chemical weapon that can attack the parasite at some vulnerable point in its life processes and impair it without similarly damaging the host. Representatives of both these approaches are members of the Chagas' Disease Chemotherapy Research Group, who have not only performed their own work along these lines, but have also encouraged similarly oriented individuals in other laboratories. In the phase involving clinical trials, the method of selecting patients to be treated is of importance. Discussions on this and criteria of cure have been another aspect of the Group's endeavors.

RESULTS TO DATE

In the phase involving the empirical search, thousands of new compounds have been screened each year, resulting in the elimination of many chemical groups from further consideration, the discovery of "leads", which have inspired the synthesis of extensive series of compounds, in the hope of obtaining greater activity or lesser toxicity, and the finding of a few substances that have merited more extensive laboratory evaluation and consideration as candidates for clinical trials. In the phase involving the basic approach, a number of areas have been explored, bringing to light metabolic characteristics of the parasite. Also, although no direct result is yet forthcoming along the lines of developing a chemotherapeutic agent, an important finding has been made that may be applied in the development of a living vaccine, attenuated by chemical means, so that the organisms, while retaining their antigenicity, are no longer infective. In the phase involving clinical trials, the clinicians of the Group have collaborated with a group of Brazilian physicians in making coordinated and systematic studies on certain candidate compounds, such as 8-aminoquinolines and nitrofurans, which has allowed more prompt and authoritative opinions on their value than would have been possible if haphazard and uncoordinated trials had been conducted by isolated practitioners.

SIGNIFICANCE

The refractoriness of the causative agent of Chagas' disease to treatment with chemotherapeutic agents available for other systemic infections poses a problem that can only be answered by further research into new chemical areas. Encouragement of any operation, therefore, designed to reveal by rational or chance methods, compounds with activity against T. cruzi are desirable and to be stimulated. A group equipped both to conduct preliminary tests and to evaluate in subsequent studies the potentialities of available substances in the treatment of Chagas' disease is a necessity for discovering and developing a chemotherapeutic agent to suppress and eradicate this infection, which stands as a major obstacle to the building of a more healthy, productive population. The problems of clinical trials and their evaluation are matters of debate and controversy that can only be resolved by the accurate, intelligent observations of the informed and critical workers who are represented in the present Group.

OTHER DATA

Grantee: Dr. Frans C. Goble, CIBA Pharmaceutical Company, Summit,
New Jersey.

Funded by: Pan American Health Organization.

Timetable: 1963

SMALLPOX VACCINATION BY JET INJECTION IN BRAZIL

PROBLEM

During the four-year period from 1961 through 1964, over 23,000 cases of smallpox were reported in Brazil. A field study was undertaken in early 1965 to evaluate the feasibility of incorporating jet injector techniques into the Brazilian national campaign against smallpox in such a way as to accelerate the completion of the attack phase. Plans were formulated to conduct such an evaluation in the Federal Territory of Amapá, with several goals in mind: (1) to vaccinate as many people as possible, in the briefest possible time, as part of the national campaign against smallpox; (2) to demonstrate and evaluate the use of jet injector equipment in field operations in Brazil under a variety of conditions; (3) to compare the cost of and the population response to the accepted house-to-house vaccination program with mass vaccination by jet injector; (4) to determine the efficacy of Brazilian-produced vaccine when administered at various dilutions by jet injection equipment; (5) to compare, by clinical and serologic means, the efficacy of the Brazilian egg vaccine with the Brazilian calf vaccine when used under field conditions.

METHODS

The vaccines used consisted of both the Brazilian calf lymph vaccine and the Brazilian chorioallantoic membrane vaccine, produced at the Instituto Oswaldo Cruz, and a U. S. lyophilized calf vaccine (Dryvax-Wyeth). For multiple pressure vaccinations, the vaccines were used undiluted, and for administration by jet injector, a so-called 1:10 and 1:50 dilution of the vaccines was prepared. For the 1:10 dilution, a standard 0.3 cc. fill of undiluted vaccine was added to 9.7 cc. of saline. For the 1:50 dilution, a 0.3 cc. fill of undiluted vaccine was added to 49.7 cc. of saline. These diluted vaccines were then administered in a dose of 0.1 cc. The U. S. Vaccine was prepackaged and dried in 10 cc. and 50 cc. containers facilitating the addition of saline directly into the lyophilized material. The lyophilized Brazilian vaccine was reconstituted in the field and diluted appropriately as described above. Samples of all lots of vaccine used were titrated before the campaign; all had titers of 10^8 or more pock-forming units per cc. of vaccine. The foot-powered jet injector (Ped-O-Jet), produced by the Scientific Equipment Mfg. Corporation and equipped with an intradermal nozzle, was used in all mass vaccination situations. The hand-model Hypospray jet injector produced by R. P. Scherer Corporation, also equipped with an intradermal nozzle, was used in special situations where mobility was extremely important.

RESULTS TO DATE

Certain clear-cut advantages of the jet injector were demonstrated by the campaign in which 48,000 persons were vaccinated in 20 work days: (1) Consistently higher take rates were observed when vaccinations were performed by jet injection as compared to multiple pressure methods. (2) Mass vaccination with jet injectors, even in the small, semirural areas, resulted in a significant reduction in the amount of time and personnel required. (3) In the city of Macapá, 30,536 persons (86% of the population) were vaccinated in two days, using 7 jet injectors. In Macapá, 300 vaccinations per man day were administered, compared to 75 in Belém, where a house-to-house campaign with multiple pressure technique was used. In addition, the cost per vaccination in Macapá was one-third the cost in Belém. (4) The Brazilian lyophilized calf vaccine compared favorably in clinical use with the U. S. lyophilized calf vaccine in performance of primary vaccinations at the 1:50 dilution. At the 1:10 dilution, both Brazilian vaccines were fully effective when administered by jet injector, either in primary vaccinations or revaccinations. (5) In the special studies comparing Brazilian egg and calf vaccines, there was no difference between the two vaccines in clinical take rates. Serologic results are not yet available.

SIGNIFICANCE

Mass vaccination using the jet injector was found to be applicable to the Brazilian campaign. Certain distinct advantages may be expected from the large-scale use of such equipment: (1) Reduction in vaccine costs: essentially 10 to 12 times as many vaccinations may be given with one tube of vaccine by use of the 1:50 dilution. (2) Reduction in man power needs: in every instance, the number of vaccinations performed per man hour expended were several times that observed when multiple pressure techniques were used. (3) Reduction in transport costs: because of the reduction in man power needs when conducting campaigns with jet injectors, the cost of transporting personnel similarly falls. (4) Increased efficacy of vaccination: throughout the study, irrespective of type of vaccine used, take rates for both primaries and revaccinees were as much as 10% higher in those vaccinated by jet injector. (5) Cessation of follow-up readings: because the jet injector has a demonstrated replicability of efficacy, and take rates under different situations vary quite minimally, a campaign based on jet injection makes it unnecessary to return to an area for follow-up readings. (6) The use of simple tally data by broad age groups was shown to be reasonably accurate in measuring the effectiveness of a mass campaign. The results of sample surveys were not significantly different from the recorded tally data.

OTHER DATA:

Grantee: Dr. J. D. Millar, Communicable Disease Center, United States Public Health Service, Atlanta, Georgia.

Funded by: Pan American Health Organization and the Communicable Disease Center, United States Public Health Service.

Timetable: January - February, 1965.

STUDIES ON ARTHROPOD-BORNE VIRUSES

PROBLEM

Arbovirus encephalitides, such as St. Louis encephalitis and Venezuelan encephalitis, and other arbovirus diseases, such as dengue fever, have caused extensive epidemics in the Western Hemisphere in recent years. The purposes of these investigations, to be undertaken in Mexico and Central America, are: (a) to study the ecologies, geographic distributions, and importance to man and domestic animals of Venezuelan encephalitis virus and several other recently discovered arboviruses and (b) to learn whether migratory birds can serve as possible intercontinental transporters of arboviruses.

METHODS

Specimens, such as mosquitoes, mammals, and birds, likely to yield arboviruses, as well as blood sera to detect arbovirus antibodies, are being collected in Mexico and Central America. Virus isolations are carried out in suckling mice and in chicken embryonic and other cell cultures. Antibody tests are performed by neutralization, hemagglutination-inhibition and complement-fixation techniques.

RESULTS TO DATE

Venezuelan encephalitis virus, a group C, a Bunyamwera group, and several unidentified arboviruses have been discovered in the state of Veracruz along the southeastern coast of Mexico. A careful search for human cases has not begun. Mexican investigators who participate in this project, however, found antibody to VE virus in a convalescent human from Campeche in 1962. A case of febrile encephalitis was found last summer at Acayucán, Veracruz. Its fourteenth day serum VE HI antibody titer was 1:640, indicating almost certainly that the disease was due to VE virus. Exposure of horses and burros has shown that VE virus in Mexico commonly produces inapparent infection rather than overt disease. To date, isolations of virus have been mostly from *Culex* mosquitoes, strongly suggesting that species within this genus are the major vectors. Various wild mammals and birds have been found to possess antibodies. Virus has been found in several of them, indicating that mammals participate in the natural cycle of VE virus.

Similar studies on the ecology, geographic distribution and importance to man and domestic animals of the other arboviruses are just beginning. The group C viruses are being analyzed to determine their

antigenic homogeneity, i.e., whether there is one or more than one virus of group C in Veracruz.

A sufficient number of specimens of organs and bloods from migratory birds has not been collected and tested to evaluate the possible role of birds in the intercontinental dissemination of arboviruses.

In addition, the program has yielded scientific data of considerable entomologic and ornithologic value; publications have appeared in each of the three major disciplines of arbovirus research (virology, entomology, and zoology), thus adding to scientific knowledge in medicine and in areas outside the health-related sciences.

SIGNIFICANCE

The research provides information on arboviruses, especially Venezuelan encephalitis virus, in Mexico and Central America, and it evaluates the possibility that migratory birds are intercontinental disseminators of arboviruses. By understanding completely the natural cycles of arboviruses, intelligent approaches can be taken to prevent and control epidemics caused by these viruses.

OTHER DATA

Grantee: Dr. William F. Scherer, Cornell University Medical College, New York.

Funded by: United States Army Medical Research and Development Command.

Timetable: 1961-1967

HERON ECOLOGY AND NORTH AMERICAN ENCEPHALITIS VIRUSES

PROBLEM

During the past decade, there has been an increasing realization of the potential importance of wild birds in the natural history and distribution of arthropod-borne animal viruses. The effectiveness of birds in these biologic roles is related to the rapidity of their population turnover, their ability to move quickly over long distances, the regularity of their migrations, and the temporal association and mixing with other animals and human populations. Despite this realization, however, there is still a critical lack of information concerning life histories of many avian species, even among the better-known groups. The purpose of this research is to study the ecology of one group of birds (herons and egrets), which is potentially important in the genesis of arbovirus encephalitis epidemics in North America.

METHODS

Heron colonies in tropical Mexico were surveyed initially, and two colonies were selected for study. These colonies have been mapped and subdivided into smaller units for detailed study to learn the date and method of arrival of herons, the time and instigation of nest building, locations of nests of one species in relation to another, egg laying, incubation periods of eggs, numbers of successfully fledged broods per year, ability to renest, length of nestling period, growth rates of young, local movements of fledged young, and, where possible by use of leg bands, information on long distance migrations. Virologic investigations have included survey of blood plasma from adult and young herons for arbovirus antibodies, and organs from nestlings for virus content.

RESULTS TO DATE

Considerable data have been accumulated, and the ornithologic aspects of the research are ready for analysis. Virologic studies have revealed Venezuelan encephalitis hemagglutination-inhibiting antibodies in several species of herons and egrets at a colony near the city of Minatitlán, Veracruz.

SIGNIFICANCE

If further studies prove that herons and egrets are important amplifying hosts for Venezuelan encephalitis and other arboviruses near human populations in Mexico a new aspect of the natural cycle of

these viruses will be uncovered. Whether control or prevention of VE and other arbovirus diseases in Mexico will be forthcoming from this information remains to be determined. From the fundamental biologic viewpoint, information concerning herons and egrets will contribute to ornithological knowledge.

OTHER DATA

Grantee: Dr. Robert Dickerman, Pan American Health Organization,
Mexico, D. F., and Department of Microbiology, Cornell
University Medical College, New York, New York.

Funded by: National Institutes of Health, U.S. Public Health Service.

Timetable: 1964-1967

RESEARCH TRAINING PROGRAM IN VIROLOGY, ORNITHOLOGY, ECOLOGY, AND TROPICAL MEDICINE

PROBLEM

During approximately the past decade, partly because of a shortage of trained investigators, little progress has been made toward the understanding of arbovirus diseases and, therefore, toward their prevention and control. Hence, the purpose of this research training program, which is conducted at the laboratory bench and in the field, is to develop competent research investigators in virology. Predoctoral and postdoctoral trainees become virologists, primarily, but they also develop a keen appreciation of entomology, ornithology, mammalogy, and other areas of zoology.

METHODS

Extensive training in virology is given at the Department of Microbiology, Cornell University Medical College, and at field training sites in tropical Mexico (Veracruz). A specialized 8-week graduate, field research-training course in virology, ornithology, entomology, zoology, and ecology has been given for the past three years to predoctoral and postdoctoral trainees. The first week of the course, which is scheduled during the summer rainy season when arbovirus activity is at a peak, has consisted of demonstrations of field techniques. During subsequent weeks, each student has carried out a research project in the field and at the field laboratory, and has attended lectures and seminars presented by the program staff and visiting scientists from Mexico.

