Research in Progress 1968

A Summary
FOREWORD

This volume supplements and brings up to date as of 30 May 1968 the information contained in the publication *PAHO Research Activities, 1961-1966*. New research projects are included and current reports are given on studies that were under way at the time the previous summary was issued two years ago.

The problem, method, results to date, significance of the research, and publications deriving from the project are individually and concisely described in the case of each activity.

In the future, this information will be kept current and made available on an annual basis.

[Signature]

Abraham Horwitz
Director
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PROBLEM

At the present time only qualitative information about the nutritional problems of Central America and Panama is available. This survey proposes to establish such information in quantitative form for a representative sample of the population of each country.

It is expected to obtain data on the social, economic, educational, cultural, environmental, dietary, agricultural, clinical, oral, anthropometric, food production, food technology, and marketing factors that may affect the nutritional status of the population.

METHOD

A trained survey team, consisting of personnel from the host country, the next country, INCAP, and the NIH Office of International Research, collects all the needed data during the course of a two- or three-month intensive survey in each country. Physicians, dentists, nutritionists, anthropologists, biochemists, statisticians, food technologists, economists, nurses, social workers, and laboratory technicians are represented on the team. The survey includes clinical nutritional examinations; oral examinations; biochemical determinations on blood and urine; dietary surveys; sociocultural interviews; anthropometric measurements; tests for intestinal parasites; immunologic studies; bone age and density determinations; and a prevalence survey for diabetes and cardiovascular disease.

RESULTS TO DATE

The laboratory and statistical analysis of the data collected has been completed, and survey reports for each of the INCAP member countries are now in preparation. The reports will describe the nutrition problems that predominate in each country and will analyze their direct and indirect causes. They will also contain recommendations for immediate and long-term action to solve these problems.
SIGNIFICANCE

The quantitative determination of the nutritional problems of Central America and Panama will provide a base line for the evaluation of future applied nutrition programs. In addition, special problems encountered will be studied in greater detail at a later date whenever advisable. Pilot applied nutrition programs in areas of special need or interest will lay the ground work for regional or national programs to improve the nutritional status of the Central American population.

PUBLICATIONS


Individual country reports are in preparation.

OTHER DATA

Grantee: Dr. Guillermo Arroyave and 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: Guatemala
Guatemala City
El Salvador
Nicaragua
Costa Rica
Honduras
Panama

February - April 1965
August 1965
September - October 1965
January - March 1966
March - June 1966
September - November 1966
January - March 1967
PROBLEM

Severe protein-calorie malnutrition has been considered to be accompanied, in the great majority of cases, by a mild-to-moderate normocytic normochromic anemia. However, the nature of this anemia has been poorly understood, particularly since malnourished children from different areas of the world present other hematological alterations, possibly due to associated deficiencies of erythropoietic factors. The response to protein therapy and other specific hematinics has been unpredictable up to now and has not helped toward understanding the relative importance of protein deficiency in producing the "anemia" of protein-calorie malnutrition.

The purpose of this study is to ascertain whether the "anemia" observed in protein calorie malnutrition is a true anemia. In other words, since malnourished children have a markedly decreased active tissue mass, the amount of total circulating hemoglobin needed to fulfill the oxygen transporting requirements, although low, may still be functionally adequate. If this is the case, there is no anemia in uncomplicated protein-calorie malnutrition.

The study also intends to define the importance of protein and other hematinics in producing the hematological alterations that accompany severe protein-calorie malnutrition.

METHOD

Severely malnourished children were studied at the INCAP Metabolic Ward under various therapeutic regimens designed to evaluate the relative importance of protein, folic acid, vitamin E, vitamin B₁₂ and iron in the development of the hematological picture in severe protein-calorie malnutrition. The hematological status of the children was evaluated not only by the usual hematological techniques but also by relating the total circulating hemoglobin and red cell mass to the total lean body mass as measured by anthropometry and creatinine excretion.

RESULTS TO DATE

It appears that the severely malnourished child, unless there is complicating disease or blood loss, does not have a true anemia. The
total circulating hemoglobin and red cell mass are normal for the amount of lean body mass and active tissue mass that the malnourished child has.

The responses to protein and specific hematinics can then be interpreted as a dynamic equilibrium between increases in lean body mass, as a consequence of protein therapy, and a corresponding increase in red cell mass and total circulating hemoglobin.

SIGNIFICANCE

The present study will provide a basic understanding of the hematological picture in the malnourished child and, consequently, indicate the various steps needed to correct it. It will also provide information that will permit a clear interpretation of the hematological data obtained in populations with a high prevalence of chronic protein-calorie malnutrition.

PUBLICATIONS


OTHER DATA

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

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

Timetable: 1964-1968
STUDIES OF ANEMIA IN TRINIDAD AND TOBAGO

PROBLEM

Hemoglobin surveys carried out in various regions have shown that anemia constitutes a public health problem of considerable importance in the developing and tropical areas of the world. In the great majority of instances it is the iron-deficiency type. Indeed, lack of iron is probably even more widespread than figures for circulating hemoglobin would indicate.

In a comprehensive nutrition survey conducted by the Interdepartmental Committee on Nutrition for National Development in the West Indies in 1961, it was found that in Trinidad and Tobago 8% of all males and nearly 40% of the female adults had hemoglobin values below 12 grams per 100 ml. Although anemia was present in adult males, it was particularly prevalent among expectant and nursing mothers and among infants and young children.

METHOD

The subjects for the present study were obtained from different parts of the country and represented various age, sex, and racial groups in the population. For the purposes of the study, they were divided into the following categories: adult men, nonpregnant non-lactating women, lactating women, children up to 5 years of age, children aged 5 to 9 years, children aged 9 to 13 years, and adolescents aged 13 to 17 years.

After a full medical history and clinical examination of all subjects, the following constituents were 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 B\textsubscript{12}.

RESULTS

The findings showed widespread anemia: 30% of the population examined had hemoglobin levels below 12 grams per 100 ml; among children and adolescents, 27% of the subjects were below 12 grams per 100 ml; among pregnant women, 31% of the subjects were below 10 grams per 100 ml.
In general, the average serum iron levels were diminished: 15% had values below 50 μg/100 ml, and 43% below 50 μg/100 ml. The serum folic acids were significantly low in the entire group: values below 3 μg/ml were obtained in 15% of the population, and below 6 μg/ml in 65%. The serum vitamin B₁₂ levels were within normal range in most of the individuals investigated: values below 120 μg/ml were observed in 11% of the population, most frequently in males and pregnant women. Microcytic hypochromic anemia was found in 59%, dimorphic in 10%, and macrocytic hyperchromic in 6% of all the subjects found to be anemic.

SIGNIFICANCE

This study has helped to establish that iron-deficiency anemia is common in Trinidad and Tobago and that an increase in iron intake through the fortification of foodstuffs, such as rice and other cereals, is desirable. This alone will not overcome the anemia, but it will help to maintain normal hemoglobin levels once other measures have been taken to bring about an elevation.

It is no doubt difficult to change the dietary habits of people, but an education program carried out over the years in maternal and child health centers, schools, and community groups will produce results.

Further investigations are required to study the availability of iron and folic acid from local vegetable food, the effect of West Indian cooking methods on these nutrients, and the incidence of intestinal malfunction in severe anemias.

PUBLICATIONS


**OTHER DATA**

**Grantee:** Dr. J. G. Chopra, Pan American Health Organization, Washington, D.C.

**Funded by:** National Institutes of Health/U.S. Public Health Service and Pan American Health Organization

**Timetable:** 1964-1967
COLONIZATION OF THE INTESTINE OF CHILDREN BY MICROORGANISMS

PROBLEM

Among the factors that influence the nutrition of the host, diet and infectious diseases can be measured more easily than others. The colonization of the intestine by parasites, bacteria (including the indigenous microbiota), and viruses bears a direct relationship to the health and nutrition of the host. Even so, however, practically no information is available on this subject.

The hypothesis is that colonization of the intestine by microorganisms is related to the quality and quantity of food ingested and to local environmental conditions. This colonization is directly related to the incidence of diarrheal disease and to the general health and nutritional state of the host.

Specifically, the aim of the research is to study in children from a poor, semi-isolated village in the tropics the following developments from the time of birth onward over a period of years: (1) the progressive colonization of the intestine by viruses, bacteria, and parasites; (2) the relative proportions of various components of the intestinal microbiota; and (3) the relationship of colonization by microorganisms to the diet, nutritional status, and health of the child, with special reference to diarrheal disease.

METHOD

A cohort of 50 children recruited during 1964-1966, representing approximately half of all the children born during that period in the selected village, has been followed up to the present date. They have been studied clinically, anthropometrically, dietologically, and microbiologically.

The subjects are measured (height, weight, and head and chest circumference) at birth, fortnightly during the first year of life, and monthly thereafter. Clinical examinations are performed every two weeks. All disease episodes are investigated and recorded. The diet is recorded every week. The microbiological studies include weekly fecal examinations for parasites, bacteria and viruses. All information is entered on precoded forms for later analysis with IBM equipment.
RESULTS

The studies of colonization of the intestine by microorganisms conducted in this Institute have indicated (1) early appearance of enteroviruses, *Candida, Shigella,* and pathogenic protozoa in the intestinal tract in infancy; (2) rapid colonization of the intestinal tract with lactobacilli, entero-cocci, and gram negative bacilli, to reach high levels \(10^9 - 10^{10}\) within the first two to three days of life; (3) persistence of *Shigella* and other enteric pathogens after a clinical attack in many children during the weaning period, particularly those exhibiting chronic recurrent diarrhea; (4) high incidence of diarrheal disease, of which approximately a fifth is chronic (15 days or more duration); and (5) significant association between high incidence of diarrhea and other infectious diseases and poor growth and nutrition.

Attack rates of diarrheal disease are high in the first three months of life, increasing progressively to reach the highest level at the end of the first year and throughout the second year of life. The rise is coincidental with the loss of maternal immunity, the increased exposure to the unsanitary environment, and the weaning process. Most diarrheas are acute, but the chronic recurrent type is unexpectedly frequent, representing about 20% of all episodes. No difference in frequency of infectious agents has been observed in the acute or the chronic form.

Potentially pathogenic agents (coxsackieviruses, other enteroviruses, *Shigella,* and pathogenic parasites) are more frequent among cases than among matched controls to a significant degree of difference. No agents have been found in about 40 per cent of all episodes.

SIGNIFICANCE

Such a close longitudinal observation of underprivileged children from birth to three years of age, with extensive studies of infectious agents, disease morbidity, and growth and development, is unique. It has already contributed significantly to the understanding of the interaction between malnutrition and infection in similar populations and has important implications for public health programs directed towards infants and young children.