RESULTS TO DATE

Twenty-seven persons have received or are receiving training under this program: six from Mexico, one from Peru, one from Jamaica, one from Iran, one from Japan, and seventeen from the United States. During the training course, students have collected specimens and recovered arboviruses. The program has not been in effect long enough for predoctoral trainees to graduate or to see possible growth of "seeds" planted in short-term trainees (mostly medical and veterinary students). However, the head of the arbovirus section of the Instituto Nacional de Virología in Mexico City, one of the initial trainees who spent three months on this program (in addition to six months at the Communicable Disease Center in Atlanta and three months at the Rockefeller Foundation laboratories), is now actively pursuing arbovirus research in Mexico. Currently under training at Cornell University are a member of the Instituto de Estudios Médicos y Biológicos de la Universidad Nacional Autónoma de México and a physician

on the staff of the Instituto de Salubridad y Enfermedades Tropicales. Upon their return to Mexico, these individuals will represent the first virologists to work at the National University and at the Tropical Medicine Institute.

SIGNIFICANCE

The significance of this research training program is that it is providing additional qualified investigators in the field of arthropod-borne virus research. Although new knowledge is already forthcoming through the research activities of the trainees (discovery of Venezuelan encephalitis and other arboviruses potentially important to man and domestic animals in Mexico), the eventual long-term productivity of the trainees, once they become independent investigators and educators, will provide the really significant rewards of the program.

OTHER DATA

Grantee: Dr. William F. Scherer, Department of Microbiology,
Cornell University Medical College, New York, U.S.A.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1962-1967

SUSCEPTIBILITY OF WILD ANIMALS TO THE VIRUSES OF FOOT-AND-MOUTH DISEASE AND VESICULAR STOMATITIS

PROBLEM

In view of the importance of foot-and-mouth disease and vesicular stomatitis, all possible factors involved in the spread of these diseases should be investigated. Among possible sources of infection are naturally susceptible wild animals which on occasion cohabit with domestic animals suffering from foot-and-mouth disease or vesicular stomatitis.

METHODS

A study was made of the susceptibility of Dasypsecta aguti to foot-and-mouth disease A and C virus and to the New Jersey virus of vesicular stomatitis. This animal, which is a rodent of the "Caydeos" family, is very common in Central and South American countries. In addition to cohabitation, the methods of infection used were: (a) intracutaneous and scarification; (b) oral; and (c) intranasal.

Virus was typed using the complement fixation method (50% hemolysis).

For the detection of antibodies in the sera the serum protection test was used in suckling mice for foot-and-mouth disease and serum neutralization in adult mice for vesicular stomatitis virus. Passages of virus were made in suckling mice by the intraperitoneal route; in adult mice, by the intracerebral route; and in guinea pigs, by intradermal inoculation of the foot pads.

RESULTS TO DATE

It has been shown that Dasypsecta aguti is susceptible to experimental infection with foot-and-mouth disease virus and with vesicular stomatitis virus and can be infected by the intracutaneous and oral routes as well as by cohabitation with animals suffering from foot-and-mouth disease. It may be infected with vesicular stomatitis both by cohabitation and by intranasal inoculation.

Dasypsecta aguti develops complement fixation and neutralizing antibodies which may also be used for the production of hyperimmune serum.

SIGNIFICANCE

The great susceptibility of Dasypsecta aguti has been shown by experimental infection, but in order to show that this animal is really a reservoir and a transmitter of foot-and-mouth disease virus and vesicular

stomatitis virus, it is necessary to demonstrate that it retains this susceptibility under natural conditions.

Similar studies on other wild animals may possibly elucidate the role played by these animals in the epizootiology of foot-and-mouth disease and vesicular stomatitis.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/ Organization of American States - Program of Technical Cooperation.

Timetable: 1960 - continuing.

IDENTIFICATION OF TYPES AND SUBTYPES OF FOOT-AND-MOUTH DISEASE VIRUS

PROBLEM

The plurality of strains of foot-and-mouth disease virus calls for the continuous typing of specimens taken from sick animals. The spread of the virus in the vast stock-raising zones of South America, where ecological conditions vary and the cattle population is of different breeds and susceptibilities, sometimes forces the virus strains to undergo major transformations in order to exist.

The identification of these strains, whose behavior may differ considerably from the prototypes used for classification, both from the serological and immunological point of view, is of great importance because of increased vaccinations in various countries.

METHODS

The methods used for identifying the strains are as follows:

- complement fixation by 50% hemolysis;
- passages in guinea pigs, suckling mice, and/or cattle;
- obtaining homologous serums in guinea pigs;
- serum protection of suckling mice;
- cross immunity in guinea pigs and cattle.

RESULTS TO DATE

In the period 1962 to 1966 a total of 2,718 specimens received from 18 countries were typed, as were 14,860 specimens obtained during the course of research at the Pan American Foot-and-Mouth Disease Center.

To date, 6 subtypes of foot-and-mouth disease virus have been identified and have been recognized by the World Reference Laboratory in Foot-and-Mouth, Pirbright, England, which has given them the following designations:

- A₁₃ - Santos, Brazil
- A₁₆ - Belém, Brazil
- A₁₇ - Guarulhos, Brazil
- A₁₈ - Zulia, Venezuela

A₁₉ - Suipacha, Argentina

O₈ - Bahia, Brazil

In addition, 4 new subtypes isolated in Argentina and Brazil are being studied.

SIGNIFICANCE

The results of this research point to the importance of continually screening virus from the field as well as the strains used for the production of vaccine. Our subtypes differ so much from one another that if specific sera are not used in the complement fixation test, it is impossible to type them and the degree of serological difference may correspond to a similar degree of immunologic difference. The appearance of a new subtype in the course of a vaccination campaign may seriously endanger the success of the campaign if it is not recognized as such and if the appropriate measures are not taken immediately. The Center is collaborating with the countries and supplying them with subtype specific sera for diagnostic tests and identifying subtypes for them.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/Organization of American States-Program of Technical Cooperation.

Timetable: 1957 - continuing.

CATTLE AS CARRIERS OF FOOT-AND-MOUTH DISEASE VIRUS

PROBLEM

Recent studies at the Pan American Foot-and-Mouth Disease Center confirmed the work of Van Bekkum et al. (1959) which demonstrated that the foot-and-mouth disease virus can be isolated from the saliva of cattle for a relatively long period of time after the illness.

Confirmation of the existence of carrier animals and the importance of this fact for epizootiological studies led to a search for a better and more adequate technique of isolating virus, sites of the survival of virus, duration of the carrier state, relationship between the carrier state and the immunological state of the animal and the biological characteristics of persisting virus.

METHODS

Specimens are collected using the apparatus described by Van Bekkum which upon being inserted into the esophagus makes possible the collection of a mixture of the muco-saliva from the esophagus, pharynx, larynx and mouth. These specimens are treated with chloroform, shaken for 30 minutes in a cold chamber, and centrifuged. The supernatant liquid is treated with 2,000 I U of penicillin and 20 mg of streptomycin per ml.

Suckling mice and monolayers of BHK-21 C-13 cells are used for virus isolation. The complement fixation test is used to verify the specificity of the death of mice.

RESULTS TO DATE

By inoculation in suckling mice and tissue cultures, foot-and-mouth disease virus can be demonstrated in material obtained from the esophagus. Foot-and-mouth disease virus can survive for several months in a great many cattle that have recovered from the illness.

Animals may become carriers without showing any clinical signs of the disease. The carrier state does not depend upon the antibody level, since it is possible to isolate the virus from animals with or without antibodies.

The pathogenicity of three strains of foot-and-mouth disease virus isolated from carrier animals 6 months after the outbreak was low for cattle and high for pigs.

SIGNIFICANCE

The role played by the carrier as a potential disseminator of an infectious virus and the immunological significance of the virus-host relationship are extremely important in the study of foot-and-mouth disease epizootiology.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/ Organization of American States - Program of Technical Cooperation.

Timetable: 1964 - continuing.

IDENTIFICATION OF TYPES AND SUBTYPES OF VESICULAR STOMATITIS VIRUS

PROBLEM

In areas in which both foot-and-mouth disease and vesicular stomatitis are present, differential diagnosis is of great importance in deciding on the veterinary control measures to be adopted. The verification of foci of vesicular stomatitis in countries in which it has not as yet been identified, such as Brazil and Argentina, provides an opportunity of demonstrating the existence for the first time of subtypes of vesicular stomatitis virus.

METHODS

Complement fixation (50% hemolysis).

Passages in guinea pigs, adult and suckling mice, embryonated eggs and tissue cultures.

Preparation of homologous sera in guinea pigs.

Serum neutralization in adult mice and embryonated eggs.

RESULTS TO DATE

From 1962 to date, 466 specimens of vesicular stomatitis virus from 12 countries in South and Central America have been typed.

Investigation of a virus isolated in 1963 from a vesicular outbreak in horses in the state of Alagoas, Brazil, showed it to be the first subtype of the Indiana strain of vesicular stomatitis virus. Another virus isolated from an outbreak in horses in Salta, Buenos Aires Province, turned out to be another subtype of Indiana virus. The World Reference Laboratory in Foot-and-Mouth Disease, Pirbright, England, confirmed the results and recognized the two viruses as subtypes of the Indiana virus.

SIGNIFICANCE

The rapid diagnosis of all specimens of vesicular diseases received from countries free of foot-and-mouth disease enables them to adopt suitable veterinary control measures. In the case of subtypes, evidence was found that some of the field viruses are so specific that they do not react at all to standard sera. The Center supplied the national diagnostic laboratories of Brazil and Argentina with a sufficient amount of the subtype specific sera to permit their typing.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro,
Brazil.

Funded by: Pan American Health Organization/Organization of American
States-Program of Technical Cooperation.

Timetable: 1957 - continuing.

USE OF THE COMPLEMENT FIXATION TEST FOR THE DETERMINATION OF
FOOT-AND-MOUTH DISEASE AND VESICULAR STOMATITIS ANTIBODIES IN
THE SERUM OF CATTLE, HORSES, AND SHEEP

PROBLEM

Almost the only tests used for the detection of antibodies in the serum of cattle, horses, and sheep are the serum neutralization and serum protection tests in suckling mice and tissue cultures. The use of the complement fixation test to replace these costly methods for detecting antibodies would mean an enormous saving in time and money. The Marucci method makes it possible to determine complement fixing antibodies in the serum of cattle infected with foot-and-mouth disease virus. The application of this method to present working conditions is seriously limited by the almost complete lack of the lymph needed as antigen in this test.

The work has been especially directed towards the search for an antigen to replace lymph in the complement fixation test and to devise a simpler method for hemolysis to be determined spectrophotometrically.

METHODS

Complement fixation test (50% hemolysis).

Multiplication of different strains of foot-and-mouth disease and vesicular stomatitis virus in tissue cultures.

Passages of different strains of vesicular stomatitis virus in the brain of adult mice and in embryonated eggs.

RESULTS TO DATE

It has been possible to demonstrate that BHK-21 cell monolayers are the best medium for the multiplication of foot-and-mouth disease and vesicular stomatitis viruses, that an excellent complement fixation antigen is obtained, and that it is thus possible to replace bovine lymph by the antigens produced in the BHK cells. The method of complement fixation (50% hemolysis), with previous incubation for 20 hours at 4° C makes it possible to obtain clear-cut reactions.

The sensitivity and specificity of the reaction can be demonstrated with subtype specific sera of convalescent cattle.

By means of this test it is possible to make a differential diagnosis and to type foot-and-mouth disease and vesicular stomatitis viruses in the sera of cattle 4 months after having recovered from the disease. It is also possible to obtain identical results with sheep serum one month after they have been experimentally inoculated.

SIGNIFICANCE

Because of its simplicity, rapidity, and cheapness the complement fixation test using cattle, horse, and sheep sera is of great practical importance whenever it is not possible to collect infectious materials for diagnosis.

It was noted that complement fixing activity of the sera does not last as long as its neutralization capacity in cattle, horses, and sheep and it might possibly be of interest to determine the relationship between the titer of the complement fixing antibodies and the actual immunity of the animals.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/ Organization of American States - Program of Technical Cooperation.

Timetable: 1960-1964

FOOT-AND-MOUTH DISEASE ANTIBODIES IN SERUM DRIED ON FILTER PAPER

PROBLEM

In addition to several studies on the use of serum dried on filter paper in vesicular stomatitis, distemper and poliomyelitis, it has been shown that it is possible to use dried blood collected on filter paper disks in complement fixation tests for adenovirus and in hemmagglutination-inhibition tests for measles.

Research was started to study this method in serum neutralization and serum protection tests in foot-and-mouth disease, with particular emphasis on their possible utilization in quantitative antibody studies.

METHODS

A paper (Whatman No. 3MM) scientifically standardized for chromatography was chosen. The separating power of the paper with regard to proteins (of known weight and volume) adsorbed in an unknown area of paper will be determined, as will be the maximum adsorption capacity of a known area. The sera of animals with known backgrounds will be broken down into their constituent protein fractions, and elution tests will be made comparing the sera and their proteins adsorbed separately on paper.

Once these results are obtained, the efficiency of sera adsorbed on paper will be compared with that of the corresponding frozen sera using the serum neutralization test in mice and in tissue culture (BHK-21 C-13).

RESULTS TO DATE

Preliminary studies on the adsorption of serum and blood on filter paper, without regard to the useful area of paper, the quality of the paper, and the amount of material adsorbed (the objective being to prove the efficiency of the method in foot-and-mouth disease) gave, over a period of 3 months, satisfactory results in serum neutralization tests in rabbits and tissue culture (BHK-21 C-13). It is now advisable to explore the method further, both qualitatively and quantitatively.

Various protein calibration curves standardized by the protein dosification method (Collowick and Kaplan) have been prepared, as have all the other materials that will be used in working out this new technique.

SIGNIFICANCE

The great advantage of this method, especially under field conditions in various Latin American countries, is the elimination of a series of problems connected with the handling and storage of refrigerated or frozen serum specimens.

Preliminary results point to its possible use in serological studies, in epizootiological surveys of foot-and-mouth disease in zones of difficult access, delayed transportation and absence of suitable working conditions for the usual techniques, and where slow and defective transportation of specimens affects the results.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/ Organization of American States - Program of Technical Cooperation.

Timetable: 1963 - continuing.

IN VITRO STUDIES OF THE KINETICS OF FOOT-AND-MOUTH DISEASE VIRUS

PROBLEM

The production of foot-and-mouth disease virus for the preparation of inactivated and modified live virus vaccines still presents many financial, technical and scientific difficulties. The use of tissue culture techniques has made many contributions, especially with respect to the isolation of the virus, the formation of clones, serum neutralization tests, and recently, the use of cell line BHK-21 C-13, as a source of the virus. There are, however, serious objections to the use of heteroploid cell lines for the modification of the virus and for the production of vaccines for use in man and in animals used as food sources. It is necessary, therefore, to study the relationships between the foot-and-mouth disease virus and the cell, using diploid strains and strains which are antigenically more potent.

METHODS

Because of the need to control the viral antigens in some of the tissues systems where there is a multiplication of virus without manifestation of cytopathogenic effects, use has been made of the fluorescent antibody technique (direct and indirect methods). This technique, with the help of acridin yellow and Jacobson stains and phase contrast microscopy, is also used in studying the virus-cell binomium. With a view to increasing the probabilities of isolating virus from specimens in which it exists in small quantities (as in the case of carrier animals), various in vitro systems are being studied to facilitate its adsorption and penetration.

In studying markers for various strains of modified virus, use will be made of cultures under different environmental conditions and from various tissues and organs such as brain, pancreas, heart, and muscle.

RESULTS TO DATE

As to the susceptibility of the various diploid strains established in this laboratory from pig and rabbit kidney and lung, and from calf embryo, it has been found that strains RS-I (derived from pig kidney) is as sensitive to the virus of foot-and-mouth disease as the cell line BHK-21 C-13.

The inoculation of virus in suspended cells (for varying periods of time) and the subsequent passage of these cells through stationary type cultures appears to increase the possibilities of virus isolation.

A start was made on series of subcultures from diploid strains inoculated with foot-and-mouth disease virus in which the virus multiplies without any cytopathic effect. Preliminary observations indicate that these cultures (carrier cultures), contrary to what happens in parallel non-inoculated cultures, undergo a series of morphological changes and do not enter into "phase III", while control cultures ceased multiplying.

Some of the morphological changes correspond to those described by Stenkvis et al. when using hyperimmune serum in tissue cultures previously inoculated with foot-and-mouth disease virus.

SIGNIFICANCE

A more thorough knowledge of the basic properties of foot-and-mouth disease virus could lead to the preparation of more antigenic vaccines that are easier to test and that are more rationally and cheaply prepared. The identification of markers is of fundamental importance, especially because of the use of modified live virus vaccines and their possible persistence in vaccinated animals. It is evident that diploid strains must be used in modifying the virus and producing vaccines in view of the above-mentioned objections to the use of heteroploid strains. Even if diploid strains have a limited period of life in vitro, in practice an unlimited number of cultures can be obtained for the large-scale production of vaccines by storing cells in liquid nitrogen. The problem of the morphological transformation induced in diploid cell strains by the foot-and-mouth disease virus can be of importance in clarifying differences existing between "complete" transformations induced by oncogenic viruses and simple morphological transformations.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/Organization of American States - Program of Technical Cooperation.

Timetable: 1965 - continuing.

STUDIES ON INACTIVATED VACCINES AGAINST FOOT-AND-MOUTH DISEASE

PROBLEM

In countries in which the disease is enzootic, foot-and-mouth disease control is based on the systematic vaccination of cattle. The vaccines most commonly used in South America are bi- or trivalent inactivated vaccines prepared from virus obtained by the Frenkel method. However, inactivated vaccines are relatively weak products, and they confer immunity for no more than four or five months. It is therefore necessary to improve these vaccines by using new methods for virus multiplication and for the production of better and cheaper antigens, by testing new methods of inactivating viruses, and by using new adjuvants.

METHODS

One of the most important factors in the culture of foot-and-mouth disease virus by the Frenkel method is the culture medium. Among the various media that have been compared with the Frenkel medium, special attention has been given to two media with a glucosol base (Parker): to one peptone (0.3%) is added, but the other is enriched with five amino acids (dl-methionine; l-leucine; dl-phenylalanine; dl-tryptophan; l-histidine). As the Frenkel method uses cattle tongue epithelium, which is often difficult to obtain in the necessary quantities in some parts of South America, studies had been made on other cellular systems for the multiplication of virus including pig-kidney and calf-kidney primary cultures and BHK-21 C-13 cell line cultures.

RESULTS TO DATE

Using the two media (glucosol plus peptone and glucosol plus peptone enriched with five amino acids) a sample of the three virus types well adapted to culture by the Frenkel method was submitted to seven serial passages in parallel cultures. In each case a sufficient amount of virus (titrated in suckling mice) was obtained for the preparation of vaccines. In addition, with the two culture media described above, good adaptation of two specimens of the Vallée O virus was observed.

When the pig kidney and calf kidney primary cultures were used, the results obtained were not very satisfactory, owing in particular to variations in the quality and susceptibility of these cultures to the foot-and-mouth disease virus.

The use of BHK-21 C-13 cells for the multiplication of foot-and-mouth disease virus and their use in the preparation of inactivated vaccines proved to be more consistent and regular than that of primary cultures. Preliminary trials showed that, to protect 50% of the cattle vaccinated, the amount of virus to be incorporated into each dose of vaccine should be $10^{6.6}$ LD₅₀/ml.

SIGNIFICANCE

For a considerable time, virus for the preparation of inactivated vaccines was obtained by inoculating susceptible cattle, generally animals intended for consumption, in slaughter houses. In view of the drawbacks connected with this method, the adaptation of new techniques for the production of virus, particularly the Frenkel method, was a great advance in the preparation of foot-and-mouth disease vaccines. Nevertheless, because of the short duration of the immunity conferred and the high cost of these vaccines, not to mention the economic, social, and geographical problems of South America, it is clearly necessary to improve these products (by using more efficient and cheaper culture media, new sources of virus production, more efficient and appropriate adjuvants, new methods of inactivation) to increase the duration of the immunity they confer, to lengthen the period of potency of the prepared vaccine, and to simplify storage.

The importance of research on inactivated vaccines is becoming more and more evident, since some South American countries cannot use live modified virus vaccines because of problems connected with the export of meat.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/Organization of American States - Program of Technical Cooperation

Timetable: 1952 - continuing.

USE OF LABORATORY ANIMALS FOR TESTING INACTIVATED FOOT-AND-MOUTH DISEASE VACCINES

PROBLEM

In the countries affected by foot-and-mouth disease, inactivated vaccines are the most commonly used vaccines in the control of this disease, despite the short period of immunity they confer and the need for revaccinations. These vaccines, like all others, must be tested in the animals for which the vaccine is intended, in this case, cattle. In countries with areas free of the disease, the vaccine can be readily tested in suitable animals. However, in countries in which the disease affects the whole territory, this test, in addition to being burdensome, is uncertain because of the difficulty in obtaining susceptible animals to test each batch of vaccine. Hence the need to intensify research on species of laboratory animals which can be substituted for cattle in the potency testing of foot-and-mouth disease vaccines.

The adaptation of the foot-and-mouth disease virus to young adult cortisone-treated mice has made it possible to test in these animals the potency of inactivated vaccine. Recently, chickens have been used for detecting foot-and-mouth disease antibodies.

METHODS

Swiss white mice between 3 and 6 weeks of age and weighing between 9 and 13 gm. were used. These mice were inoculated subcutaneously with different doses of vaccine. A certain number of mice of the same weight and age as those vaccinated are used as unvaccinated controls. Twenty-one days after vaccination the immunized and control mice are challenged with a virus adapted to young adult mice. From the difference in their titers the protection index of each dose of vaccine and the 50% protective dose are calculated. The serum neutralization test in suckling mice is used for determining the antibodies produced by inactivated vaccine in chicks.

RESULTS TO DATE

Although it is not possible to compare the protective dose for mice with that for cattle it has been shown that the subcutaneous inoculation of foot-and-mouth disease vaccines in young adult mice will confer protection against intraperitoneal inoculation of virus strains adapted to these animals. In addition, a direct relationship has been found between the viral content of the vaccines and the protection they confer.

SIGNIFICANCE

Although these results cannot be compared with the potency test in bovines, it is necessary to continue the search for a technique which will make possible the establishment of a relationship between the two tests.

OTHER DATA

Grantee: Pan American Foot and Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/ Organization of American States - Program of Technical Cooperation.

Timetable: 1954 - continuing.

RABBIT-MODIFIED LIVE VIRUS VACCINE AGAINST FOOT-AND-MOUTH DISEASE

PROBLEM

Many authors have shown that it is possible to immunize animals susceptible to foot-and-mouth disease with modified live virus vaccines. The multiplication of the virus in the organism generally causes a solid and durable immunity. Because of their wide antigenic spectrum, modification enables these vaccines to be successfully used against strains of the same type found in South American countries affected by foot-and-mouth disease and with very special characteristics that make it difficult to control.

METHODS

Materials: Passages in rabbits at graded age intervals from 1 to 50 days. Rabbits are inoculated by the intraperitoneal route, and the skeletal muscles are collected as passage material. To control the purity of the viruses at each passage and their infective titers, complement fixation and intraperitoneal inoculation of suckling mice are used.

Myotropism, especially of the cardiac type, is being studied in guinea pigs. The muscles of suckling mice killed by inoculation with rabbit modified virus and suspended in 50% buffered glycerin are used for the preparation of vaccines.

RESULTS TO DATE

The Vallée O (Deodoro) type virus has undergone 111 passages in rabbits of increasing age. Pathogenicity and antigenicity tests have yielded irregular results and for that reason this virus has been abandoned.

The Waldmann C type virus has been passaged in rabbits of increasing age and has reached the 66th passage. Its antigenic behavior is satisfactory, and protection conferred in cattle varies between 80 and 100% when the quantity of virus used is greater than $10^{7.6} \text{LD}_{50}/\text{ml}$.

The Vallée A (Cruzeiro) type virus is at present in the 29th passage but its pathogenicity and antigenicity in cattle is not yet known.

SIGNIFICANCE

Research along these lines would make possible more efficient vaccines from the standpoint of immunity, longer duration, and wider antigenic

spectrum; cheaper and more easily prepared vaccines; and vaccines that can be used in different species of animals such as cattle, sheep, goats, and pigs.

OTHER DATA:

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/ Organization of American States - Program of Technical Cooperation.

Timetable: 1952 - continuing.

DEVELOPMENT OF MODIFIED LIVE VIRUS VACCINES AGAINST FOOT-AND-MOUTH DISEASE IN EMBRYONATED CHICKEN EGGS

PROBLEM

The immunization of cattle susceptible to foot-and-mouth disease has been repeatedly shown to be possible through the use of modified live virus vaccines. Virus multiplication in the organism generally produces a solid and durable immunity which, in the absence of pathogenicity, greatly improves the possibility of foot-and-mouth disease control in South American countries.

METHODS

The three types of foot-and-mouth disease virus existing in South America (Vallée A and O, Waldmann C) were adapted to embryonated eggs by intravenous inoculation for 14 days, alternating, when necessary, with passages in suckling mice, tissue cultures, and one day old chicks. Initially the incubation temperature is approximately 35°C but is lowered to 30 to 32°C in advanced passages. Samples are studied in susceptible animals from the standpoint of safety and immunogenic power as follows:

in guinea pigs, by intramuscular or intraplantar inoculation in order to observe loss of infectivity, post-vaccinal disorders (cardi-tropism) and immunity response, by studying antibodies and the absence of generalized infection when homologous pathogenic viruses are inoculated;

in cattle, by intradermal inoculation of the tongue (IDT), intramuscular inoculation, and cohabitation so as to assess possible post-vaccinal lesions, viremia, immunity response and contagiousness;

in pigs, inoculated subcutaneously and intramuscularly, and by cohabitation so as to study the immunity response, pathogenicity, and contagiousness.

The vaccines were prepared with hearts and livers of the chicken embryos of the muscles of 4 to 6 day-old suckling mice inoculated intraperitoneally or intramuscularly with virus modified in embryonated eggs. The materials are triturated and suspended in 50% buffered glycerin, treated with chloroform, and then centrifuged. The supernatant liquid is used as immunizing material. The vaccinating dose normally used in between $10^{7.0}$ and $10^{8.0}$ suckling mice LD₅₀.

RESULTS TO DATE

The vaccines produced with virus modified in chicken embryos have shown themselves to be capable of inducing an adequate level of protec-

tion and to have low pathogenicity for cattle. Preliminary results appear to indicate that revaccination produces a firmer and more durable immunity. The immunity conferred by these vaccines appears to be less efficient in young cattle than in adult animals.

Laboratory data with 2 strains of modified virus confirm immunity through IDT inoculation of pathogenic virus and through cohabitation. The data with the Waldmann C type virus are incomplete but low pathogenicity and good protection was observed in a single test in cattle on the 21st passage.

SIGNIFICANCE

Research with modified virus may lead to vaccines that, from the standpoint of immunity, duration, and antigenic spectrum, are better, are less expensive and more easily prepared, and are effective in a variety of animal species.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization, Organization of American States - Program of Technical Cooperation, and the National Research Council of Brazil.

Timetable: 1957 - continuing.

USE OF MODIFIED FOOT-AND-MOUTH DISEASE LIVE VIRUS VACCINES IN SOUTH AMERICA

PROBLEM

Foot-and-mouth disease is enzootic in all South American countries, where it mainly affects cattle. Susceptible cattle are protected through systematic and periodic vaccination on a relatively large scale. The most commonly used type of vaccines are inactivated vaccines, three doses a year being required. These vaccines, despite their limitations, are the only used in the meat-exporting American countries.