The longitudinal observation of colonization of the intestine by viruses, bacteria, and parasites and of changes in the intestinal bacterial flora permit a better understanding of this basic phenomenon and of its relation to nutrition and health, especially diarrhea. The knowledge that will be gained will be fundamental in the understanding of the etiology of diarrheal disease.
This information is basic to the establishment of adequate preventive measures in areas of low socioeconomic development with poor sanitation and nutrition. Diarrheal disease is by far the most frequent cause of death among small children in underdeveloped areas. Its high prevalence contributes to the poor nutritional status of these populations, which in turn aggravates morbidity and mortality due to diarrhea and other diseases.

The study is particularly significant because it has been conducted in the field without removing the subjects from their environment and without notably disturbing the ecosystem.

PUBLICATIONS


OTHER DATA

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

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

Timetable: 1962-1968
POSSIBLE EFFECTS OF AUROFAC ON THE INTESTINAL FLORA
AND ON GROWTH OF CHILDREN

PROBLEM

The over-all purpose of this investigation is to demonstrate the effect of Aurofac, when added to the diet, on the growth of children, and to ascertain whether the effect is mediated through changes in the characteristics of the intestinal microflora. The specific objectives are to determine the development of the intestinal microflora of breast-fed children of low socioeconomic status; to study whether the administration of Aurofac under a controlled situation has any effect on the components of the intestinal microflora; and to determine whether Aurofac has any demonstrable effect on the growth of children during the first year of life.

METHOD

Fourteen children of low socioeconomic status were recruited at birth in an Indian village of Guatemala. All children are breast-fed and live under poor sanitary conditions. The infants are measured (height, weight, head circumference) at birth and every two months during the first year of life. A physical examination is made concomitantly. All disease experiences are properly studied and recorded. Dietary records are prepared at weekly intervals.

Seven children were picked at random for administration of Aurofac syrup by mouth beginning at two months of age. No side effects have been recorded.

Examination of the intestinal microflora is made by means of weekly fecal cultures. Most bacterial groups - aerobic, microaerophilic, and anaerobic - are investigated quantitatively. Feces are also examined for the common intestinal parasites, enteric bacteria and viruses.

RESULTS TO DATE

Breast-fed children born and living in a heavily pathogen-seeded environment are rapidly colonized by aerobic and anaerobic bacteria. Anaerobes outnumber the aerobic flora by one to three. The predominant flora consists of bifidobacteria \(10^{11} \text{ to } 10^{12}\) bacteria per gram of wet feces), whereas gram negative aerobic bacilli (Enterobacteriaceae) reach
titers of not more than \(10^9\) to \(10^{10}\) per gram. Children with this flora rarely become infected with *Shigella* or other pathogenic agents. When infections occur, the tendency is to spontaneously eliminate the pathogen. This evidence points to a possible defense mechanism provided by the particular intestinal flora developed as a result of breast feeding.

No results on the effect of feeding with Aurofac are yet available because of the recent initiation of these studies.

**SIGNIFICANCE**

There is solid experimental evidence that Aurofac, a low-cost by-product in the fermentation of chlortetracycline, has a growth-promoting effect in several animal species, including man. Until recently, the growth-promoting capacity of Aurofac was thought to reside on the small amounts of antibiotic remaining in the product, which probably control undesirable effects of fermentative intestinal bacteria.

Additional experiments have shown that Aurofac improves growth even after destruction or neutralization of the antibiotic capacity. It is important to test whether Aurofac has any effect on the growth of the children in the critical first year of life, and to see if any such effect is accompanied by changes in the characteristics and composition of the intestinal flora.

**PUBLICATIONS**


**OTHER DATA**

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

Funded by: American Cyanamid Co.

Timetable: 1966-1968
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 that in preschool children lack of nutrients at various levels will produce modifications in physical growth and mental development. Accordingly, the project, working with an undernourished population of preschool children, set out to perform the following tasks:

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 relationship 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.

After numerous schemes and approaches were tried, it was decided that the individual state of nutrition could not be properly defined and quantified. However, the following design is being applied to evaluate the nutritional condition and mental development of individuals in the populations under study:

"In the experimental villages, habitual family diet plus food supplementation should cover protein-calorie-vitamin needs to make adequate physical growth possible and probably to bring about changes in mental development."
METHOD

The experimental design calls for comparing two groups of children six 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.

Owing to certain growth characteristics of the central nervous system, it is necessary to supply required nutrients during critical periods - namely, pregnancy and the first two years of extra-uterine life. Based on these general principles, food supplementation is directed principally toward the pregnant woman, the lactating mother, and the child in its first two to three years of life. On the basis of findings in the populations under study by the Dietary Surveys Section, the habitual diet covers approximately 50-70% of nutritional needs. It is therefore necessary to provide the rest by means of food supplementation. However, if the stress factor, due principally to contagious and emotional diseases, is taken into account, over and above the existing nutritional deficit, it may be found necessary to provide at least 130% of the allowances recommended by INCAP/FAO (INCAP Scientific Publication 5:75, 1966) for the three groups under study.

In the experimental populations, preschool children, pregnant women, and lactating mothers receive a daily food supplementation that provides 130-150% of the recommended needs. The results are compared with similar populations subsisting on their habitual diet.

These children and all women of child-bearing age will be followed longitudinally with demographic studies, dietary surveys, clinico-nutritional examination, neurological testing, anthropometric measurements, psychological testing and biochemical determinations.

RESULTS TO DATE

Surveillance of over 250 rural villages has been conducted in order to find proper research sites. In twenty of these, which appeared to fulfill most of the basic requirements for the study, a general population census has been performed; in ten, a cross-sectional anthropometric survey of parents and preschool children has been conducted. The methodology is being tested and perfected and team members of each discipline are being standardized for data collection.
Several preliminary cross-sectional studies of different communities have been conducted. Thus, the groundwork is nearly laid for the definitive longitudinal studies.

SIGNIFICANCE

All available data show that great numbers of children from developing countries are subject to varying 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 the populations under study during most of the preschool years when children's growth rates are high. As a consequence of this interaction, many investigators have shown that the physical growth, mental development, and maturation of children at this age are being seriously impaired for an unknown period of time and are likely to be permanently arrested. The proposed study will attempt to determine and quantify the relative effect 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 hypotheses are correct, it is mandatory to apply immediate measures in this area to prevent protein-calorie malnutrition as well as infectious diseases during the preschool age, in view of present difficulties in obtaining proper amounts of good quality animal proteins for all people in general in the developing nations.

It is expected that the results from this study will emphasize the importance of developing inexpensive protein in sufficient amount to meet the physical growth needs of preschool children and to prevent the onset of mental retardation among the millions of children now being exposed to the dangers of undernutrition.

PUBLICATIONS


OTHER DATA

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

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

Timetable: 1964-1970
THE INFLUENCE OF NUTRITIONAL STATUS ON
PHYSICAL WORKING CAPACITY

PROBLEM

The purpose of the present investigation is to obtain scientific data regarding body composition and physical working capacity in the natural environment of young adult males with different nutrure and to study cardiovascular, respiratory, and metabolic responses to standardized work situations in a physiology laboratory. From this research, the relative importance of nutritional background, present dietary intake, and physical exercise regimen as determinants of body composition and capacity to perform physical work is being studied. The calorie and protein intake necessary to maintain adequate protein nutrition in terms of normal body composition and nitrogen equilibrium is also being explored.

METHOD

Groups of young adult males from different socioeconomic groups, whose dietary intake is being measured, are studied in their own environment on the basis of time-motion records. Energy cost of work is also determined in these subjects in order to obtain total caloric expenditure, caloric intake, and caloric balance. Insensible weight loss and total sweat is also determined, as well as nitrogen balance. In the laboratory, body composition is determined by means of anthropometry, hydrometry, body density determinations, and oxygen consumption. The subjects are also studied as they perform submaximal and maximal exercises on a treadmill.

RESULTS TO DATE

People who consume between 60 and 110 grams of protein per day have essentially normal lean body mass for height. Caloric intakes ranging between 3400 and 2800 a day seem to determine the degree of physical exercise that the people in a given population group perform, since those with a higher intake, when allowed to work in the field ad libitum maintain the same degree of adiposity as those who consume low-calorie diets. However, if these people consume more calories but have a restricted physical exercise regimen, they become relatively obese.
Sweat appears to be a very important avenue of nitrogen loss in males who engage in a high degree of physical activity. Nutritional background seems to be of little importance in determining body composition and physical working capacity. The effect of improved nutrition — namely, increase in both caloric and protein intake — appears to be similar to that of athletic training among people who develop a high degree of physical activity to maintain caloric balance, since their lean body mass increases and their ability to perform work before exhaustion is also increased.

SIGNIFICANCE

These studies will provide the first scientific data on the relative importance of nutritional background, present nutritional intake, and physical exercise regimens on body composition and capacity to perform physical work. As a consequence, recommendations on dietary intakes for young adult males, based on optimum body composition and maximal working capacity, will come out of this study.

PUBLICATIONS


OTHER DATA

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

Funded by: U.S. Army Medical Research and Development Command

Timetable: 1964-1968
The investigation is concerned with the relative importance of protein and calorie deficiencies in producing the functional alterations in children that lead to the well-known clinical pictures of protein-calorie malnutrition (PCM). It is also intended to study the process of nutritional recovery in functional terms.

Specifically, the research will attempt to quantify the magnitude of protein and calorie deficiencies and to correlate their various degrees of severity with functional impairment in the areas of erythropoiesis, intestinal absorption, and endocrine function.

Malnourished children are admitted to the INCAP Metabolic Ward where a quantitative estimation of their degree of protein and calorie deficiency is obtained by means of clinical, biochemical, and physiological techniques. The children are then submitted to various dietary regimes that allow the researchers to judge their recovery in terms of protein and calories in a quantitative way. The main tools used for this purpose are physical anthropometry, nitrogen balance, and the creatinine-height index. The functional exploration of the erythropoietic system is investigated by means of the usual hematological techniques plus the determination of total circulating red cell mass and hemoglobin.

Intestinal absorption has been explored by means of specific absorption tests for glucose, D-xylose, total fat, purified $^{131}$I triolein and oleic acid, vitamin A palmitate, and total nitrogen. The fate of the absorbed glucose and fat has been studied by means of intravenous glucose disappearance tests, lipid radioactivity patterns in plasma, and inorganic $^{131}$I excretion in urine. Nitrogen retention has also been measured.