Modified live virus vaccines are preferable to the inactivated type in that they confer longer immunity, are simpler to prepare, cost less, and are more easily stored, transported, and handled in the field. Under the special conditions existing in South America which make large-scale systematic vaccination difficult, this type of vaccine is of considerable immediate interest for countries which are not meat exporters and for those which maintain large stockraising areas whose products are used for domestic consumption.

METHODS

Once a virus strain has been modified in the laboratory, its behavior in small groups of susceptible cattle in a given country and in representative classes of the population is ascertained. The susceptibility of the animals is determined by an examination for their serum antibodies. Each animal is inoculated by the intramuscular route with a 2 ml dose of vaccine with a titer of about $10^{7.0}$ suckling mice LD_{50} per ml.

Reactions to the vaccine, temperature, vesicular lesions, erosions, cardiac effects, milk production, gestation, etc. are individually observed for a period of two weeks. After 21 days, antigenicity is tested in a group of 8 to 10 cattle by inoculating them intradermally in the tongue with a dose of homologous virus of $4 \times 10,000$ suckling mice LD_{50} . The results are observed for two weeks, during which blood antibody titrations are made. Subsequently, the vaccine is administered in the same dosage and by the same route, in pilot areas with approximately 5,000 cattle, which are subject to overall observations and, in addition, individual observations in a representative sample. Immunity is measured by means of periodical examinations, usually on a monthly basis, of the blood antibodies in selected groups, by virus elimination and through recording the morbidity in natural outbreaks of the disease. Subsequently, vaccinations may be extended throughout the country according to its own needs.

RESULTS TO DATE

Strain O, modified in chicken embryo, has been administered in the field to about 200,000 cattle, and the pathogenicity index has been observed to be low. There has been no significant fall in immunity during observations which have lasted up to 10 months.

Strain A modified in chicken embryo is the most widely used in the field. In Venezuela about 10 million doses have been given since 1962 to a cattle population of some 6 million head. In a period of two years Ecuador has used about 300,000 doses, Brazil 30,000 doses, Colombia 10,000, Chile 5,000 and British Guiana 5,000. In all these countries the pathogenicity observed was low and benign.

Since these vaccination programs were begun, none of the countries have reported the presence and spread of the disease caused by the virus concerned, in the cattle population vaccinated with this strain.

Rabbit-adapted strain C has been used only on an experimental scale in Brazil. Pathogenicity in general is low, except in very young animals and the immunity conferred has been satisfactory.

Since 1963 polyvalent modified live virus vaccines have been used; bivalent OA in Colombia, Ecuador, and Venezuela; and bivalent AC and trivalent OAC in Brazil. It appears that the pathogenicity of the individual strains is not being modified and the same applies to the antigenicity observed in laboratory and in the field. Some preliminary results in calves seem to indicate that young cattle need revaccination.

SIGNIFICANCE

The use of these foot-and-mouth disease modified virus vaccines in epizootic outbreaks in British Guiana (Rupununi, 1961), Venezuela (Zulia, 1962), and Ecuador (Sur de la Sierra, 1965) showed it to be highly effective in halting and controlling the disease. The outbreak in British Guiana was quickly controlled, and no new cases have since appeared. Since intensive campaigns with these vaccines have been undertaken a pronounced fall in the number of typings of the virus concerned has been observed in Ecuador and Venezuela. These countries conduct their campaigns with vaccines prepared locally from their own modified strains as well as from those supplied by the Pan American Foot-and-Mouth Disease Center. These results and the difficult environmental conditions in most of South America (socio-economic problems, vast ranches, poor communications, topography and adverse climate, low degree of development of official health services, etc.) indicate the importance of these vaccines in controlling foot-and-mouth disease. In order to achieve confidence in, and the general use of, these modified live virus vaccines, specific and additional studies must be made of the vaccine's behavior in the field, in various

cattle populations and under variable environmental conditions as well as of such factors as the pathogenic effects for different breeds, ages and situations, age at the first dose, need for revaccinations in order to achieve an adequate level of protection, spread of the virus in the species and in other animal species, virus carriers and the survival of the virus in meat and other subproducts, duration of immunity, and vaccination of pigs and sheep.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/ Organization of American States - Program of Technical Cooperation.

Timetable: 1962 - continuing.

SURVIVAL IN CATTLE OF MODIFIED LIVE VIRUS VACCINE FOR THE CONTROL OF FOOT-AND-MOUTH DISEASE

PROBLEM

The survival in cattle of modified live virus vaccine is being studied because of the importance of this type of vaccine in the control of foot-and-mouth disease in South American countries. The innumerable advantages of this type of vaccine were described in other projects of the Center.

One of the possible obstacles to the use of live vaccine by exporting countries is lack of knowledge about the duration of survival of the virus in vaccinated cattle, which is reflected in the international meat trade in the form of restrictive measures by importing countries. It is therefore necessary to ascertain the exact duration of the survival of the virus and the danger it may represent in the export of the meat prepared from vaccinated cattle.

METHODS

Cattle vaccinated with live modified virus are slaughtered at different times, and their organs and tissues are examined for the presence of the virus. The organs and tissues are divided into two groups: one of commercial interest for export - such as muscles, congealed blood, ganglions, synovia and marrow, which can raise problems for the importing countries- and a second group of little commercial importance: hearts, livers, pancreas, kidney, tonsils and skin.

Suckling mice and monolayers of BHK-21 C-13 cells were used for the isolation of the virus, and the complement fixation test for identifying its type.

RESULTS TO DATE

It was possible to isolate C type virus but never A type virus in cattle slaughtered between 18 and 64 days after vaccination with bivalent modified live virus vaccine.

Up to the present time, in the group of organs and tissues of commercial interest, virus has been isolated solely from congealed blood, 32 days after vaccination. In the group of no commercial importance the virus was isolated from the kidney, pancreas, tonsils and skin up to 34 days after vaccination.

SIGNIFICANCE

It is of great importance to ascertain how long live modified viruses survive in cattle; vaccines prepared from these viruses will confer better

and longer immunity and their use will help improve the control of foot-and-mouth disease in Latin America.

The fact that it has hardly been possible to isolate modified virus, and then only for a relatively short time, leads us to believe that possibly the type of modification may affect the duration of the persistence.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/Organization of American States-Program of Technical Cooperation.

Timetable: 1966 - continuing.

HEAT INACTIVATION OF NATURAL AND MODIFIED STRAINS OF FOOT-AND-MOUTH DISEASE VIRUS

PROBLEM

The object of this study is to learn, by applying the principles of modern food science and technology, how to destroy the foot-and-mouth disease virus and how to inactivate it in meat so as to make exportation to non-enzootic areas possible. Its immediate objective is the study of the characteristics of thermal inactivation of natural and modified strains of the virus types present in South America.

The first step is to obtain comparative data on the different types and samples of virus under identical environmental conditions and then to study the effects of various environmental factors on the characteristics of thermal inactivation.

METHODS

The effect of temperature on the antigenicity and infectivity of 3 types of foot-and-mouth disease virus, natural and modified, in a buffered phosphate solution is being determined. Subsequently, the thermal effects on the virus will be studied, as affected by suspension, pH and other factors.

Viral suspensions of known concentration are made in a buffered phosphate saline solution at pH 7.5, sterilized by filtration, titrated in white suckling mice and in monolayers of BHK-21 C-13 cells, types and antigenic titers being confirmed by complement fixation. The suspensions are placed in glass ampules or capillary tubes, heated to a known temperature several times and again titrated. The rate of the reduction in infectivity and antigenicity is then determined by complement fixation.

RESULTS TO DATE

The rate of thermal inactivation of viral infectivity per unit time is decimal. Modified strains show decimal reduction times significantly higher than the natural strains of the same type of virus A (Cruzeiro) with identical temperature, suspension medium, and pH. The suckling mouse appears to be the most sensitive test medium for modified and natural strains of the A type foot-and-mouth disease virus and consistently shows a higher infectivity titer and a higher decimal reduction time than that obtained in monolayers of BHK-21 C-13 cells. The complement fixation activity of the antigen is much more resistant to thermal action than is its infectivity.

SIGNIFICANCE

This project implements a recommendation made in 1962 by the Joint Argentina-United States Foot-and-Mouth Disease Committee, in collaboration with the Pan American Foot-and-Mouth Disease Center. An international agreement exists whereby an animal food technologist of the U. S. Air Force is serving at the Pan American Foot-and-Mouth Disease Center for a period of 3 years.

The development of economical methods for completely inactivating the foot-and-mouth disease virus present in meat and in other products of animal origin would be of great benefit both to the countries in which the disease is enzootic and to countries free of the disease.

OTHER DATA

Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.

Funded by: Pan American Health Organization/ Organization of American States-Program of Technical Cooperation and the U. S. Air Force.

Timetable: 1964-1967

RICKETTSIAL ZONOSSES IN SOUTH AMERICAN DOMESTIC
ANIMALS: SURVEY FOR EVIDENCE OF INVOLVEMENT IN
ENDEMIC AREAS OF HUMAN TYPHUS

PROBLEM

In Ethiopia and Egypt, significant numbers of domestic animals are reported to have specific antibodies against epidemic and murine typhus and some against tick typhus and Q fever. Isolates of all of these agents have been reported from ticks off Ethiopian cattle or goats, and of Rickettsia prowazekii from blood of these two hosts. Some of these isolates have been confirmed at the Rocky Mountain Laboratory, Montana. Experimental typhus infection of lambs and of ticks has also been reported. If there is a digression of human and tick typhus into livestock, study is needed on how this happens, how prevalent it is, and whether it occurs in other areas, such as in endemic areas of human typhus in South America. There appear to be seasonal variations in the levels of typhus antibodies in Egyptian animals, which should be considered in surveys elsewhere.

METHODS

Initial evidence of rickettsial involvement of livestock and their ectoparasites in a given area is best indicated by serological surveys using specific antigens against the 4 agents. Samples are collected on "typhus premises" or in local abattoirs. Complement fixation (CF), toxin neutralization, and microagglutination supply appropriate tools, supplemented by capillary agglutination and radioisotope precipitation for determination of Coxiella burneti antibodies. Epidemiological and serological evidence is also sought among local residents. Isolation attempts in guinea pigs and chicken embryos then become paramount. Identification of any isolates is accomplished by suitable immunological and serologic procedures and by experimental infection of young livestock and suitable ticks.

RESULTS TO DATE

In collaboration with respective health authorities, animal and some human blood samples were collected from the following areas in each of which the presence of human typhus was confirmed serologically: Puno, Peru, Sept. 1963; along the Altiplano, Ecuador, July 1964; Cautín, Chile, Nov. 1965; and La Quiaca area, Argentina-Bolivian border, Dec. 1965. By a new technique, a few suspect burros, cattle and sheep CF reactors for typhus were proved nonspecific, so that, on the dates of collections, none was found to be positive among 683 serums, mainly from cattle, sheep, goats, donkeys, llamas, a few horses, pigs, alpacas, and pet guinea pigs.

A few CF reactors for tick typhus were found among sheep, a cow and persons in northern Ecuador, and in 4 persons on the Argentina-Bolivian border; for Q fever among people in both areas but, peculiarly, only among animals in Ecuador and Peru.

At the Rocky Mountain Laboratory, susceptibility of a young donkey and 2 young goats to R. prowazekii, recently isolated from Ethiopian cattle ticks, was demonstrated, but the agent could not be recovered from their blood. Specific epidemic typhus antibodies (CF and toxin neutralizing) reached high titers in one month, but by the third month had dropped to low titer in the donkey and to zero in the goats.

SIGNIFICANCE

Observations in northeast Africa of extrahuman cycles of epidemic typhus have focused attention on possible involvement of domestic animals in rickettsial zoonoses, in addition to Q fever. Extension of these observations could significantly influence epidemiologic concepts.

In view of the rapid decline of demonstrable typhus antibodies in experimentally infected animals, the present brief serological surveys in South American livestock are not conclusive as negative evidence of such involvement. More convincing data should be obtained in the vicinity of human outbreaks at different seasons.

OTHER DATA

Grantee: Drs. Cornelius B. and Robert N. Philip, Rocky Mountain Laboratory, Hamilton, Montana.

Funded by: Pan American Health Organization and the Rocky Mountain Laboratory.

Timetable: 1963

NATURAL BRUCELLA INFECTION IN WILD FOXES OF ARGENTINA

PROBLEM

Foxes are common predators in many areas of Argentina. The purpose of this study was to investigate the natural occurrence of brucellosis in the grey foxes of the Pampa and north Patagonia regions.

METHODS

A total of 728 foxes were examined by the agglutination test, 410 being specimens of Dusicyon gymnocercus antiquus of the Pampa region and 318 Dusicyon griseus griseus of the north Patagonia region.

Bacteriologic examinations were performed on 31 pools composed of organs of 77 D. gymnocercus specimens, as well as on 34 individual specimens of the same fox species.

RESULTS TO DATE

A high rate of reactors with titers of 100 I. U. or more was found in both fox species: 13.9% in D. gymnocercus and 7.9% in D. griseus. Males and females were found to be equally infected. When results of agglutination tests of cattle and foxes originating from the same ranches were compared, it was found that the percentage of titers of 100 I. U. or more were higher in foxes than in cattle from four of the five ranches where both species were examined.

Brucella strains were isolated from 5 pools and 3 individual specimens. One of the latter had a titer of 25 I. U. and the other two 100 and 200 I. U. The eight fox strains were found to be Br. abortus biotype 1.

SIGNIFICANCE

The natural occurrence of brucellosis in wild foxes in Argentina was recognized. Their role and that of other wild species in the epizootiology of brucellosis is still controversial. It is not known to what extent the infected individuals might contribute to inter-species spread of the disease.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization and the Government of Argentina.

Timetable: 1962-1964

BRUCELLOSIS IN HARES FROM AZUL AND ADJACENT AREAS

PROBLEM

Hares (Lepus europeus), which are important reservoirs of brucellosis in Europe, are abundant in the farm areas surrounding Azul, Argentina. The purpose of the study was to investigate the natural occurrence of the infection in these animals.

METHODS

From 694 hares processed during the year, cultures were made on 135 pools of organs, and plate agglutination tests on 275 samples.

RESULTS TO DATE

Br. suis was isolated in only one of the samples. Agglutination tests were negative for all but one sample with a titer of 1:50.

SIGNIFICANCE

Work done to date indicates that very little brucellosis occurs in hares in this area. Epidemiologically, it is interesting that, in an area where Br. abortus is prevalent, hares maintain the original Br. suis infection, probably from Europe.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization,
and the Government of Argentina.

Timetable: 1965

NATURAL INFECTION OF SHEEP BY BRUCELLA MELITENSIS IN ARGENTINA

PROBLEM

Ovine brucellosis due to Br. melitensis is an important cause of economic loss and a source of infection in man in several countries of Europe, Africa, and Asia. Serologic studies of sheep by other investigators have revealed brucellosis reactors in various countries of the Americas, but attempts to isolate the etiologic agent have been unsuccessful. The work reported is an attempt to determine whether brucellosis due to Br. melitensis occurs in the Americas in sheep exposed to infection under natural conditions.

METHODS

Serologic surveys were made on sheep and goats in three Argentine provinces. Bacteriologic examinations were limited to 3 flocks where sheep with serologic reactions to the plate agglutination test were found. Milk and vaginal mucus samples were obtained and cultures made on Brucella agar (Albimi). Cultures that had characteristics of the genus Brucella were typed by biochemical and serologic methods.

RESULTS TO DATE

Brucella melitensis was isolated from 3 serologically reacting ewes kept on a ranch with infected goats.

SIGNIFICANCE

The natural occurrence of Br. melitensis in sheep in western Argentina constitutes a potential economic and public health danger for other sheep-raising areas of the country. Similar bacteriologic examinations are necessary in other countries of the Americas.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization and the Government of Argentina.

Timetable: 1961-1962

INVESTIGATION FOR THE PRESENCE OF BRUCELLA IN THE MILK SUPPLY FOR THE CITY OF AZUL, ARGENTINA

PROBLEM

As in many other small Argentine cities, retailed milk is not pasteurized in the city of Azul. The object of the investigation was to determine whether Brucella organisms were present in milk, and if so, to identify species and biotypes.

METHODS

The brucellosis ring test (BRT) and guinea-pig inoculation were employed on herd milk samples of 37 dairy farms. Isolated strains were typed, using biochemical, serological, and phage tests.

RESULTS TO DATE

The samples of 33 (89%) herds were BRT positive. Milk samples from 34 dairies were bacteriologically examined and Brucella organisms were isolated in 14 (41%). Twelve were identified as Br. abortus biotype 1 and two as Br. abortus biotype 4. Classification of biotype strains was confirmed by oxidative metabolic tests.

SIGNIFICANCE

The presence of Brucella in the milk supply of Azul demonstrates the danger to consumers of such dairy products as butter and fresh cheese, which are produced locally from raw milk. It is of epidemiological importance to know how each of the biotypes of Brucella species is distributed by country and region, as well as the frequency of infections therefrom.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization and the Government of Argentina.

Timetable: 1961-1962

STANDARDIZATION OF DIAGNOSTIC METHODS FOR BRUCELLOSIS IN THE AMERICAS

PROBLEM

On the basis of many years of experience it is widely accepted that the seroagglutination test is of great value in the diagnosis of brucellosis in man and animals. In practice, however, difficulties have been encountered because of lack of standardization of antigen, techniques used, and criteria in interpreting the test. For these reasons, there is often little agreement on diagnoses made in different countries, or even between laboratories in the same country. These differences tend to discredit the test, to adversely affect control programs, and lead to serious export and import problems for the cattle industry.

The purpose of this study is to measure progress toward standardization, define existing differences, and investigate possible causes of such differences.

METHODS

Ten countries provided a total of 38 antigens (14 for the tube test and 24 for the plate test). The first part of the study consisted in analyzing data provided by the producing laboratories on the technique used for preparing the antigens, on methods for conducting the test, and on criteria for interpreting results. The second half consisted of laboratory tests of antigens, including those of purity, sterility, pH, cell volume, and sensitivity as compared to standard antigens.

RESULTS TO DATE

A tendency was noted toward standardization of antigens and techniques in animal use but not in man. As compared to the standard antigen, 71.4% of the tube test antigens and 47.3% of the plate test antigens for animal use were satisfactory. Only 14.3% of the tube test antigens and none of the plate test antigens for human use were acceptable when tested against the standard.

SIGNIFICANCE

The findings suggest that appropriate government agencies have not taken suitable measures to ensure the standardization of both the antigens and the seroagglutination test techniques in the diagnosis of brucellosis.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization and the Government of Argentina.

Timetable: 1962-1963

EFFECT OF FOOT-AND-MOUTH DISEASE VACCINATION ON
THE SEROAGGLUTINATION TITERS FOR BR. ABORTUS

PROBLEM

The object of this study is to ascertain whether the application of foot-and-mouth disease (FMD) vaccine in calves previously vaccinated with Br. abortus strain 19 vaccines can cause a rise in seroagglutination titers for brucellosis. The diagnosis of brucellosis and control programs could, thereby, be affected.

METHODS

Seventy-eight young heifers, previously vaccinated with strain 19 vaccine, were divided into 3 groups to receive 3 different types of FMD vaccine. A group of 24 heifers did not receive FMD vaccine and served as control. Each animal was bled 11 times at intervals of 3 days before vaccination and equally after vaccination. Average Br. abortus titers were determined for each animal before and after FMD vaccination.

RESULTS TO DATE

Comparing results for the periods before and after vaccination, there was no significant difference in the average titers among the three vaccinated groups, nor between the vaccinated and control animals.

SIGNIFICANCE

Any effect that FMD vaccination may have on the agglutination titers for brucellosis is so slight that it would be of no importance in diagnosis and have no effect in control and eradication programs.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization and the Government of Argentina.

Timetable: 1962

ECHINOCOCCOSIS IN FOXES AND IN THE GRISON IN SOUTHERN SOUTH AMERICA

PROBLEM

Although the dog, infected with Echinococcus tapeworms, is generally recognized to be the source of the relatively high prevalence of human hydatidosis in southern South America, there is new evidence of the involvement of wild animals in the life cycle of this parasite. These studies are designed to produce additional evidence.

METHODS

The intestinal tract of Pampas grey and Patagonian grey foxes was examined for Echinococcus tapeworms. Specimens of the weasel-like mustiline, the grison, were also examined.

RESULTS

A total of 16 out of 442 Pampas foxes (3.6%) and 56 out of 360 (15.5%) of the Patagonian foxes were found infected. Natural infection was also found in the grison.

SIGNIFICANCE

While this study confirmed the presence of echinococcal infection in foxes and the grison, there is need for additional studies of echinococcosis in South America to clarify the natural history of the disease and define the extent of the control problem.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization and the Government of Argentina.

Timetable: 1962-1964

SCREENING OF DRUGS FOR THE TREATMENT OF CANINE ECHINOCOCCOSIS

PROBLEM

The spread of canine echinococcal infection to man and domestic animals in the form of hydatid disease is of great public health and economic importance in many countries. The purpose of this study is to screen a number of drugs for a highly efficient, nontoxic taenicide for treatment of infected dogs and to study its therapeutic range. The relationship between resistance to treatment and the stage of maturation of tapeworms has also been investigated.

METHODS

Dogs are infected by oral administration of specified numbers of Echinococcus granulosus scolices in sheep hydatid cysts, obtained from local abattoirs. Various drugs are tested against these infections, with approximately half of the dogs in any one trial serving as controls. Observation of post-dosing effects are made along with examination of evacuated fecal material. All dogs are sacrificed and their intestinal contents and walls are examined. Counts of E. granulosus and proglottids are made.

RESULTS TO DATE

The most promising and efficacious drug tested thus far is the naphthalene compound (N:N-di-butyl-4-hexyloxy-1-naphthamidine hydrochloride), which is giving highly significant results in ten separate trials with a large number of dogs. Studies to determine an optimal dosage and its action on immature stages of the tapeworm are under way.

SIGNIFICANCE

Although the life cycle of hydatid disease is known, and, theoretically, it is simple to control by preventing the feeding of infected offal to dogs, education and enforcement measures have not been successful in most infected areas. An effective dog taenicide will be a practical means of breaking the cycle of this important parasitic zoonosis.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: National Institutes of Health/U.S. Public Health Service,
The Wellcome Foundation, Ltd., and the Pan American Health
Organization/World Health Organization.

Timetable: 1960-1969

A MODIFIED SLIDE LATEX SCREENING TEST FOR HYDATID DISEASE

PROBLEM

A tube and slide latex agglutination test for hydatid disease has been described by Fischman (1960). The purpose of the present study was to devise a more rapid test, using nondiluted sera and a stable antigen which can be stored in the refrigerator for ready use.

METHODS

The batch of antigen used in the study had the following composition: hydatid fluid (3-fold concentration), 5 ml.; glycine saline buffer, 15 ml.; and polystyrene latex particles suspension (Hytex) (1:4), 3 ml. The latex antigen was kept in the refrigerator at 4° C when not in use. Tests were performed on different days in order to ascertain the repeatability of the test. One drop each of inactivated serum and antigen are placed side by side on a glass plate and mixed with an applicator stick. The plate is tilted continuously for 2 minutes and read by the naked eye.

All samples used were human sera from individuals suffering from hydatid disease or other infections.

One battery of sera from 221 patients was processed for specificity and sensitivity of the test. A second battery of 60 was used to evaluate relative sensitivity of the test and correlation with hemagglutination and bentonite flocculation procedures.

RESULTS TO DATE

A total of 221 serum samples containing 23 specimens of surgically proven hydatid cases were tested for sensitivity and specificity. Twenty-nine sera gave positive results. All 23 hydatid sera were positive by this test and 6 (3%) were false positives. According to these results the test was 100% sensitive and 97% specific. In the second battery of 60 sera, the sensitivity of the latex test in detecting proven hydatid cases was 93%. Good correlation was obtained with the hemmagglutination and flocculation tests.

SIGNIFICANCE

Results indicate that the modified slide latex agglutination test may be sufficiently sensitive and specific to be used as a screening test for hydatid disease. The latex test may be also a useful, inexpensive,

and rapid tool for epidemiological survey of large numbers of serum specimens from endemic areas. The procedure, however, requires further evaluation in the field.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina and the Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia.

Funded by: Pan American Health Organization/World Health Organization.

Timetable: 1962

THE CASONI REACTION IN THE DIAGNOSIS OF HYDATIDOSIS

PROBLEM

Although the Casoni test is considered a valuable aid in the diagnosis of hydatidosis, discrepancies exist between the test and clinical and surgical findings, due mainly to lack of standardization. Unsuccessful attempts have been made to improve the original technique, making changes principally in the preparation of the antigen, amount to be inoculated, and reading time, or by regulating the sensitivity or specificity of the reaction.

METHODS

The sensitivity and specificity of three different antigens were compared for use in the intradermal (Casoni) test for hydatid disease. Two antigens were obtained by defreezing sheep hydatid fluid. One of these fractions had a high concentration of carbohydrates and the other one of albumins. The third antigen consisted of total hydatid fluid and was used for reference purposes.

The study subjects were 23 surgical hydatid disease cases, 27 patients with diseases other than hydatid disease, and 26 normal persons. Both early and delayed reactions were recorded and compared.

RESULTS TO DATE

All three antigens, although highly sensitive and specific, gave no statistically significant differences in response.

SIGNIFICANCE

This study has shed some light on the problems in the use of the Casoni skin test for diagnosing hydatidosis, particularly with regard to the sensitivity and specificity of different antigens.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization and the Government of Argentina.

Timetable: 1960 - 1962

A VACCINE TRIAL AGAINST OVINE HYDATIDOSIS

PROBLEM

Because hydatidosis constitutes a serious economic and public health problem in many countries, it is desirable to strengthen and improve available methods for the control and eventual eradication of the disease.

Since 1937, various workers have investigated the use of hydatid liquids, scolices, etc., as vaccines against the cystic form of the disease in animals. This study was undertaken to assess the value of a vaccine recommended for immunizing sheep against hydatidosis.

METHODS

The vaccine tested consisted of a fraction of hydatid cyst fluid with high protein content. Three-to-6-month-old lambs of Corriedale stock born at the Farm Annex of the Pan American Zoonoses Center were used. The 118 lambs were randomly separated into two equal groups: one group received subcutaneously, at 30 day intervals, 3 doses of 2 cc. each of the product; the lambs in the other group were treated as unvaccinated controls. Lambs in both groups were kept together under the same conditions throughout the study. Forty-five days after the last dose of vaccine, each animal in both groups received an oral dose of 30 to 100 mature Echinococcus granulosus eggs.

At varying intervals during the 4 to 21 month period after challenge, equal lots of vaccinated and control animals were killed and examined.

RESULTS TO DATE

Macroscopic and histopathologic examinations were made of lesions encountered in the viscera. Since, by coincidence, exactly 51 vaccinated animals and 51 controls had lesions attributable to hydatidosis, it was concluded that the product conferred no demonstrable protection against the disease.

SIGNIFICANCE

The study clearly demonstrated the lack of immunological activity of the hydatid cyst fluid fraction tested.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization
and the Government of Argentina.

Timetable: 1962-1963

NATURAL LEPTOSPIRA INFECTION IN THE PAMPAS CAVY AND IN THE OPOSSUM

PROBLEM

The purpose of this research is to study the natural occurrence of Leptospira infection in wild animals in Argentina and to assess their role as reservoirs of the infection in domestic livestock and in humans.

METHODS

An outbreak among cattle on a large ranch in the central part of the Province of Corrientes, Argentina, was due to Leptospira pomona infection. Several species of wild mammals, the commonest being the native cavy (Cavia pamparum), were examined for their possible role in the perpetuation and dissemination of the disease.