Endocrine function has been measured by means of 17 hydroxysteroid excretion before and after the administration of Metapyron and ACTH. Cortisol production and turnover has been measured by means of the administration of $^{14}$C-cortisol, determination of plasma cortisol specific activity, and excretion of tetrahydro cortisol and tetrahydro cortisone. The endocrine factors involved in utilization of glucose and liberation of fatty acids have been explored by means of responses to intravenous
glucose, insulin, and epinephrin administrations. Blood glucose and free fatty acid levels have been estimated in serial samples after the administration of each one of these substances.

RESULTS TO DATE

With respect to the quantitative characterization of protein and calorie deficiency, it has been found that severely protein-calorie malnourished children of the kwashiorkor of kwashiorkor-malasmus type have a deficit in lean body mass ranging from 30 to 70% when brought to the hospital. Marasmic children, on the other hand, present extreme caloric deficiency, though the creatinine-height indices ranging from .75 to .85 would indicate that the protein mass can be preserved. These marasmic children have normal serum proteins and albumin. Other groups of marasmic children might show differing degrees of protein depletion, since the creatinine-height indices can be as low as those observed in kwashiorkor. From these studies it has been quantitatively established that both kwashiorkor and marasmic children form a continuum in terms of both calorie and protein nutrition. Upon nutritional recovery the kwashiorkor child replenishes his caloric nutrition at a fast rate (judged by weight for height), whereas protein nutrition takes a long time to recover despite the attainment of normal serum proteins and albumin within two to three weeks of therapy. Marasmic children, on the other hand, can replete their protein nutriture at a faster rate than their caloric nutriture.

It has also been established that intestinal malabsorption occurs in children who show more than 20% lean body mass deficit and do not return to normal until their protein nutriture is replenished, as evidenced by creatinine-height indices above .80. Fat absorption and glucose absorption are mostly affected.

The hematological alterations in protein-calorie malnutrition are primarily adaptative, since it appears that the primary determinant for the total red cell mass is the amount of active tissue mass, independent of hemoglobin concentration. This is primarily due to a smaller decrease in plasma volume.

Both kwashiorkor and marasmic children appear to have adequate hypophysary and adrenal functional reserve. On the other hand, the utilization of glucose is delayed, which suggests a deficiency of insulin production.
SIGNIFICANCE

From these studies a dynamic nutritional picture has been obtained by which the changes leading to the various clinical syndromes of severe protein-calorie malnutrition can be understood. This also applies to the changes observed during nutritional therapy in protein-calorie malnutrition.

The importance of various degrees of protein and calorie deficiency in terms of functional impairment has been studied and defined for the first time. Protein malnutrition produces alterations in intestinal absorption that are intimately correlated with the degree of defect in lean body mass. The so-called "anemia" of protein-calorie malnutrition seems to be primarily an adaptation phenomenon. There is little evidence that primary abnormalities in adrenal function due to protein deficiency are primarily responsible for some of the clinical characteristics of the malnourished child. It appears that endocrine function is altered as an adaptation phenomenon to protein and calorie deficiency.

PUBLICATIONS


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: 1956-1967
METABOLIC ADAPTATION TO NUTRITIONAL STRESS

PROBLEM

In spite of continuous efforts to better evaluate the characteristics of protein-calorie malnutrition, little emphasis has been placed so far on the study of the essential biochemical mechanisms that are altered and that may eventually hinder normal development in children. A biochemical handicap developed during chronic or acute malnutrition may quite possibly be overshadowed by apparent physical improvement during recovery, and even when such children are clinically cured they may still present drastic biochemical alterations. It is considered of the utmost importance to gain more knowledge on the biochemical adaptive changes that develop during protein-calorie malnutrition. Once this information is gathered, better evaluating procedures can be developed for diagnosis and treatment.

The principal aims of this investigation are (1) to study the adaptive enzymatic changes produced by protein-calorie malnutrition in exudate polymorphonuclear leucocytes obtained experimentally from children suffering from either kwashiorkor or marasmus; (2) to relate the enzyme patterns observed to those produced in experimental animals subjected to conditions designed to reproduce the same types of malnutrition as observed in human beings; (3) to develop practical evaluating procedures for a more accurate characterization of the nature and severity of the different types of protein-calorie malnutrition; and (4) to separate the biochemical changes observed in uncomplicated malnutrition from those due mostly to infection interactions.

METHOD

Exudate polymorphonuclear leucocytes are obtained from malnourished or completely recovered children using the technique of Boggs et al. (Amer J Pathol 44:61, 1964), as modified in our laboratories.

Enzymes responsible for amino acid oxidation, glycolysis, and respiration are measured in whole cell homogenates and also in subcellular fractions obtained by differential ultracentrifugation after breakage by sonification. The specific enzymes being studied are aldolase, isocitric and malic dehydrogenases, aconitase, fumarase, glutamic-oxalacetic and glutamic-pyruvic transaminases, and the lysosomal enzymes - acid phosphatase, B-glucuronidase, RNA'ase, and DNA'ase.
RESULTS TO DATE

In comparing the distribution of enzyme activity in subcellular fractions of PMN-leucocytes, it has been found that fumarase is 100% in the cytosol both in kwashiorkor and in normal cells, with significantly higher activity in kwashiorkor. Cells obtained from marasmic children have the enzyme distributed throughout all the different compartments, and show a total activity below the normal level.

Aconitase varies in exactly the same manner as fumarase. Isocitric and glutamic dehydrogenases are significantly lower in kwashiorkor and higher in marasmus. Aldolase, the enzyme used as an indicator of glycolysis, is also increased in marasmus but not in kwashiorkor.

In summary, it may be said that most of the enzymes studied so far show opposite activity for kwashiorkor and for marasmus.

SIGNIFICANCE

The variations discovered in enzyme activity provide a starting point toward the development of new and more dynamic evaluating techniques, which could be adapted to field studies by using PMN-leucocytes derived from blood by differential sedimentation.

The differences observed with respect to lysosomal-related enzymes, at least in the case of kwashiorkor, may indicate some degree of physical alteration in the lysosomes. Such changes could account to a certain extent for the inability of these cells to cope with the stress of phagocytosis and thus for their metabolic handicap during active infections.

It is believed that with further work in this area a practical and sound system for diagnosis and monitoring during recovery will be at hand, which will undoubtedly improve our knowledge of the basic metabolic phenomena associated with different types of protein-calorie malnutrition.

PUBLICATIONS


OTHER DATA

Grantee: Dr. Oscar Pineda, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

Funded by: Nutrition Foundation, Incorporated

Timetable: 1966-1968
AMINO ACID METABOLISM IN CHILDREN

PROBLEM

The limited availability of protein in adequate quantity and quality is a universal problem that is receiving increasing and renewed attention throughout the world. The literature, both scientific and popular, contains many references that define the problems and suggest logical solutions. Among the solutions proposed are the improvement of the protein quality of cereal grains by the addition of the limiting essential amino acids and the development of formulated protein-rich food mixtures. Such proposals have promoted increased activities in this field of research.

Protein malnutrition is a serious health problem today among children in developing countries. There is an urgent need to learn how the foods that are available can be improved nutritionally without inducing other deficiencies and how useful reference patterns for formulating new foods or improving others can be developed. More needs to be known about the nutritional significance of deficiencies, excesses, and the balance between essential and nonessential amino acids.

METHOD

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 protein intake as a means of protein quality evaluation of formulated foods, and of altering the ratio of essential to nonessential amino acids in specific foods.

RESULTS TO DATE

The protein quality of opaque-2 corn protein has been determined in children. The results indicate that opaque-2 corn protein fed at 1.5 g protein/kg/day gives nitrogen retention values slightly lower than those obtained from equivalent amounts of skim milk protein but significantly higher than those from common corn, even when the latter is fed at increased levels of intake. The reason for this is that opaque-2 corn has higher lysine and tryptophan levels than common corn, which are the limiting amino acids in the protein of this cereal grain.
Studies have also been carried out to determine the effect of adding nonessential amino acids to skim milk. At a protein intake level of 2 g/kg/day no effect in decreasing nitrogen balance resulted, but a definite effect in decreasing nitrogen retention was observed when nonessential amino acids were added to skim milk protein fed at an intake level of 1 g/kg/day.

Similar results have been obtained in studies of dogs and rats that were fed other protein sources. The effect observed is conditioned by the quality of the protein being studied and the level of protein fed. A greater effect in decreasing protein quality is obtained from inorganic nitrogen than from nonessential amino acid nitrogen.

SIGNIFICANCE

The ultimate objective in research programs of this nature is to obtain information that can be applied to the establishment of protein requirements and reference amino acid patterns and to the development of protein foods of better quality.

PUBLICATIONS


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and Nutritive Value of INCAP Mixture 15, Based on Soybean and
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8. Bressani, R. "La calidad protéica del maíz con gen opaco-2." 
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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: 1960-1967
ELECTROLYTES IN PROTEIN-CALORIE MALNUTRITION

PROBLEM

Severely protein-calorie malnourished children have an associated potassium depletion. The purpose of this work is to try to ascertain the importance of protein in inducing this depletion.

METHOD

Malnourished children are admitted to the INCAP Metabolic Ward and kept on a maintenance protein diet while potassium and other electrolytes are administered in the required amounts. After 10 to 12 days, the protein intake is increased to therapeutic levels. Muscle biopsies are obtained during both phases of treatment and potassium is measured in intracellular and extracellular water.

Total muscle mass is measured by creatinine excretion, and total muscle potassium is obtained by multiplying the muscle potassium concentration times the total muscle mass.

RESULTS

The results indicate that, in the absence of a protein intake that will increase muscle mass, potassium administered during the treatment of malnourished children does not go into muscle until protein is provided in amounts sufficient to induce a replenishment of the muscle mass.

It appears that the total muscle-binding sites are decreased in protein-calorie malnutrition and that these must be brought back to normal with protein in order to obtain potassium repletion.

SIGNIFICANCE

The present investigation will provide an understanding of why potassium depletion occurs in protein-calorie malnutrition, and it will indicate the steps necessary to correct this phenomenon. Furthermore, it will provide bases for understanding the problems observed in potassium metabolism during the treatment of severely malnourished children.
PUBLICATIONS


OTHER DATA

Grantee: Dr. Jorge Alvarado, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

Funded by: Baylor University

Timetable: 1967-1969
RESEARCH UNDER THE DIVISION OF AGRICULTURE AND FOOD CHEMISTRY

PROBLEM

Inefficient food production, in the face of rapid rates of population growth, is creating a situation in Central America that requires immediate attention. INCAP has been well aware of this situation for many years, since the Division of Agriculture and Food Science was created for the purpose of applying science and technology to the solution of this problem. The need for agricultural research on the nutritional problems of these countries and the application of the results continues.