A total of 282 cavies and 25 opossums were captured, autopsied, and examined serologically and culturally for leptospirosis. Serums were tested against live antigens for 12 serotypes, using the microscopic agglutination (lysis) technique. Kidney tissue was cultured, using Wolff's procedure. Urine obtained aseptically by bladder puncture was cultured, using the dilution technique. Fletcher's medium was used for isolation.

RESULTS TO DATE

Of the 282 cavies captured and examined on three occasions in one year, 25 (9%) were serologically positive for L. pomona. The organism was isolated from urine and kidney of 11 (4%). None of the cavies showed clinical signs of the disease.

Serum samples of 2 of the 25 opossums showed an agglutination titer. One had a titer of 1:800 for L. pomona and another 1:200 for L. bataviae. Two strains of Leptospira were isolated and found to belong to the bataviae group, with the highest titer for L. paidjan antiserum. Agglutination-absorption tests confirmed that the isolated strain belonged to the L. paidjan serotype.

SIGNIFICANCE

This is the first reported isolation of L. pomona from wild cavies and the first evidence that these animals play a role in the epizootiology of bovine leptospirosis. The study also produced the first isolation of a serotype of the bataviae group in Argentina, and the first finding of L. paidjan in marsupials.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization
and the Government of Argentina.

Timetable: 1961-1962

SEROLOGICAL SURVEY OF BOVINE LEPTOSPIROSIS IN ARGENTINA

PROBLEM

It was planned to conduct a survey on farms in Central Argentina to determine the rate of leptospiral infection in cattle, due to serotypes L. pomona and L. sejroe.

METHODS

A total of 1,533 bovine serum samples from 38 farms were tested by microscopic agglutination techniques.

RESULTS TO DATE

In this study, 926 (60%) of the samples tested gave titers of 1:200 or greater for L. sejroe and 7 (0.4%) for L. pomona. The number of cows with serological reactions to the Hebdomadis group is very high. It has not been possible to isolate any strain of this serogroup in Argentina, and attempts are continuing, by culture and animal inoculation, to isolate the responsible agent.

SIGNIFICANCE

Additional information has been obtained on the epidemiology of bovine leptospirosis in Argentina.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization and the Government of Argentina.

Timetable: 1965

CULTURE AND MULTIPLICATION OF RABIES VIRUS ON BHK-21 C-13 CELLS

PROBLEM

The purpose of the research is to describe the culture and multiplication of rabies virus in BHK-21 C-13 cells.

METHODS

Bottles and tubes of baby hamster kidney cells batch 21, clone 13 (BHK-21 C-13) were used in all the experiments. Cells were grown in Eagle's medium with 10% tryptose phosphate broth and 10% calf serum.

Virus suspension for infection of tissue culture BHK-21 C-13 cells were street and fixed strains of rabies, previously adapted to cell cultures; mice brain suspension infected with street strain; and rabbit brain suspension infected with fixed Pasteur No. 2019 rabies strain. Virus was harvested every 4-5 days.

Virus multiplication was demonstrated by intracerebral inoculation in mice (14-16 gm.), fluorescent antibody technique, Mann stain, gel precipitation test, and immunoelectrophoretic analysis.

RESULTS TO DATE

Street rabies strains. Using rabies brain suspension to infect BHK-21 C-13 cells, intracytoplasmic inclusions appear on the 10th day, the virulence is low, and there is no cytopathogenic effect. Tissue culture adapted virus antigen visualized by specific fluorescence appears as early as 12 to 15 hours, fluorescence increases with time and all cells become fluorescent around the 10th to 12th day. Titer in mice is between $10^{3.5}$ to $10^{4.5}$ per 0.03 ml. obtained at the 4th and 10th days after infection.

Fixed rabies strains. With passage of infected brain suspension or virus adapted previously to tissue cultures, virus antigen appeared on the 4th day after infection of BHK-21 C-13 cells and a titer of $10^{2.5}$ per 0.03 ml. in mice was obtained. By gel precipitation and immunoelectrophoretic analysis, results were obtained after the 12th day of infection of the BHK-21 C-13 cultures. Two lines of precipitation appeared, one with all the immune sera used, and the other with only some of them.

SIGNIFICANCE

The clone 13 of BHK-21 cells is easily grown and maintained as a continuous line. This cell has been shown to be more sensitive to the multiplication of strains of fixed and street rabies virus, already adapted to tissue culture, than to the direct isolation of this virus.

These properties have made possible the study of various characteristics of the virus, such as its multiplication, purification, presence and composition of inclusions, and the examination of ultrathin sections of these infected cells by the electron microscope.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization
and the Government of Argentina.

Timetable: 1964

TRANSMISSION OF RABIES BY THE RESPIRATORY ROUTE IN LABORATORY ANIMALS

PROBLEM

The purpose of the research is to study in adult and newborn laboratory animals the effect of exposure to rabies virus strains by the respiratory route. Included are observations on the site of virus multiplication and the route of elimination.

METHODS

Three rabies virus strains were inoculated into newborn and adult animals (mice, hamsters and gerbils), using nasal droplet and aerosol methods. Brain, trachea, kidney, and urine were examined by direct fluorescent antibody methods. Virus titration was done by intracerebral inoculation in mice. After exposure to the virus, newborn animals were returned to the cage with their mothers.

RESULTS TO DATE

Out of 308 animals exposed, 159 (52%) contracted rabies. After the brain, the lung is the most important site of virus multiplication. Free virus is found in trachea and kidneys but not in urine of paralyzed animals. Newborn hamsters infected their mothers by cohabitation.

SIGNIFICANCE

The data add to knowledge on the transmission of rabies in animals and confirm earlier observations made on the aerosol transmission of the virus. (Constantine, D. G., Publ. Hlth. Rep., 77: 287, 1962)

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/ World Health Organization and the Government of Argentina.

Timetable: 1965

STUDY OF RABIES VACCINES FOR BOVINE USE

PROBLEM

Hundreds of thousands of cattle die annually in the Americas from bat-, fox- or canine-transmitted rabies. The sylvatic vectors are extremely difficult to control, and hence, emphasis must be placed on protection of exposed domestic animals. Various types of vaccine are currently being used to protect cattle in enzootic areas. Vaccine protection, however, is generally deficient in degree and duration. Vaccine safety is also a problem.

The aim of this research is to evaluate rabies vaccines, particularly some of the newer ones that are being developed for use in cattle.

METHODS

Controlled studies of rabies vaccines are being conducted in cattle at the Center's farm annex. Five vaccines are currently under study: (1) the suckling mouse brain U.V. killed vaccine; (2) a modified live swine kidney tissue culture vaccine (Connaught); (3) a live virus vaccine of chicken embryo origin (Cyanamid); (4) the same vaccine (Cyanamid) with aluminum hydroxide adjuvant; (5) two killed sheep brain vaccines inactivated with beta-propiolactone, alone or in combination with phenol (CEPANZO).

Small groups of young cattle were vaccinated with the above-mentioned vaccines, and controls were maintained. Revaccination with the first two vaccines took place after 30 days. Blood samples are collected periodically for serum neutralization tests, 100 days, 200 days, and one year after the first vaccine inoculation. Direct challenge with live virus strains may also be carried out, depending on the availability of funds for isolation facilities.

RESULTS TO DATE

All vaccines provoked detectable rabies sero-neutralization antibodies one month after vaccination. Two vaccines (Nos. 1 and 2, above) demonstrated antibodies 100 and 200 days after the primary vaccination. In both these vaccines, the revaccination (booster inoculation) resulted in a rapid increase in antibody titer.

SIGNIFICANCE

It is highly important that a safe, potent vaccine be developed and used to provide cattle with long-lasting immunity against rabies. The partial

success of the rabies vaccines tested has demonstrated the urgent need for more intensive studies.

OTHER DATA

Grantee: Pan American Zoonoses Center, Azul, Argentina.

Funded by: Pan American Health Organization/World Health Organization
and the Government of Argentina.

Timetable: 1965-1968

THE INTER-AMERICAN INVESTIGATION OF MORTALITY

PROBLEM

A campaign of the statistics of various countries shows wide differences with respect to mortality for many diseases. To some extent, these presumably reflect differences between peoples in exposure or response to causal factors. However, the interpretation of variations in mortality between countries is complicated by differences in language, terminology, and certification practice, as has been shown for diarrheal diseases and for diseases of the circulatory system. Before international comparisons can be accepted as indicating the effects of biological, social, or environmental determinants of disease, these obstacles to interpretation must be removed.

The primary objective of the investigation is, therefore, to provide a comprehensive account of adult mortality in the selected populations, which will be as accurate and comparable as possible. Such an account serves to define more sharply than heretofore the scope for preventive action and to indicate those differences in mortality that might profitably be investigated further by epidemiological methods.

METHODS

Small teams of physicians and home visitors collect in a standard form all available information relevant to establishing the cause of death of residents dying between the ages of 15 and 74 years during a two year period in 12 cities: Bogotá, Bristol, Cali, Caracas, Guatemala City, La Plata, Lima, México City, Ribeirão Preto, San Francisco, Santiago, and São Paulo. About 4,000 deaths have been investigated in each city consisting either of all deaths of residents in the age range, or a sample systematically drawn. The completed questionnaires are sent for review to the central office in Washington so that the causes of death can be assigned in a uniform manner following established international practice. For deaths from cardiovascular conditions or deaths where more than one disease seemed to be involved, the final assignments of cause are made by two medical referees working independently, and their separate opinions are combined by using weights.

RESULTS TO DATE

Questionnaires for 43,298 deaths in the 12 cities are being used in the analysis. The central review procedure has been completed, tabulations are being prepared from the punch cards, and analysis of the results is proceeding. A report will be published in book form, in both English and Spanish, in late 1966. Some of the results now available are summarized.

1. Death rates per 1,000 at ages 15-44 years show marked differences: the high rate in an age and sex group in one city is as much as four times the rate in another city. From 45 to 74 years, however, the disparities are much less. Age adjusted death rates range from 3.2 to 5.6 per 1000 for females and from 6.2 to 9.8 per 1000 males.

2. In studying the principal causes, the age adjusted death rates were grouped according to established principles. The two leading causes were diseases of the heart and malignant neoplasms. For males, diseases of the heart were found to be the leading cause in eight cities, malignant neoplasms in three, and cirrhosis of the liver in one. In contrast, in females, malignant neoplasms held first place in nine cities and diseases of the heart in three.

3. Mortality from tuberculosis shows a 15-fold range, being particularly high in Cali, Lima and Santiago.

4. Although mortality from malignant neoplasms as a group does not vary as much as some other causes, the death rates for individual sites of cancer show marked variation: cancer of the stomach is high in Bogotá, Cali, Lima and Santiago; cancer of the lung (males) is high in Bristol and La Plata; cancer of the cervix uteri is high in Cali, Guatemala City, Lima, and Mexico City; cancer of the bladder (males) is high in La Plata.

5. In San Francisco, the death rate in males from diseases of the heart is three times higher than in Guatemala City, but the rate in females is only 39 per cent higher in San Francisco than in Guatemala City.

6. Mortality from bronchitis among males in Bristol is over twenty-four times as high as the rates in Guatemala City and Lima. By contrast, death rates for females are low.

7. Mortality from cirrhosis of the liver is unusually high in Mexico City, San Francisco, and Santiago, in both males and females. In Mexico City and Santiago, the male mortality from this cause exceeds that of malignant neoplasms of all sites.

8. Maternal death rates of one or more per 1,000 births are found in all the Latin American cities except Ribeirão Preto and São Paulo. The highest rate is 3.2 per 1,000 (Santiago). The importance of abortion as a cause of death is clearly evident in the data.

9. The highest death rates from motor vehicle accidents (males) are noted in Guatemala City and Santiago. For females, the rates are generally much lower, with the highest rate noted in San Francisco. San Francisco has the highest death rate from suicides, in both males and females.

SIGNIFICANCE

Certain populations will be distinguished by unusually high death rates from many specific causes, thereby indicating those areas where preventive measures may be undertaken and others where further research may be profitable for the purpose of testing defined and pointed hypotheses. The investigation demonstrates the feasibility and value of cooperative research on an international scale.

OTHER DATA

Grantee: Dr. Ruth R. Puffer, Pan American Health Organization,
Washington, D.C.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1962-1966

RESEARCH PLANNING ON THE ETIOLOGY OF CONGENITAL MALFORMATIONS

PROBLEM

There are no standard procedures in Latin America for reporting congenital malformations and for analyzing current data on their frequency and distribution, so that medical authorities might be alerted to unusual changes in the secular trends. The establishment of effective control measures is dependent upon research into underlying causes. Reliable data, therefore, are urgently needed from many areas as clues to research into the causative factors of congenital defects.

METHODS

The Planning Conference for Research on Congenital Malformations, held between January 3 and 7, 1963, advised the Organization on how it should plan a collaborative program for the Americas and put it into effect. It was generally agreed that, by detecting unusual groupings of congenital defects in time and space, the proposed research would be significant as a step toward recognizing detrimental effects of newly introduced potentially teratogenic agents.

Several widely dispersed study areas in the Americas would be selected for participation in the research program. The additional inclusion of selected hospitals or of areas where efforts were needed to strengthen existing services would be justified on the basis of special situations. Among these might be an unusually high frequency of a specific malformation or exceptional environmental circumstances, such as high altitude or poor nutritional status of the population.

RESULTS TO DATE

An application for a research grant was submitted on February 20, 1963, but approval was not obtained. Interest continued, and several exploratory activities were carried on, supported in part by the planning grant.

1. Since the problem of congenital malformations is not unrelated to the problem of mental retardation, the Special Assistant on Mental Retardation to the President of the United States on November 22, 1963, called a meeting on Research in Congenital Malformations to discuss how progress could be made in this field. Three suggestions were made: a) the original proposal could be redesigned, emphasizing demography in Latin America and community centered research on congenital malformations in the United States; b) the development of a research program on deaths in infancy and childhood (similar to the Inter-American Investigation

of Mortality) would provide comparable data on the fatal congenital malformations; and c) a communication center on congenital malformations could be established in order that information collected in various areas would become available to all concerned with research in this field.