METHOD

A wide variety of research projects have been carried out in the areas of food crops, industrial raw materials and their by-products, animal nutrition, processing of oil seeds, and the improvement of the quality of foodstuffs.

RESULTS TO DATE

Evaluation of the protein quality of opaque-2 corn in rats: Opaque-2 corn fed raw and as lime-treated corn produced excellent growth in rats and had protein efficiency ratio values only slightly inferior to those from casein. Its performance was about 2.5 times greater than that from common corn.

Use of opaque-2 corn in vegetable protein mixtures: The various mixtures developed at INCAP contain 58% corn in their formulations. It was therefore of interest to learn whether the protein quality of such mixtures would increase by using opaque-2 corn for common corn. The results indicated a definite improvement only when the protein concentrate of the mixture was low in lysine. Therefore, Mixture 9 (containing 38% cottonseed flour) was improved in quality when mixed with opaque-2 corn, but Mixtures 14 and 15 (containing soybean flours) were not.

Studies on the chemical composition and use of shrimp meal: It was considered that the shrimp meal by-product of the relatively large quantities of shrimp being exported by the Central American countries might be of use locally. Chemical analysis indicated this material to be high in protein and lysine. Studies were carried out to determine
whether it could be used in poultry rations to replace fish meal. Because of its quality, the results showed that it can replace between 20 to 30% of the fish meal without any decrease in the performance of the poultry.

**Effect of various nitrogen and phosphorus fertilizer levels on the nutritive value of beans:** Various levels of N and P fertilizer caused changes in the yield of beans and in the nutritive value of the protein. The results need to undergo further statistical analysis before any recommendations can be made.

**Quality of immature corn:** The protein quality of immature corn has been studied by chemical and biological techniques. Corn soon after the milking stage has more lysine, tryptophan, and methionine than mature corn. Its protein quality is about twice as high as that of mature corn.

**Other studies conducted:** The value of various methods for determining the protein quality of cottonseed flour; vegetable protein mixtures for human consumption; development of INCAP vegetable Mixture 17 based on leguminous seeds; amino acid and protein supplementation of cottonseed flour protein concentrate.

**SIGNIFICANCE**

Because of the nature of the grant supporting the investigations reported, the Division's studies were providing information of practical value to the development of the countries of the area. Furthermore, many of them were carried out by university students under the guidance of the professional staff of the Division. These students were exposed for the first time to the meaning of research and the benefits that it brings to the welfare of people and of a country in general.

**PUBLICATIONS**


**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

It is already a well-accepted fact that in order to be able to properly feed the population of the world of today and of the future, foods other than the conventional ones must be developed and tested. Many such foods have been tested chemically and biologically and some are already in use - for example, flours from cottonseed, soy bean, and sesame. Microbial proteins, such as yeast, have also been used to a limited extent. For example, INCAPARINA formulations contain 3% torula yeast, which supplies both protein and vitamins to the mixtures. Protein sources of this type could make an even greater contribution toward solving the problem, but their use is limited by the lack of sufficient nutritional and toxicological information, particularly since they are being produced from different media.

Protein WO-100 is a microbial protein source produced from petroleum. Very little information on its nutritional quality and potential is available.

The purpose of this investigation, therefore, is to obtain sufficient information so that its use can be extended in human nutrition.

METHOD

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

The following biological methods have been employed: 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; long-term feeding tests and gross pathological and biochemical studies; and supplementary value tests of cereal proteins in relation to other vegetable proteins and mixtures.

RESULTS TO DATE

Several rat feeding experiments have been carried out with material produced over the period 1964-1966. The quality of the material was poor,
and adverse physiological factors were found to be present.

Four studies have been completed with a new batch of WO-100 protein produced in 1967. The results show that its protein quality is higher than that of previous batches but still quite inferior to casein. The limiting amino acid in such protein is methionine, which when added to WO-100 protein in an amount of 0.2% of the diet significantly increases the protein quality of the product.

The new batch caused allergic reactions - skin rash and excitement of the respiratory system - among people handling the material.

SIGNIFICANCE

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

PUBLICATIONS

None, because of the nature of contract between INCAP and the company making WO-100.

OTHER DATA

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

Funded by: ESSO Research and Engineering Company

Timetable: 1965-1968
NUTRITION SERVICES FOR THE EDUCATION OF MOTHERS AND RECUPERATION OF MALNOURISHED CHILDREN

PROBLEM

Protein-calorie malnutrition among preschool children is one of the most serious problems in Guatemala. The scarcity of hospital beds for the care of acute cases, and the little success met by the Health Centers in preventing these cases, has led to this study of the possibility of establishing Nutritional Services attached to the Health Centers as another approach to the prevention of protein-calorie malnutrition. These services, operating on a daily basis, have two main purposes: to treat moderate cases of malnutrition, and to educate mothers in child feeding methods.

The project has the following specific aims: (1) to establish nutritional services in different areas of Guatemala in order to determine whether or not such services are effective and practical and whether they merit further extension, (2) to seek possibilities for local support of these services in order to guarantee their permanent operation, (3) to study the effectiveness of such services in the recuperation of malnourished children, and (4) to ascertain whether the methods used at present for the education of mothers are effective in changing current feeding practices.

METHOD

Practical measurements of the children in the study were taken at the level of the Health Centers, and certain clinical signs were investigated. Height and weight were taken when they first attended the service and at the end of their stay. Improvement in these measurements and disappearance of clinical signs were used to evaluate the benefits of the services.

To investigate changes in feeding practices resulting from the education of the mothers, a follow-up of children four months after their discharge from the service was arranged. Their height and weight were compared with their measurements when they left the service.

Finally, the possibility of obtaining local support for these services was determined by the growth of new services and by the help given at the community level.
RESULTS

At the end of December 1966, 12 services were in operation, with a daily attendance of 300 children. As of the date of the present report, the number of services had increased to 17, with a daily attendance of 510 children.

Expected normal increments in height and weight were compared against the real increments in these measurements over a four-month period while the children attended the services. During the year 1967, the average real weight gain among 354 children studied was 1.20 kgs, constituting a difference of 0.52 kgs over the average expected gain of 0.68 kgs. The average height increase was 3.00 cms, or 0.90 cms more than the expected 2.10 cms.

By the way of follow-up, the height and weight of 129 children were investigated over a period ranging between four months and one year after their departure from the services. According to the results, 55.8% of the children gained more than their expected weight increase, 25.6% experienced normal weight gains, and 18.6% failed to gain the amount expected for the corresponding age increase. With regard to height, 63.5% grew more than the expected amount, 29.5% had normal height increases, and 7.0% grew less than the expected amount.

SIGNIFICANCE

This work must be classified as a type of operational research. The results, if they are positive, could contribute to a decrease in morbidity from kwashiorkor and marasmus. At the same time, they may help to the decrease of the high mortality rates among children from one to four years of age.

Furthermore, the education of mothers may help to prevent new cases of protein-calorie malnutrition in their other children.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Dr. Romeo de León, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

Funded by: Williams-Waterman Fund

Timetable: 1965-1971
FEED COMPOSITION TABLE, WITH INSTRUCTIONS FOR ITS USE IN FORMULATING ANIMAL RATIONS

PROBLEM

During the last 10 to 15 years significant advances have been made in animal husbandry, particularly in the poultry industry, in the Central American countries. The two main problems at the moment have to do with the quality of the various components of the diet, and the relatively high cost of the specific rations being used. The latter is particularly serious for the swine industry, in which sources of both protein and calories are involved.

There is need, therefore, not only to know about the variability in the chemical composition of the ingredients being used but also to seek, through chemical analysis, ingredients that can replace others that are either costly or available in limited amounts. Furthermore, feed manufacturers, as well as farmers and students in agricultural sciences, need a reference feed composition table, which is not yet available for the animal industries in Central America.

METHOD

The procedure followed in this program has been to collect representative samples of all the ingredients used in the formulations of animal rations. Such samples have been collected in each of the Central American countries. Together with the sample, information is obtained, for purposes of standardization, on the availability of the product, how it is prepared, amounts used in the formulations, and the producer(s)' definition of the product.

When the sample arrives at the laboratories it is analyzed for its proximate composition, using official methods, and for Ca, P, and iron. The products are also analyzed for thiamine, riboflavin, niacin, carotene, and vitamin A. If the product constitutes a significant portion of the animal's diet, or is a protein source in the ration, it is analyzed for its essential amino acid content.

In some cases, feeds have been tested on poultry or swine to determine the possibilities of their use as substitutes for other components that are either costly or not easily available.
RESULTS TO DATE

During the last two years, approximately 500 samples have been analyzed, including forrage crops, by-products of the cereal industry, by-products of the oil seed industry, and by-products of various other industries. Samples of various cereal grains have also been analyzed, as well as other sources of carbohydrates, such as banana, yucca, molasses, and the like.

Approximately 30 products have been analyzed for their essential amino acid content.

A preliminary feed composition table has been prepared covering all the products analyzed so far. There are some, however, that need additional chemical analysis, which is presently being carried out. A limited number of feeding tests have been performed with poultry and swine. There is a need for more intensified research in this field. Each experiment takes time, and at present only a limited number of people are involved in the project.

SIGNIFICANCE

The availability of tabulated data on the chemical composition of the rations used in the animal industries will be of great help to both commercial producers and to stock raisers who mix feeds with their own facilities. It is hoped that with such data in hand the producers of the various feed ingredients will attempt to standardize their by-products according to chemical composition, thus offering to the consumer a product of guaranteed standard composition and making for more efficient feed preparation as well.

The information will also permit the exchange of products in a formulation on the basis of gross chemical composition, and on amino acid composition in the case of protein. The feeds will, therefore, be more efficiently utilized by the various animals consuming them.

Finally, the table will serve as a reference to evaluate new ingredients being placed on the market, as well as those animal rations that are now based on tables from other areas and countries.
PUBLICATIONS


OTHER DATA

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

Funded by: U.S. Agency for International Development/Regional Office for Central America and Panama

Timetable: 1966-1968
LOW-COST FOOD FOR CALVES (TERNERINA)

PROBLEM

The present-day practice in the dairying areas of the Central American countries is to slaughter young calves when they are between 3 and 10 days old. This results in a great economic loss, since the utilizable and edible sections of the carcass are relatively small. Although the young calves could be fed imported milk replacers, the cost would be too high to make the practice profitable. Even female calves to be kept as replacement of cows do not perform well enough with these imported milk replacers to justify their use. Requests were therefore made for INCAP to develop a feeding system using economical milk replacer formulations of sufficiently high nutritive value to enable calves to grow and attain ages and weights at which their product can be more efficiently utilized. In order for such foods to be economical, they should be made from locally produced materials. Moreover, it is important that they be applied in such a way that they can be utilized as sources of nutrients by the calf. In the early days of its life, the animal is still monogastric and cannot digest foods of vegetable origin. Once the rumen develops at about five to seven weeks, coarse foods of lower protein content and nutritive quality can be fed to them.

METHOD

Formulas were developed on the basis of biological tests carried out with young growing rats, using materials available in the area. In view of the limitations of the animal's digestive system, the cost of feeding, and other considerations, four types of formulations were developed. The first group (four in number) were defined as milk replacers and consisted of formulations with a minimum of 30% skim milk powder. The second group (also four in number) were defined as supplements and were formulated with less than 30% skim milk. The next group, to be fed at a later age, were defined as concentrates. The last group were the finishing rations.

Starting at 3 days of age, the calves were fed 57 liters of milk over a period of 22 days. From the 11th to the 63rd day they were given a milk replacer, along with a 20% protein concentrate. Two finishing rations were then fed for 16 weeks, after which time the animals were free to go to pasture.
The quality and benefits of the four types of formulations were tested on the basis of weight gain in various groups of calves, using whole milk as control. In some studies blood parameters were used. Digestibility trials of the formulations were also carried out.

RESULTS TO DATE

After intensive experimental work, several feeding systems were studied as well as the nutritional quality of the various foods developed.

Weight gains with limited amounts of whole milk, the replacers, and the various supplements ranged from 0.5 to 0.7 kg/day. These values did not differ significantly from whole milk feeding alone. With the concentrates fed at 63 days of age for 16 weeks, gains as high as 1.5 kg/day were recorded.

In general, the results have been very satisfactory, but there is need to carry out additional studies to upgrade the quality of the milk replacer formulations and to improve the feeding system so that it can be applied more efficiently. Also the system needs to be tested in female calves.

SIGNIFICANCE

The practical application of research on the formulation and development of milk replacers for calf feeding is of great importance for the economic and nutritional development of the area. These formulations will permit the meat from calves to be more efficiently utilized for export or for local consumption. Furthermore, if the system proves to be effective for female calves, their production costs will be reduced. The use of milk replacers will also make milk available to the population that would otherwise be used to feed female calves.

PUBLICATIONS

OTHER DATA

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

Funded by: Government of Costa Rica

Timetable: 1966-1967
PROBLEM

The purpose of this project was to explore the hygienic condition of some of the food products, including ice, manufactured and sold in the area of Azul, Province of Buenos Aires, Argentina. As an indication of the sanitary state of these products, samples were collected and examined for the presence of enterobacteria and *Staphylococcus aureus* from several fresh and cured products commonly consumed in the area.

METHOD

The following food products were examined: ice cream manufactured in Azul and in Buenos Aires, ice blocks, cooked ham, pasteurized butter, cheese, and beef.

In the case of each product, 22 samples were tested for the presence of bacteria. The ice cream came from five manufacturers and ten stores in Azul and from three manufacturers in Buenos Aires. An assortment of 16 ice blocks was tested. The cooked ham, pasteurized butter, and cheese came from eighteen stores, and the beef was taken from the municipal market and from two private slaughterhouses.

Acceptable standards for the products were based on the following requirements prevailing in the United States and in Europe: for raw products, less than 1,000 enterobacteria and *S. aureus* per gram and no evidence of Salmonella in ten grams of the product; for food products cooked or cured and for ice, less than 10 enterobacteria and less than 100 *S. aureus* per gram of the product.

Bacteriological examinations of specimens were performed according to the accepted techniques for the isolation and identification of enterobacteria and *S. aureus*.

RESULTS TO DATE

By the standards stated in the previous paragraph, only the beef products were satisfactory in all the samples examined with respect to enterobacteria, including Salmonella and *S. aureus*.
With respect to \textit{S. aureus} only, all the manufactured ice cream products and the cheese were satisfactory.

\textbf{SIGNIFICANCE}

The investigation provides useful information on the sanitary condition of several commonly consumed food products from the area of Azul. It indicates that fresh and cured food products are perhaps not handled and stored in the best hygienic manner.

\textbf{PUBLICATIONS}


\textbf{OTHER DATA}

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina

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

Timetable: 1966-1967
ENDEMIC GOITER AND RETINISM 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 present 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 region to the laboratory at the Institute of Biophysics in Rio de Janeiro.

METHOD

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

The total population under study (3,049 families; 9,590 individuals) has been divided into homogeneous samples in which all factors interfering with goiter prevalence are the same, and genetic studies of these groups are also in process.

RESULTS

The data have shown little, if any, relationship between endemic goiter and genetic inbreeding, and no indication of a relationship to any other factor.

Thyroid function tests were performed on 360 of the patients studied in the region. They showed a significantly higher mean 24-hour $^{131}I$ thyroid uptake, conversion ratio, and $PB^{131}I$ than subjects from a nonendemic area (Rio de Janeiro). However, the mean $PB^{127}I$, BEI, BII, and TI were significantly lower than the values obtained in a nonendemic area. $T_3$ resin uptake was in the normal range.

Cretins in Mato Grosso showed a lower mean uptake and $PB^{127}I$ than noncretins from the same region. No significant differences were found between cretins and noncretins living in the Goiás area.
The increase in the 24-hour thyroid uptake after TSH stimulation was significantly lower in the cretins studied in Mato Grosso than in normal controls with low uptakes. In all but one case, cytogenetic studies showed normal karyotypes. Spasticity is the typical neurological finding in these cretins. Pneumoencephalograms revealed no noteworthy abnormalities. EEG showed diffuse anomalies with slow waves.

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.

The analysis of family data showed the following results:

1. Masculinity ratio: 1.06.

2. Fertility: 5.6 pregnancies per couple. If one or both parents had goiter, the average number of pregnancies was significantly higher than when both parents had no goiter. This fact may be explained by the lower age of nongoitrous couples.

3. Prenatal mortality: 9.5% when the inbreeding coefficient F was zero, and 15.2% when coefficient F was greater than zero. The presence of goiter in one or both parents did not significantly change these figures.

4. Proportion of liveborn siblings having died unmarried: 23% when the inbreeding coefficient F was zero, and 34% when coefficient F was greater than zero. The occurrence of goiter in one or both parents did not alter these figures significantly.

5. Prevalence of goiter and deafmutism: 0.98%.

6. Prevalence of congenital malformation: 2.56% (2.37% when F was zero; 5.71% when F was greater than zero). No significant differences were observed when one or both parents had goiter.

A higher incidence of goiter 1, 2, and 3 was observed in older patients, and a higher prevalence in general was found among Negroes than among the white inhabitants of the area. Also, the incidence was higher when the social status of the family was lower (as determined by a variety of indexes—housing conditions, density of individuals per room, level of education attained, and profession of the spouse). Although a higher proportion of Negroes live under poor social and sanitary conditions, an analysis of Negroes and whites living in different environments revealed that in fact both ethnic and socio-sanitary factors interfere with goiter prevalence.
A higher prevalence of goiter was found among persons born and married in the rural areas or villages in the endemic zone than among persons from outside. Persons from the outlying rural areas showed higher prevalence of goiter than those living in towns. Although there are more Negroes living in rural areas, socio-sanitary conditions are worse there also, and both factors probably interfere with goiter prevalence.

SIGNIFICANCE

The findings of this study should be helpful in describing the overall world problem of endemic goiter and its impact on a given population. In particular, endemic goiter and possible genetic relationships are being explored.

PUBLICATIONS


OTHER DATA

Grantee: Dr. Luis Carlos Lobo, Faculdade de Ciências Médicas, Universidade de Brasília, Brasília, Brazil

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

Timetable: 1962-1968
EPIDEMIOLOGICAL STUDIES OF ENDEMIC GOITER IN THE STATE OF SÃO PAULO AND THE EFFECT OF IODINE SUPPLEMENTS

PROBLEM

Endemic goiter is recognized as a problem in Brazil - in the State of São Paulo and elsewhere. However, the role of genetic and environmental factors in the etiology of the disease has never been fully studied.

METHOD

Endemic goiter surveys have been conducted over the past several years on thousands of elementary schoolchildren 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 iodine under various dosage schedules. Urinary iodine excretion, protein-bound iodine, and antibody determinations have been made in blood.

Studies on the concentration of iodine in water supplies and the analysis of samples of enriched salt have been carried out.

RESULTS TO DATE

In many of the rural and semirural areas of the state of São Paulo, the incidence of goiter in schoolchildren is about 15 to 30 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.

Although the goiter endemic has been reduced by the iodine enrichment of salt, it is still considered a public health problem, since almost 20% of the schoolchildren have goiter.

The concentration of iodine in the water supplies of 151 cites in the State of São Paulo was too low ($X = 2.4 \mu g/liter$) to give any protection against thyroid hypertrophy.

The analysis of 869 samples of enriched salt collected from 153 cities revealed an average iodine content ($X = 4.6 \pm 3.6 \text{ mg/kg of salt}$) lower than 50% of that prescribed by federal law.

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The average iodine excretion was 84.5 µg/day. The average radioiodine uptake among children with goiter was higher than among nongoitrous children, the difference being statistically significant. All the cases with an uptake higher than 50% above the average occurred in children who had goiter.

The results of PB$_{127}$I were within physiological limits, and the differences between the groups were not statistically significant. The average of PB$_{131}$I was within normal values, but the schoolchildren with goiter had a smaller average value which was statistically different from that of the children without goiter. The same difference was found in the four cities studied.

Both the schoolchildren and their relatives presented low hemagglutination titers for antithyroglobulin antibodies. There was no significant difference between children with and without goiter. Females rendered the majority of positive sera. Low hemagglutination values for antithyroglobulin antibodies were found in some of the schoolchildren with and without goiter, as well as in their relatives.

Results of this type suggest a relative iodine deficiency - moderate and physiologically compensated - for the schoolchildren with goiter.

The results also suggest that, in addition to a relative iodine deficiency, intrinsic factors may be related to the prevalence of regional endemic goiter. Possible dishormonogenensis could perhaps be shown to exist in people with goiter selected in areas of mild endemicity.

In the tests, the urinary excretion of iodine was related to that of creatinine per centimeter of height, after the periodic variation of both have been corrected. Correction factors for both were established for each six-hour period of the day. An average of 84.5 ± 50.0 µg of iodine per 24 hours was obtained. There was no difference between the groups with and without goiter as far as the urinary excretion of iodine was concerned.