2. An informal meeting on Research in Congenital Malformations was held on January 7, 1964, to discuss the projects proposed for California, Minnesota, and New York City. A research project was developed for New York City and is presently under consideration for funding.

3. At the January 7, 1964 meeting, a need had been expressed for a manual on congenital malformations. The Organization is to develop diagnostic standards of congenital malformations and promote their international acceptance. A consultant prepared a draft manual on congenital malformations, which was reviewed by a working group on January 18 and 19, 1965. The manual will be aligned with the Eighth Revision of the International Classification of Diseases of the World Health Organization, whose contents will be finally determined in 1966.

4. Research on deaths in infancy and early childhood is under consideration.

5. An inter-American investigation of abortions has been proposed.

SIGNIFICANCE

The problem of congenital malformations in different geographical areas can be most clearly defined through accurate description and comparable tabulations. These studies and those in related fields, if undertaken, will yield valuable data for planning future research in congenital defects.

OTHER DATA

Grantee: Dr. Ruth R. Puffer, Pan American Health Organization,
Washington, D.C.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1962-1965

STUDIES IN COLOMBIA ON HEALTH MANPOWER AND MEDICAL EDUCATION

PROBLEM

The prime objective of the research is to assist countries in obtaining a comprehensive understanding of the projected quantity and quality of their health services and the role of the physicians therein. This would help to identify and, insofar as possible, to measure the controlling factors influencing contemporary practices in medical education. Information thus provided will help in planning medical education programs and, in general, all programs for education in the health field, in relation to needs, resources, and attitudes relevant to the requirements of a specific country.

METHODS

The national health survey is the most complex part of the project and is considered essential to the objectives of the study. Sampling is done with the assistance of experts from the U.S. Public Health Service and the U.S. Census Bureau.

For sampling purposes, the country was divided into 200 districts of approximately equal population. These 200 were then grouped into 40 strata, using such criteria as urban-rural, growth rate of health services, infant mortality, altitude, and average income. From each stratum, one district was selected for study. This gave 40 districts, proportionately representing the population of different parts of the country. From these districts small areas were selected for household and clinical surveys. In each of the 40 districts a total of 240 household interviews and 90 clinical examinations are being conducted. This sample size will provide reliable estimates for the nation and, in 3 or 4 regions of the country, for characteristics which appear in 10% of the population. Somewhat less reliable estimates of characteristics appearing less frequently will be obtained. Since these 40 districts will represent the entire country, they will be the best choice of areas for other investigations that are to be part of the total study of resources - including hospitals, health centers, physician and nurse activities.

RESULTS TO DATE

Data on medical and nursing education as well as on physicians and nurses have been obtained and are being processed for final analysis. The national household survey has been carried out successfully. Of the 40 districts in the sample, 18 had been surveyed by February 1966. A sample of health facilities is being selected for intensive studies to terminate in August 1966. The economic studies are expected to be completed by that date.

The study has proved an excellent experience in team work for health authorities and medical educators. Medical school faculty and students have been involved in various aspects of the national household survey, which has been a valuable educational experience for both.

SIGNIFICANCE

The study is of importance:

a. In the development of a method that can be used in other Latin American countries to obtain data for more rational planning of health personnel education and training.

b. 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.

c. In the development of a closer understanding between health authorities and those responsible for medical education to achieve more effective joint action in the attainment of the common goal of raising health standards in Colombia.

OTHER DATA

Grantee: Dr. Alfonso Mejía Vanegas, Ministry of Health, Colombia,
and Dr. Raúl Paredes Manrique, Colombian Association of
Medical Schools, Colombia.

Funded by: The Milbank Memorial Fund.

Timetable: 1964-1966

PAHO REFERENCE LABORATORY AND TRAINING CENTER FOR IODINE
DETERMINATIONS IN ENDEMIC GOITER RESEARCH

PROBLEM

To accelerate the PAHO-sponsored research program directed toward solving the endemic goiter problem in Latin America, a Reference Laboratory and Training Center was established in Santiago, Chile, to provide facilities for standardizing laboratory procedures and iodine determinations and to train personnel in these techniques.

METHODS

Duplicate samples of biological fluids (blood and urine), food, water, soil and other materials from collaborating laboratories will be checked at the Center for iodine content. Standardized test samples will be circulated periodically by the Reference Center to the collaborating laboratories for iodine determinations.

Personnel from the collaborating laboratories and from public health departments in countries where iodine prophylaxis of endemic goiter is currently under way or is being planned will receive training at the Center in the laboratory techniques to be used in the study.

RESULTS TO DATE

The biochemist in charge of the Reference Laboratory received training in the methodology of iodine determinations at the Boston Medical Laboratory between March and May, 1965.

Equipment and supplies for the Reference Laboratory have been purchased and shipped to Santiago, and it is expected that the Center will be fully functioning by mid-1966, when the first trainees will be received.

SIGNIFICANCE

The Reference Center will considerably facilitate current research on endemic goiter being conducted, under PAHO sponsorship, by the 12 collaborating laboratories. The Center will also provide training for personnel participating in national goiter prophylaxis programs, thereby making possible careful control of iodine levels in the vehicle used (such as salt), or in biological fluids of the populations receiving the prophylaxis.

OTHER DATA

Grantee: Dr. John J. Kevany, Pan American Health Organization,
Washington, D.C.

Dr. José Barzelatto, Hospital del Salvador, Santiago,
Chile.

Funded by: Williams-Waterman Fund.

Timetable: 1965-1967

PAHO/WHO REFERENCE LABORATORY AND TRAINING CENTER FOR
APPLIED RESEARCH IN NUTRITIONAL ANEMIAS

PROBLEM

In view of the limited data on nutritional anemias prevalence in Latin America and the Caribbean and of the desirability of conducting PAHO/WHO sponsored surveys using recently developed techniques for measuring such deficiencies, a Reference Laboratory and Training Center was established in Caracas, Venezuela, to provide facilities for the standardization of vitamin B₁₂, folate and iron determinations and for training participating personnel in these techniques.

METHODS

Techniques for determining iron, vitamin B₁₂ and folic acid in blood have been set up within the Reference Laboratory and are used on a routine basis. Duplicate samples, suitably stabilized, are received from collaborating laboratories and analyzed, and the results are forwarded to the respective investigator. In this way, he is able to maintain a check on his own methodology and results.

At a later stage, standardized test samples will be distributed by the Reference Laboratory to investigators so that they will be able to make further checks within their own laboratory.

Both the principal investigator and the laboratory technician from collaborating laboratories will receive orientation and training in the study design and laboratory methodology, respectively, by means of short training visits to the Reference Laboratory.

RESULTS TO DATE

All the basic determinations are operational in the laboratory. To date, 645 determinations of vitamin B₁₂ and 777 determinations of serum folate have been carried out.

A physician from the collaborating laboratory in Mexico City received training in laboratory methods during May and June 1965. An investigator, now in INCAP's anemia laboratory, and a biochemist, participating in the Trinidad anemia study, also received orientation and training on methodology for determinations of vitamin B₁₂, folic acid and serum iron.

Scientists from Johannesburg and London, who are cooperating in the WHO Nutritional Anemias Study, visited the Reference Laboratory and later sent duplicate samples from their own laboratories for analyses of serum iron, per cent saturation of transferrin, folic acid and vitamin B₁₂. In this way the Laboratory remains standardized with others collaborating in the WHO study.

SIGNIFICANCE

This project is a research service and has greatly facilitated the work now being conducted by five laboratories collaborating in the PAHO/WHO Nutritional Anemias Study. The results of this epidemiological study of nutritional anemias in Latin America will provide the information necessary to establish simple, effective public health measures for combating these nutritional diseases.

OTHER DATA

Grantee: Dr. John J. Kevany, Pan American Health Organization
Washington, D.C.

Dr. Miguel Layrisse, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

Funded by: Williams-Waterman Fund.

Timetable: 1965-1967

PAHO/WHO IMMUNOLOGY RESEARCH AND TRAINING CENTER

PROBLEM

Immunology is assuming increasing importance in a variety of fields of biology and medicine. Rapid developments are in progress in transplantation, immunopathology, knowledge of structure and function of the immunoglobulins, and in cellular differentiation and genetics. It is clear that immunology now deserves attention as a central part of biology and medicine and that the subject is no longer restricted mainly to serologic techniques and immunization procedures. In spite of the broadening of the scope of immunology, the phenomenon of immunity to infectious diseases is poorly understood, and vaccination remains an empirical procedure. Pertinent knowledge will probably be gained by a closer study of the immunology of parasitic diseases. In order to develop harmoniously, progress in immunology requires cooperation and awareness of research developments all over the world.

METHODS

The Institute of Microbiology, Escola Paulista de Medicina, in São Paulo, Brazil has been designated a PAHO/WHO Immunology Research and Training Center. A research program on the biological significance of the heterogeneity of immunoglobulins has been initiated with the purpose of determining which ones are active in protecting against infectious agents and which contribute to tissue damage by hypersensitivity reactions (allergy and immunopathology). A course in the theory and laboratory techniques of immunology has also been prepared. WHO and PAHO are facilitating visits by foreign immunologists to participate in the course and in the research.

RESULTS TO DATE

The Center has just begun operations, but the course in immunology has started with a visit by a specialist in immunofluorescence who will also investigate the incidence of auto-antibodies in a form of pemphigus seen in Brazil.

SIGNIFICANCE

Experienced scientists agree that applied research will flourish only if it is constantly being nourished by ideas, techniques, and findings derived from basic research. In any region of the world, it is therefore shortsighted not to devote a reasonable part of the available

resources to this supporting role of basic research, particularly in immunology, where there is often only a short step from basic findings to their application in therapy and prophylaxis. A laboratory for basic research can be maintained at relatively small expense and can prove a tremendous asset as a continuing source of inspiration and intellectual support for those who are applying immunology in public health.

There are three avenues of immunology research that can be expected to make valuable contributions to solving some of the public health problems which call for pressing action in the developing countries: the development of immuno-diagnostic tests, studies in immunopathology, and research in immunoprophylaxis. For all these reasons, the Organization by establishing centers for research and training in immunology is helping to create the trained scientific manpower essential for promoting regional and local studies in the countries where tropical diseases exist.

OTHER DATA

Grantee: Dr. Otto Bier, Escola Paulista de Medicina, São Paulo, Brazil.

Funded by: World Health Organization/Pan American Health Organization.

Timetable: 1966 - continuing.

CENTER FOR THE PRODUCTION AND BIOLOGICAL CONTROL OF
ANTIGENS IN THE LABORATORY DIAGNOSIS OF CHAGAS' DISEASE

PROBLEM

The complement fixation test is the method of choice for the serologic diagnosis of Chagas' disease. Since several types of antigens have been used for this test, comparison of results is made difficult.

The need for obtaining comparable data in epidemiological surveys of various endemic areas requires the establishment of regional centers responsible for the preparation of standard antigens and for testing antigens prepared by different laboratories.

METHODS

Following the report of the PAHO Advisory Group on Research in Chagas' Disease which had met to evaluate the present status of knowledge of the disease, define the most important fields for study, and indicate the type of research best suited for the solution of the problem, the PAHO Advisory Committee on Medical Research recommended the establishment of centers for the production and control of antigens for the serologic diagnosis of Chagas' disease.

RESULTS TO DATE

PAHO signed in 1962 an agreement with the Faculty of Medicine of the University of Chile, by which the Department of Parasitology would undertake studies for the preparation of a standard antigen for use in the complement-fixation test and assume responsibility for distributing the antigen to countries that request it. Up to the present, a standard antigen has been distributed free of charge to seven countries in the Americas.

The Organization will continue to provide this type of assistance and is studying the possibility of a similar agreement with another institution, which would also serve as a reference and training center for the serological diagnosis of Chagas' disease.

SIGNIFICANCE

Chagas' disease is known to occur in most of the countries of the American continent but its true incidence has not yet been determined. The number of persons exposed to infection by Trypanosoma cruzi is estimated at 35 millions. If the average of the infection rates obtained in

epidemiological surveys carried out in several countries is taken at 20%, it may be calculated that at present there are at least seven million individuals infected with T. cruzi.

To assess the health importance of this disease and organize and carry out control programs, it is essential to have as accurate information as possible on its distribution, as well as data on variations in such distribution and frequency, and on the changes it undergoes in the course of time. The polymorphism of Chagas' disease in man and consequently its faulty reporting and the rareness in which it appears in mortality statistics clearly show the need to carry out epidemiological surveys in endemic areas.

The serologic diagnosis of the disease by the complement fixation test constitutes a most important element in those surveys and in order to obtain comparable results in various areas it is essential that the antigens used be standardized.

OTHER DATA

Grantee: Faculty of Medicine, University of Chile, Santiago, Chile.

Funded by: Pan American Health Organization.

Timetable: 1962

MENTAL HEALTH INFORMATION CENTER ON LATIN AMERICA

PROBLEM

The lack of exchange of information and of communication among professionals in mental health in Latin America and the need of a central point for collecting and disseminating such information led to the establishment of the Mental Health Information Center on Latin America (MHICLA).

Established on January 1, 1963, the Center has the following aims:

- a. To obtain information about mental health problems, resources, and facilities available in Latin America.
- b. To establish and develop an international clearinghouse of information on mental health for the Latin American countries.
- c. To function as a permanent instrument for international cooperation in the mental health field.
- d. To promote and coordinate research activities in the field of mental health in Latin America.
- e. To work in close cooperation with the National Clearinghouse for Mental Health Information (NCHMHI) and with the International Research Programs Section of the National Institute of Mental Health, USPHS, in order to draw up plans for the collection and dissemination of information in this field.