SIGNIFICANCE

This investigation serves to point out the prevalence of endemic goiter in the State of Sao Paulo and its good but incomplete response to the administration of prophylactic iodide. It also shows that a residual group of patients failed to respond to this program and must have etiological factors other than iodine deficiency. The results indicate and measure the slow fall in iodine avidity occasioned by the administration of iodide. In this region endemic goiter does not seem to be related to autoimmunological factors.
PUBLICATIONS


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
ABNORMAL IODOPROTEINS IN ENDEMIC GOITER

PROBLEM

All the iodine in the normal thyroid gland is not in the form of thyroglobulin. There are small amounts of iodinated albumin, and in some pathological states there are large amounts of this component plus a highly dense insoluble iodoprotein. The purpose of this research is to define the distribution of iodine among the various iodinated proteinaceous components of the thyroid in endemic goiter.

METHOD

At the Hospital das Clínicas large numbers of patients from surrounding areas where endemic goiter has been well documented in the past are operated on annually. The operated glands are available for homogenization and fractionation by routine centrifugal methods. They are prelabeled with radioactive iodine, and the distribution of components is measured both in terms of radioactive iodine and stable iodine.

RESULTS TO DATE

The grantee has published earlier studies on the distribution of iodoproteins in sporadic goiter in the United States. He is now accumulating data on similar patients from endemic goiter areas in Brazil, and parallel studies are being conducted on rats made goitrous by iodine deficiency. In both the human subjects and the rats an increase in the highly dense insoluble iodoprotein component has already been noted. The research activities in Brazil are just now getting into full operation.

SIGNIFICANCE

This investigation promises to provide more information on alternative pathways for iodine metabolism in the thyroid gland affected by the hyperplasia of endemic goiter. The results suggest that some of the iodine is sequestered - in some instances, large amounts. This may constitute a drain on the available iodine supply. Further definition of the abnormalities of iodine distribution in endemic goiter is badly needed. The absence of this information is hampering the approach to the problem.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Geraldo A. Medeiros-Neto, Hospital das Clínicas,
School of Medicine, University of São Paulo, São
Paulo, Brazil

Funded by: General Research Fund of the Hospital das Clínicas,
São Paulo, Brazil

Timetable: 1967-1970
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.

METHOD

A clinical survey of 85% of the population of Pedregoso has been conducted, with special emphasis on demographic, anthropological, genetic, nutritional, and medical aspects. Subsamples of the population have been used for studies in reference to endemic goiter, including radiiodine kinetic studies and chemical determinations of iodine in organic fluids. Items of the diet have been tested in rats for goitrogenic activity. The distribution of different genetic markers have been looked for (PTC, blood groups, dermatoglyphs, etc.).

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. Metabolic studies show a great inefficiency of the compensatory mechanisms, expressed by a considerable "iodine leak." In many subjects there was some impairment of dehalogenase activity, and in the blood increased NBE-^31^I, considerable amounts of triiodothyronine, and small amounts of endogenously labelled iodothyrosines.

The variability of endemic goiter, as related to size and nodularity seems to depend on multiple factors, including dietary variations and possibly also genetic influences.

Experimental studies in rats show a goitrogenic activity of piñon, the nut of the Araucaria araucana, which is an important and permanent component of the diet in Pedregoso.
SIGNIFICANCE

Iodine deficiency appears to be the fundamental etiology of endemic goiter in Pedregoso, though the variability of its expression is influenced by multiple factors.

PUBLICATIONS


OTHER DATA

Grantee: Dr. José Barzelatto, Hospital del Salvador, University of Chile, and Dr. Edmundo Covarrubias, School of Medicine, University of Chile, Santiago, Chile

Funded by: International Atomic Energy Agency, University of Chile, The Population Council, and the Chilean National Commission on Scientific Research and Technology

Timetable: 1961-1969
The aim of this project is to define more precisely the epidemiology of endemic goiter in the Cauca Valley of Colombia, to explore possible etiological factors, and to study the pathophysiology of the thyroid glands of patients from this area.

Straightforward surveys are conducted, and an attempt is made to correlate the data collected with such factors as diet and water - its hardness, source and bacteriology.

Evidence accumulated over the past eight years indicates that the goiter present in the Cauca Valley is not due to iodine deficiency. This assertion is supported by longitudinal epidemiological studies conducted over this period and cross-sectional surveys that have demonstrated a 20-30% prevalence of goiter among school-age and adult populations in the presence of high urinary iodide excretions (> 300 μg/day). Other functional parameters of iodine metabolism in vivo (thyroidal 131I uptake, PBI, PBI31I, and conversion ratio) and in vitro (studies of intrathyroidal iodine metabolism) also indicate an adequate or even high iodide supplementation. Kinetic studies of iodine metabolism in goitrous and nongoitrous individuals have shown high plasma inorganic iodide (9.08-13.38 μg/liter), iodide space of 15-20 liters, normal thyroidal and renal clearances, low-rate constant of movement of 131I from pool 1 to pool 2 (uptake), normal thyroidal absolute uptake, and normal hormone secretion rate. The thyroxine degradation rate is also normal. The variety of water supply systems used in the Cauca Valley and in the town of Candelaria have depicted a complete spectrum of quality, from "soft" (<50 ppm) to "very hard" (>300 ppm), and from heavily contaminated with bacteria (940,000 E. coli/100 ml) to "completely potable." "Goitrogenic activity" of these waters was tested in female albino rats. On a low-iodine diet, marked enlargement of the thyroid gland (27.7 with a range of 18.8-35.2 mg/100 gms rat) was observed in animals given water from the city of Cali, considered "soft and potable," and very little enlargement in rats given water from the Parraga River considered "moderately hard" and having 240,000 E. coli/100 ml (11.9,
with a range of 10.8-13.4) and Candelaria's Well B, considered "hard" and having 240 $E.\ coli/100\ ml$, (12.5 with a range of 7.2-16.1; $P<.01$). Intermediate "goitrogenic activity" was found with "demineralized" water from Cali (17.0 with a range of 15.4-18.7) and water from Candelaria's Well D, considered "hard" and having 240 $E.\ coli/100\ ml$, (17.9 with a range of 12.2-26.4). Little activity was observed in rats taking water from a "private well" in Candelaria considered "very hard" and having 940,000 $E.\ coli/100\ ml$. "Hard" waters contained Ca, Mg., Mn, sulfates, chlorides, and nitrates in high concentration. Enlargement of the thyroid gland was accompanied by depressed thyroidal $^{131}I$ uptake, high MIT/DIT ratio, and decreased proportion of thyroidal $^{131}I$ thyroxine. $PB^{131}I$ and conversion ratios were unaffected. The enlargement and functional behavior of the thyroid gland in rats given water from Cali was comparable to that observed in animals given Methimazole 30 $\mu g$/day/rat diluted in "demineralized" water from Cali (31.0 with a range of 20.1-48.8). No correlation was found between the degree of "hardness" or bacterial contamination and the degree of "goitrogenic activity." Therefore, other factor(s) must account for the "goitrogenicity" of these waters. None of the above changes were observed on Purina Laboratory Chow. Even the effect of Methimazole was masked by the Purina diet. Replicate factorial analysis of these experiments are currently under way.

**SIGNIFICANCE**

The evidence to date indicates that goiter in the Cauca Valley of Colombia has a different origin than simple iodine deficiency. The significance of the project rests in the ability to define the nature of the epidemiological factor.

**PUBLICATIONS**


OTHER DATA

Grantee: Dr. Eduardo Gaitán, School of Medicine, Universidad del Valle, Cali, Colombia

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

Timetable: 1963-1969
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 genetic and environmental factors, 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 has been started to assess effects on physical and mental deficiency states that coexist with endemic goiter.

METHOD

A complete inventory of several villages in Ecuador has been made. Data on many aspects of the health of the individuals 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 development, cardiovascular 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

Results continue to accumulate regarding the distribution and impact of endemic goiter in rural Ecuador. Frequent surveys are made by the grantee and his colleagues. The principal effort at the present time is aimed at appraising the effectiveness of iodinated oil (see "Iodinated Oil in the Prevention of Endemic Goiter and Cretinism in Ecuador," p. 71). A recent study conducted jointly with the Harvard Medical School in two villages in rural Ecuador has shown that subjects with short stature as well as control subjects react at least as well as normal subjects from the U.S.A. to insulin stimulation of growth hormone secretion. In some instances there seemed to be a response above normal.
SIGNIFICANCE

The growth hormone study indicates only that, in spite of short stature in association with endemic goiter in the rural villages, the secretion of growth hormone in response to insulin stimulation ranges from excellent to excessive. This indicates that the short stature is not the result of an inadequate supply of growth hormone.

PUBLICATIONS


OTHER DATA

Grantee: Dr. Rodrigo Fierro, National Polytechnic Institute, Quito, Ecuador

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

Timetable: 1962-1970
IODINATED OIL IN THE PREVENTION OF ENDEMIC GOITER
AND CRETINISM IN ECUADOR

PROBLEM

The aim of this project is to establish the effectiveness of iodinated oil as a prophylactic measure for endemic goiter and attendant endemic deafmutism, cretinism, mental retardation, and short stature.

METHOD

Two villages in rural Ecuador have been chosen, each with a population of approximately 900. The incidence of endemic cretinism, goiter, deafmutism, mental deficiency, and short stature is extremely high in both places. One of the villages has been given iodinated oil prophylactically and the other has served as a control. Frequent surveys are made in both groups with a view to ascertaining the prophylactic effectiveness of the oil. A resident physician, established by the project, carefully observes all pregnancies and newborn infants in these two villages.

RESULTS TO DATE

After one year of iodization, in Tocachi the endemic goiter incidence has had a 30% reduction. After a year and half, there has been a 46% reduction among children up to twelve years of age, including those born to iodized mothers. Moreover, not a single one of the 60 children born to iodized mothers presents thyroid enlargement. On the other hand, 8% of the 150 children born in La Esperanza after March 1966 present thyroid Grade 0-6.

After one year of iodization, there was a striking difference in thyroid function in the two villages studied: in La Esperanza the values were PBI 2.7, thyroxine iodine 2.4, and average urinary iodide excretion per 0.9 grams creatinine 10.4 mg, whereas in Tocachi in patients previously given iodinated oil the values were 12, 8, and 292 respectively. Thus there appears to have been restoration of thyroid function to normal in those persons of Tocachi from whom detailed physiologic data have been obtained after one year and half of iodization.

Though the data have not yet been statistically analyzed, it would appear that neuromotor development among children born to iodized mothers from Tocachi is better than that among children from La Esperanza.
The results so far show not only that this is a reliable prophylactic measure against endemic goiter and cretinism but also that it will continue to produce favorable effects in the immediate future. This is demonstrated by the iodine levels presented by the Tocachi population 18 months after administration of the oil.

SIGNIFICANCE

It has never been established with scientific certainty that iodide prophylaxis will prevent cretinism, mental retardation, or short stature associated with the presence of severe endemic goiter. The present study is designed to answer this question.

PUBLICATIONS

See publications listed under "The Pathophysiology and Prophylaxis of Endemic Goiter and of Cretinism in Ecuador" p. 69.

OTHER DATA

Grantee: Dr. Rodrigo Fierro, National Polytechnic Institute, Quito, Ecuador

Funded by: Pan American Health Organization and the Mental Retardation Foundation

Timetable: 1966-1970
PROBLEM

Endemic goiter may be regarded as a dynamic condition with constantly changing characteristics. A gradual increase in iodine intake seems to be the cause of slow disappearance of endemic goiter in a small town that has been under observation for a five-year period. Current average iodine intake of schoolchildren from this community is around 50 µg/day. These iodine intake levels which vary according to the age of the individual and to the season of the year, have been enough to bring down to normal levels the 24-hour radioiodine thyroid uptake and to considerably diminish the prevalence of goiter. Although these finding may help to answer the fundamental question of how much iodine should be supplied to assure normal iodine nutrition, the study has been limited to an area of endemic goiter. Goiter surveys and iodine nutrition studies have been started in several communities over the country and they should be continued on a long-term observation schedule.

Although iodine deficiency seems to be the only cause of endemic goiter in Mexico, little study has been made of other factors such as goitrogenic substances. The rising levels of iodine nutrition in the country as a whole and the decrease of goiter prevalence offer a unique opportunity to study the goitrogenic action of common foodstuffs in areas that have low or barely normal iodine intake levels.

METHOD

A small field laboratory and metabolic unit was established in a village in rural Mexico where endemic goiter has already been recognized, and its extent and severity has been measured in the school population over several years. A nutritional and goiter survey was carried out during the summers of 1962, 1965, and 1967 and 24-hour 131I uptake was measured in samples of goitrous children every year. Different methods have been used to evaluate the iodine intake levels of these children. Iodine balance studies in the field metabolic unit or in the child's home and estimates from basal urinary excretion rates gave different approaches to determine the changes in iodine intake.

The evaluation of iodine nutrition levels in school-children from rural villages throughout the country and some central studies in Mexico City and other non-goitrous areas have been carried out.
Some preliminary steps toward the identification of goitrogenic factors have been made in the endemic area of Tepetlixpa. Ten-day iodine balance studies were carried out in 5 children on a constant iodine intake close to their customary ingestion, with foodstuffs completely different to the usual Tepetlixpa diet. The effect of the different diet was evaluated by PBI$^{131}$I at 72 hours, and the results were compared to that of a 0.1 tds dose of Methimazole.

The sensitivity of thyroid hormone synthesis to small doses of antithyroid substances in relation to different levels of iodine intake is being studied. Thirty children divided into three groups according to their iodine intake had a measurement of $^{131}$I concentration in the liver by external scintillation counting as an indirect measurement of PBI$^{131}$I at 72 hours. The purpose in using this indirect method was to avoid large doses of $^{131}$I and withdrawal of blood - a procedure that is deeply disliked by rural Mexican children.

RESULTS TO DATE

The goiter disappearance rate is in relation to a natural improvement in the iodine nutrition levels in the school population of Tepetlixpa. Goiter prevalence has come down from 93% in 1962 to 68% in 1965 and 50% in 1967. Nodularity has also diminished from 30% to 13% and 5%. The 24 hour $^{131}$I thyroid uptake has come down to normal values and the iodine excretion in 24-hour urine samples increased from a mean of 9 µg/day in 1962 to a mean of 33 µg/day in 1966. Iodine balance points (the amount of iodine intake to obtain intake equal to excretion) also showed an increase from 21 µg/day to 70 µg/day in a small number of children.

Areas surveyed along the Pan American Highway showed a negative correlation between 24-hour $^{131}$I thyroid uptake and basal urinary iodine excretion. According to this relation, the uptake should be over 50% when the urinary iodine excretion is below 30 µg/day.

Our current search for goitrogens gave negative results in children that were in iodine balance with a iodine dietary intake of 100 µg. The 24 hour radioiodine uptake of these children was within normal limits, as were their PBI$^{131}$I values at 72 hours. The administration of a different diet or Methimazole in small doses did not produce any significant change.

When external scintillation counting over the liver was used as an indirect measurement of PBI$^{131}$I concentration at 72 hours in 30 children, there was no significant correlation between estimated average iodine intake and estimated PBI$^{131}$I (r=0.03) nor between 24-hour $^{131}$I thyroid uptake and basal urinary iodine excretion.
uptake and estimated PBI$^{131}$I, (r=0.10), but the usual correlation between iodine intake and thyroid uptake was shown (r=0.88). These results suggest that the conversion rate from inorganic to hormonal $^{131}$I is independent of iodine intake and thyroid uptake in the children under study.

SIGNIFICANCE

Although endemic goiter has been known since ancient times, we are still not certain of its etiology. The role of iodine in the pathogenesis of endemic goiter is well established, and iodine deficiency has been extensively studied by direct and indirect methods. Nevertheless, long-term observation studies have not been made in goitrous and nongoitrous populations living on similar iodine intakes. For the past five years the school population of an endemic goiter area has been studied and a correlation between goiter disappearance and improvement of the iodine nutrition levels has been clearly shown. Experience in reliable field work methods to evaluate iodine nutrition will be used throughout the country in regions previously known as endemic goiter areas.

Since this project is a continuation of work done during past years, it is felt that the results will provide supporting evidence on optimal iodine requirements as well as indicate a useful way to evaluate supplementation programs in iodine-deficient areas that can be applied in the Mexican public health program.

Recent literature has stressed the need to look for other goitrogenic factors in endemic goiter areas with current iodine intake levels similar to those in nonendemic areas.

This work will give information about goitrogenic factors interfering with the normal biosynthesis of the thyroid hormones without modifying the thyroid uptake or inorganic iodine turnover. Most of these latter studies will be done on people living under native conditions and may satisfy in part the lack of epidemiological data on goitrogens.

PUBLICATIONS


OTHER DATA

Grantee: Dr. Jorge Maisterrena, National Institute of Nutrition, Mexico City, Mexico

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

Timetable: 1962-1968
ENDEMIC GOITER IN THE HIGHLANDS OF PERU
AND ITS CONTROL WITH IODINATED OIL

PROBLEM

The principal aims of this study are to define the endemicity of endemic goiter in the highlands of Peru, where it has been known to exist for many centuries; to study the impact of this disease on the health of the population; and to determine the effects, both beneficial and unwanted, of iodinated oil administered as a prophylactic measure. In addition, the study is concerned with the pathophysiology of these thyroids and with the effect of slow-release oil on thyroid function.

METHOD

Three towns in the highlands of Peru and the area surrounding Tarma were chosen for the study in October 1966. With the assistance of a team from the Pan American Health Organization, a prophylactic program was undertaken and several hundred persons were injected with iodinated oil according to varying schedules. The program was controlled by administration of a placebo oil having no iodine. Full personal data were obtained on all the subjects. As part of the program, injected individuals will return and be re-examined as frequently as possible over the next several years. As far as possible, data on new births, deaths, and incidental illnesses will be obtained. Studies are contemplated for the measurement of thyroid function by radioactive iodine uptake and turnover among patients who have received the iodinated oil.

RESULTS TO DATE

No definite information has yet been obtained regarding the effectiveness of the iodinated oil in this population group, a large amount of epidemiological data on the disease has been obtained. These have not yet been collated for publication.

SIGNIFICANCE

This is another attempt to define in precise detail the endemicity of endemic goiter in an isolated area of the world where the disease is known to occur. The principal significance of the investigation relates to the
benefit of prophylactic oil in reducing goiter among those who have it, in keeping it from developing among those who do not, and in preventing occurrence of the afflictions that accompany endemic goiter, such as cretinism and endemic deafmutism.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Eduardo Pretell, Institute for High Altitude Studies, Cayetano Heredia University, Lima, Peru

Funded by: Institute for High Altitude Studies, Cayetano Heredia University, and the Public Health Service of Peru

Timetable: 1967-1971
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.

Information now available has established the effectiveness of small amounts of fluoride in the diet during the years of teeth formation in increasing the resistance of teeth to caries attack. There is also considerable evidence 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 given 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.

METHOD

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 (the control population) is receiving its normal supply of common table salt as used in Colombia. Another community is receiving common salt with added sodium fluoride calcium pyrophosphate mixture on a regular basis, and a third community is receiving common salt with added calcium fluoride tricalcium phosphate mixture. The fourth community is being provided with water fluoridation. Comparable data on caries prevention and urinary fluoride excretion will thus be available.

A basic dietary survey, with special attention to the consumption of salt in the younger ages, was planned on a 10% randomly selected sample from each community, using a seven-day observation weighing technique. Clinical nutrition studies are also to be conducted, as well as X-ray examinations 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, from both urban and rural areas. Each inhabitant was identified by name, age, sex, residence, and socioeconomic status. Determination was then 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 subjects in the 12-14 age group is used for dosage adjustments of fluorides in the community fluoridated water and in the experimental communities.

Surveillance of dental conditions and the level of fluoride excretion in the urine is being maintained.

SIGNIFICANCE

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

PUBLICATIONS


OTHER DATA

Grantee: Dr. Hernán Velez, 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.

METHOD

Turnover of manganese in normal subjects and manganese-poisoned individuals was measured, as was the absorption of radio manganese after inhalation and ingestion. Simultaneous determinations of intestinal absorption of radio iron in both normal subjects and patients with chronic manganese poisoning were also made.

Patients were transported a distance of approximately 400 miles from the mining district to the Catholic University Hospital in Santiago, Chile, where they were hospitalized. In addition to an extensive routine chemical and laboratory work-up, detailed neurologic and psychiatric evaluations were conducted 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 in the United States.

L-Dopa was administered orally to patients with manganism and to healthy miners. The effects were documented by motion pictures, clinical observation, and muscular efficiency tests.