METHODS

Sources for obtaining needed data are: libraries (both in Washington, D.C. and in Latin America), professional associations, government agencies, mental health institutions, medical schools, psychiatrists, and PAHO field staff.

The scientific reference analyst of the Center does research in the Washington area libraries. Communication with other sources is established by circular letters, questionnaires, and other types of correspondence.

Because it is difficult and time consuming to obtain information from Latin American sources by mail, two individuals in Latin America have twice been engaged to compile information for the Directory of Psychiatrists. This method has been most successful, but unfortunately budgetary restrictions prevent its more frequent use.

RESULTS TO DATE

A. The following material has been compiled: A selected bibliography on psychiatry and mental health in Latin America (1950-1962) and an annotated bibliography of articles published in Latin American psychiatric and mental health periodicals (1960-1962). Lists of: (a) journals on mental health, psychiatry, and related subjects published in Latin America; (b) international meetings on mental health, held in Latin America (1928-1962); (c) governmental mental health agencies in Latin America; (d) mental health organizations in Latin America; (e) professors in charge of graduate training in psychiatry; and (f) mental health facilities and institutions. Card files listing names of key mental health personnel and bibliographic material on mental health available in the 72 medical libraries in Latin America that replied to the request for information have also been compiled.

B. A preliminary survey of mental health legislation in Latin America has been made in the Washington, D.C. libraries. The data will be tabulated to show areas of law affecting mentally disabled persons in each country.

C. A provisional Directory of Psychiatrists is being prepared. The sections on the following countries have been completed: Argentina, Bolivia, Brazil, Chile, Colombia, and Costa Rica.

D. A survey of existing programs for education in psychiatry and related sciences has been started.

SIGNIFICANCE

Information that could not be obtained heretofore in compiled form will be made available to psychiatrists and other mental health workers. Communication and exchange of information among them will be facilitated, resulting in less duplication of effort and better coordination of mental health activities.

OTHER DATA

Grantee: Dr. René González, Pan American Health Organization,
Washington, D.C.

Funded by: National Institutes of Health/U.S. Public Health Service.

Timetable: 1963-1966 (Renewable).

RESEARCH TRAINING IN THE HEALTH SCIENCES

PROBLEM

The purpose of the grant was to carry out feasibility and planning studies for establishing a faculty and research training center in Latin America in the fields of medical demography, epidemiology, and preventive medicine, and for preparing faculty for such centers.

METHODS

For the 18-month period from July 1, 1964 to December 31, 1965, staff and consultants of the Pan American Health Organization were active in exploring the feasibility of education and training programs in population dynamics, with emphasis on training for research and on population studies.

The initial exploratory visits - made by the staff of the Organization to Santiago, Chile, São Paulo and Ribeirão Preto, Brazil, and Caracas, Venezuela, July and August 1964 - were concerned with medical research training in biosocial studies, including preventive medicine, medical statistics, and demography, as part of a larger program of the Organization for graduate training in medical research.

The PAHO/WHO Conference on Population Dynamics, on January 7, 1965, agreed that much more training and stimulation of interest in population dynamics is needed in Latin America. The Organization was advised to explore the availability of qualified candidates and of rewarding job opportunities in Latin America.

The Working Group on Research Training Centers in Medical Demography, São Paulo, Brazil, March 18 and 19, 1965, attended by professors of preventive medicine and statistics and others interested in population dynamics, was held at the School of Hygiene and Public Health of the University of São Paulo, Brazil. Interest was expressed in a training program in medical demography at the School of Public Health of the University of Chile and at the School of Hygiene of the University of São Paulo.

RESULTS TO DATE

During the two-week period, August 2 to 13, 1965, a senior research demographer of the Office of Population Research of Princeton University, serving as consultant to PAHO, met with staff of the School of Public Health of the Faculty of Medicine of the University of Chile regarding a

training program. The document prepared as the basis for the program, Health and Population Dynamics, was discussed on August 12 and 13, and general agreement was obtained on the program and on the participation of the Organization. The agreement between the Government of Chile and the Organization was signed on November 30, 1965, and an announcement of the program was issued in December 1965.

The Consultant spent the two weeks from November 8 to 20, 1965 in the School of Hygiene and Public Health of the University of São Paulo, to assist on planning for a training center. The Head of the Department of Applied Statistics, in the above mentioned School of Public Health, who had been in Chile for discussions there (August 10 to 13), had conceived the idea of a population center, the first stages of which were developed by her department and by the Department of Sociology of the School of Philosophy. The opportunities for training and positions in such a center had been discussed with several potential members of the staff, including a pediatrician, two sociologists, and an economist, among others. A document was prepared presenting the needs, the multidisciplinary problem, present trends and new dimensions, goals and plans, the phasing of developments, organization and staff, and research and teaching of the Population Center.

SIGNIFICANCE

The Pan American Health Organization is assisting in the development of research training programs in health and population dynamics by the School of Public Health of the Faculty of Medicine of the University of Chile and by the School of Hygiene and Public Health of the University of São Paulo.

In Chile, the first four month course on health and population dynamics is scheduled for August 1 to November 30, 1966. An announcement of the program was released in December 1965, in order to ascertain the interest of members of faculties of medical schools, principally of departments of preventive medicine, in participating in this first scheduled course of this nature. Beginning in 1967, the course previously given for specialization in biostatistics would be rearranged and divided into a principal course and courses in health and population, health statistics and research. This will provide flexibility in the selection of courses for specialization in research, population, or health statistics. Some students may be admitted for the four-month course in health and population dynamics.

In São Paulo, during the first 18 months, the new staff added would undergo training both in the Department of Applied Statistics in São Paulo and in courses in the United States, to prepare them for their specific roles in the population center. It is hoped that a course in population dynamics can be offered in the School of Hygiene in the fourth

quarter of 1966 and annual courses thereafter. If the time schedule for training can be met, four quarters in statistical methodology and demography can be offered in 1967, and the one-year program for population specialists can be initiated in 1968.

OTHER DATA

Grantee: Dr. Ramon Villarreal, Pan American Health Organization.

Funded by: Agency for International Development (AID).

Timetable: 1964-1967

RESEARCH, EDUCATION AND TRAINING IN SANITARY ENGINEERING

PROBLEM

The increasing awareness of the relationship of man's physical environment to the state of his health is creating a growing need for engineering services essential to an effective public health program. Modern phenomena such as accelerated population growth and urbanization and the consequential need for water supply, sewerage, air and water pollution control, solid wastes disposal and the like, raise many technical and managerial problems that cannot be satisfactorily resolved by conventional methods.

All too often sanitary engineers have relied on these conventional methods instead of developing new techniques fitted to the problems and the conditions they encounter. Training and applied research programs emphasizing the value of seeking new and imaginative solutions to technical and managerial problems are particularly necessary.

METHODS

In order to foster research and training activities in engineering schools in Latin America, a network of cooperating universities is being developed. Agreements are being signed with Member Governments establishing the basis of cooperation with the Organization. These agreements provide for technical assistance through consultants, fellowships for teaching staff, and grants for the conduct of short courses and for implementation of research.

The aim of the project is to develop the capability of local institutions for research and training and to attract potential talent into this field of activity.

The best stimulant to research is the actual conduct of research projects. Proposals are to be submitted by the interested institutions to the Organization, which will provide advisory services and a small grant to help defray local costs. The proposal should state the nature of the problem, objectives of the research, results expected, facilities available, previous work done, and any other pertinent information; and include a detailed timetable and budget for the activity. The development of a good proposal and plan of operation is considered an important aspect of research training and therefore consultants may be provided to give assistance in this phase.

Grants awarded cover only part of the costs, and are intended to help meet local expenses. They should not normally cover more than 50% of the total cost of the activity. This requirement is considered essential in order to establish research activities on a regular and continuous basis in the institution.

The research projects should be of the close-end type and should produce results in a relatively short time. The purpose is to emphasize planning for the activity and to establish, as soon as possible, a feed-back mechanism that will hopefully improve planning for future activities.

RESULTS TO DATE

The approach used was highly successful in establishing the network of cooperating universities. Up to now, 25 agreements have been signed with 16 countries and established the basis of cooperation with about 35 engineering schools in the region.

In order to strengthen the network, greater emphasis was first given to short courses. The course subjects were selected by the schools themselves in consultation with the national interested agencies. In 1965, a total of 40 courses were offered covering a wide range of sanitary engineering subjects. There were more than 300 lectures and about 900 students.

These training activities are encouraging the necessary liaison between the university and national agencies which will provide the basis for applied research.

Some specific requests for research grants were received and are under consideration. Assistance is being provided in developing plans of operation before the award of grants.

Applied research projects in the following areas have been proposed: stabilization ponds, hydraulic flocculators, plastic pipes, water disinfection, water pollution surveys, algae in water supplies, ground water development, sewage treatment, and socio-economic evaluation of water supply programs.

SIGNIFICANCE

The project is primarily concerned with the establishment of sanitary engineering research as an organized, regular, and continuous activity in the cooperating universities. At the initial stage, emphasis will be put on research training, the actual results of the research projects being of secondary importance. However, through a careful selection of research projects closely tied to pressing national problems, the results are expected to prove useful from the very beginning.

OTHER DATA

Grantee: Environmental Sanitation Branch, Pan American Health Organization, Washington, D. C.

Funded by: Pan American Health Organization.

Timetable: 1961 - continuing.

INTER-AMERICAN PROGRAM OF EDUCATION AND RESEARCH
IN BIOSTATISTICS, HEALTH, AND POPULATION DYNAMICS

PROBLEM

The objectives of this research and training program are to expand the traditional field of health to include the interrelations of health and population dynamics; to disseminate knowledge on the process of population dynamics and its implication for health programs and activities among medical and other personnel in the health field; to develop a Latin American center to cooperate with and advise health institutions on matters of health and population dynamics; and to promote interdisciplinary research in health and population dynamics, making such studies available to the governments for intersectorial planning.

METHODS

A first four-month course in health and population dynamics, primarily for medical school faculty, is scheduled to start August 1, 1966. The course announcement has been distributed to medical schools, which will propose participants. Included are courses on the design and conduct of research and on methods of analyzing health and demographic data. The staff will include visiting professors from the Universities of Harvard, North Carolina, Princeton, and São Paulo. Guidelines for the teaching of demographic statistics in medical schools will be developed during the course.

In 1967, the four and six-month courses for specialization in biostatistics at the School of Public Health of the University of Chile will be supplemented by a basic course in health and population dynamics. There will be an additional course, either in health statistics, taught in the Department of Statistics, or in the design and conduct of research, coordinated by the Department of Research.

The 15-month course starting in March 1967 will thus permit greater flexibility in the selection of course subjects.

RESULTS TO DATE

The program for a four-month course beginning on August 1, 1966 is now being developed through consultants. A senior member of the faculty of the University of Chile visited population study centers in England and the United States between February and April 1966.

SIGNIFICANCE

This research training program will prepare medical school faculty in Latin America for teaching and research in health and population.

OTHER DATA

Grantee: Dr. Guillermo Adriasola, School of Public Health
University of Chile, Santiago, Chile.

Funded by: United Nations Technical Assistance Program,
World Health Organization/Pan American Health Organization.

Timetable: 1966-1970 (with probable extension).

RESEARCH AND TRAINING IN POPULATION DYNAMICS AND HEALTH

PROBLEM

Information on population size, distribution, and growth are basic to the fields of medicine and health, among others. The rate of population increase is a highly complex variable, dependent upon mortality as related to the availability of scientific and technical developments, to economic productivity, and to education; upon fertility as related to educational, social, and economic levels; and upon reproduction.

It is reasonable to assume, therefore, that developmental planning will view economic, social, and human advances as an interrelated process in which population trends are deserving of a most intensive and scientific survey.

The proposed center for studies in population dynamics and health at the University of São Paulo calls for research, teaching, and consultation in these fields, with courses to be given in the medical, health, social, economic, and mathematical sciences. Responsibilities for planning and organization within the university will be shared initially by the health and social sciences schools.

METHODS

The University of São Paulo plans to conduct one and two year programs for qualified students who wish to specialize in population. These programs will include basic courses required for all students and diversified and specialized courses designed to meet the needs of those with different backgrounds and interests. The graduates of one and two year programs will be qualified as teachers or research workers in demography or population dynamics in universities, institutions, or government departments. To provide students or professional staffs in other fields with knowledge and understanding of population will require the development of special short training programs.

It is expected that the university will give graduate degrees to those with satisfactory educational requirements for admission who successfully complete courses of one and two years' duration.

A course in population dynamics is planned in the School of Hygiene for the fourth quarter of 1966, and annual courses will be given thereafter. Four quarters in statistical methodology and demography are to be offered in 1967. The one-year program for population specialists is scheduled to begin in 1968.

At the University of São Paulo, the School of Philosophy, through its Department of Sociology, and the School of Hygiene and Public Health, through its Department of Applied Statistics, will cooperate in initiating the program. The Department of Sociology is large, consisting of approximately 30 sociologists. The Department of Applied Statistics has five full-time members, who are specialists in mathematical statistics, vital statistics, sampling, and biostatistics. Additional staff members are studying in the United States and Chile. All are unusually well qualified in statistical methodology.

The designated members of the staff will receive training during the first 18 months in the Department of Applied Statistics in São Paulo and in the United States to prepare them for their specific roles in the Population Center. Several persons are being considered for such training: a pediatrician to specialize in medical demography, a sociologist to study demography and its health applications, and an economist.

RESULTS TO DATE

An agreement for this program has been signed recently between PAHO and the University of São Paulo, and initial planning for faculty is under way.

SIGNIFICANCE

This research training program will prepare faculty for teaching and research in medical schools in Brazil and in other Latin American countries. As a result of it, courses on health and population will be conducted in other medical centers, and research will be encouraged.

OTHER DATA

Grantee: The Faculty of Hygiene and Public Health, University of São Paulo, São Paulo, Brazil.

Funded by: World Health Organization/Pan American Health Organization

Timetable: 1966-1970 (with probable extension).