RESULTS TO DATE

At Brookhaven the relationship of aromatic aminoacids and Parkinsonism was extensively investigated. Several metabolic maneuvers were attempted, some of which produced marked changes in the Parkinsonian state. Parkinsonian tremor became aggravated after administration of the melanophore-stimulating hormone PMSH. Since the skin became darker as well, it was postulated that precursors of melamines and catecholamines were being diverted from the brain to the skin. This made it desirable to study the effects of aminoacids which are precursors in the synthesis of melamines and of catecholamines. Sudden administration of DL phenylalanine in large amounts tended to aggravate the signs and symptoms of Parkinsonism. Slow and progressive administration of DL dyhydroxyphenylalanine (Dopa) and later on L-Dopa improved the Parkinsonian
state - in some cases, dramatically - and the improvement was sometimes sustained for months.

From the beginning of the research, certain neurological similarities between chronic manganese poisoning and Parkinsonism had been noted. Indeed, in one of the latest publications it was indicated that a metabolic approach recently developed for Parkinsonism by the project might be serviceable in the study of chronic manganese poisoning (Neurology 18:377, 1968).

One of the principal investigators took a pretested supply of L-Dopa to Santiago, Chile, in February 1968, and was granted appropriate authorization to proceed with investigations there. It was decided to follow as elastic a protocol as had been followed for the Parkinsonians at Brookhaven. This decision has already led to the following exciting observations:

Two healthy manganese miners have developed significant, sustained muscular relaxation upon receiving 6.0 g of L-Dopa a day. Three days after abrupt termination of the L-Dopa the hypotonia disappeared.

Three young ex-miners crippled by severe, hitherto untreatable muscular rigidity showed striking alleviation or disappearance of the rigidity.

Patients who had hitherto been constantly contemplating suicide began to discuss their marital prospects.

These observations would lead to the following conclusions:

1. Dopaminergic receptors have been shown for the first time to play a cardinal role in a disease other than Parkinsonism.

2. The induction of hypotonia might well signal the diminution of brain serotonine by administration of L-Dopa. This highly plausible hypothesis can be readily tested by administering 5-OH Tryptophane, which is a precursor of serotonine, as Dopa is for dopamine. This step will be taken in the very near future in Santiago. If the relationship between hypotonia and 5-OH Tryptophane should become verified, extensive studies will be carried out both in Santiago and at Brookhaven on these and possibly other neurological disorders. As in the case of Parkinsonism, it is quite possible that other "degenerative" neurological disorders are, at least in part, deficiency diseases.

Studies on factors of susceptibility to manganism have shown that the classically accepted route of entry of manganese ore dust is the lung. However, the work in Santiago showed that inhaled manganese salts were eventually mobilized into the gastrointestinal tract. The gastrointestinal absorption of manganese
was linked linearly to the absorption of iron; that is to say, increasing demand for iron increased the demand for manganese. The demand for iron seemed to be potentiated by the manganese overload, which induced significant excretion of iron. Thus a complete vicious cycle, which promotes the absorption of manganese by apparently depleting the iron stores, seems to be operating in these individuals. A role of this mechanism in determining individual susceptibility to manganese poisoning seemed plausible. Furthermore, even these studies might be pertinent to Parkinsonism, since iron concentrations are found to be high in Parkinsonian brains.

SIGNIFICANCE

The present findings, viewed together with data on animals, suggest the following conclusions: (1) normal, working miners must be living with a significant tissue burden of manganese; (2) since patients with chronic manganese poisoning lost their manganese burdens after removal from the mines but did not lose the symptoms and signs of chronic manganese poisoning, chelation therapy is not indicated in these cases; (3) the relationships between chronic manganese poisoning and metabolism of aromatic aminoacids is established; (4) factors of susceptibility to chronic manganese poisoning appear to be determined by the following sequence: (a) inhalation of ore dust; (b) swallowing of the inhaled material; and (c) intestinal absorption of manganese as a linear function of absorption of iron.

PUBLICATIONS


OTHER DATA


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 ON AREAS OF HIGH NATURAL RADIATION IN BRAZIL

PROBLEM

The increasing use of nuclear energy for civilian purposes will expose a growing number of people to ionizing radiation levels over and above those normally encountered. The biological effects of ionizing radiation are delayed and, in the case of genetic effects, 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.

METHOD

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, investigations are made to determine how many people are living in these areas, the sources of their food, and the duration of their residence.

The frequency with which somatic mutations are occurring is being studied by chromosomal analyses of peripheral blood lymphocytes. Epidemiological studies on the incidence of leukemia, bone cancer, and other known delayed radiation effects may be undertaken if justified by the level 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 in the field, who are cataloguing the native species and selecting certain ones for special studies.

Grasshoppers and scorpions have been selected for chromosome aberration studies. Several plant species are being investigated with a view to their suitability for radioresistence studies.
RESULTS TO DATE

It has been determined that in Guaraparí, a coastal community in the State of Espirito Santo where the population is exposed to radiation from monazite sands, chromosomal aberrations are found in peripheral blood at a slightly higher frequency than in the control population.

Some aberrations noted are those normally associated with internal emitters. Since the levels of radioactivity in local foodstuffs are within or slightly above the normal range, and most of the food consumed by the population is imported from other regions, other vehicles of internal contamination of man have been searched. Some of the Guaraparí homes do not have paved floors and monazite sand has been used as a building material. Inhalation of thoron and daughters or radioactive dust are currently under study as a possible source of internal exposure.

Attempts to estimate radioactive body burdens in some of the Guaraparí inhabitants have been made by measuring the thoron content of the expired breath and by whole body counting. Elevated results have been found in some people, but the proper interpretation of data is still dependent on the improvement of equipment and methods.

External radiation exposure at Guaraparí has been measured in samples of the population who have cooperated by wearing thermoluminescent dosimeters for periods of several months. The average dose rate for the 317 people measured was 636 mR/yr with a range of 100 mR/yr to 3200 mR/yr.

At Araxá, in the State of Minas Gerais, the principal source of exposure is radium in food. The radium content in many of the foods is 20 to 300 times greater than normal. A dietary inquiry was conducted in a sample of the local population to estimate the daily intake of the natural radionuclides. The average daily intake of alpha emitters ranged from 22 to 505 pCi. The maximum daily intake of radium was 42 pCi for $^{226}$Ra and 242 pCi for $^{228}$Ra.

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 5 to 10 grams of ash, and arrangements have been made with midwives to obtain placentas from representative mothers. Those are analyzed for $^{226}$Ra and $^{226}$Th, and a few have shown contents elevated by a factor of as much as 7.
The third radioactive anomaly is found at Poços de Caldas, also in the State of Minas Gerais, where the Morro do Ferro has external radiation levels up to 300 times greater than 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, ranging from 3,000 to 30,000 rem a year, and that in one plant, of the genus Melastomataceae, radium tends to concentrate in far greater amounts than in other plants. Studies to estimate the dose received by the various tissues of Melastomataceae are being carried out.

SIGNIFICANCE

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

PUBLICATIONS


Annual Reports


OTHER DATA

Grantee: Dr. Eduardo Penna Franca, University of Brazil, Rio de Janeiro, and Father Thomas Cullen, Catholic University of Rio de Janeiro, Rio de Janeiro, Brazil

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

Timetable: 1963-1969
STUDIES OF CESIUM-137 IN JAMAICAN MILK

PROBLEM

In April 1964 the results from an environmental surveillance program initiated by the Jamaican Ministry of Health and the Pan American Health Organization to study fall-out from nuclear weapons tests showed that cesium-137 was elevated in a number of samples of Jamaican milk. Of samples taken at similar latitudes elsewhere in the Caribbean and Central America, the only others that showed such a high elevation of $^{137}\text{Cs}$ were from an area in Florida in the United States. Interestingly enough, although the $^{137}\text{Cs}$ was elevated, the strontium-90 concentration was not high. This indicates that a selective mechanism is operating in the complicated passage of $^{137}\text{Cs}$ from the air to milk.

The surveillance program sampled Jamaican milk from three of the main milksheds on the island. The samples from the western end of Jamaica were found to be more elevated than those from the middle or on the eastern ends. Accordingly, a field research program was initiated to study the possible factors responsible for the pattern of cesium-137 concentration.

METHOD

In the western end of Jamaica, a sampling program of milk from a number of farms in a north-south transit was carried out. By October 1965 milk from over one hundred farms and pooled milk at the local cooling station had been tested. From the results, it was possible to identify 59 farms that had $^{137}\text{Cs}$ concentrations in excess of 100 pCi/liter. Work undertaken since then has been concerned with intensive sampling of individual farms identified in this study.

The $^{137}\text{Cs}$ content of pooled milk samples from three milksheds representing the whole island has been analyzed weekly, and seasonal variability was studied in 1966 and 1967.

Further analysis of milk from several of the individual farms identified in the October 1965 survey has also been carried out. Many of the farms that had elevated $^{137}\text{Cs}$ in milk in 1965 were also high when sampled in 1966 and 1967. At present, seasonal variation is being investigated in milk from individual farms and in the pooled samples from the whole milkshed.
In addition, a program has been undertaken to study $^{137}$Cs in grass, drinking water, and supplemental feed in selected farms where high $^{137}$Cs contents have been identified in milk.

RESULTS TO DATE

The $^{137}$Cs in pooled milk from the western end of Jamaica showed a consistent seasonal variation in 1966 and 1967 that roughly corresponded to the rainfall. The highest concentration was observed in the month of highest rainfall, normally October, and the lowest concentration was found in the month of March. It appears that $^{137}$Cs is decreasing in Jamaican milk much more slowly than would be expected from experience in other areas of the world. Between October 1966 and October 1967, $^{137}$Cs in pooled milk from the western portion of Jamaica decreased only 10 per cent. The failure for it to decrease at the rate it has done on the North American continent suggests that the levels in milk may depend on the total amount of fall-out rather than rate of fall-out, as has been shown to be the case for many areas of the world. In such event, one would expect the milk to remain elevated for years. The sampling program that has been undertaken will be continued to see whether or not this is so.

The ratio of $^{137}$Cs to $^{90}$Sr in milk is an indicator of the different behavior of these two radionuclides. In Jamaican milk the ratio of $^{137}$Cs to $^{90}$Sr is much higher than the ratios observed in the continental United States. Clearly, these two radionuclides are being treated differently by the environment.

Farms with elevated $^{137}$Cs in milk and farms with low $^{137}$Cs in milk have been identified within several miles of each other. Study of $^{137}$Cs deposition in the soils of these farms has shown much less variability than that observed in the milks. Thus, differences in deposition of $^{137}$Cs could not be responsible for elevated $^{137}$Cs in milk.

Grass has been analyzed from the various farms, but no one grass has been found to contain excessively more $^{137}$Cs than any of the others. Pangola, for example, was not elevated above paragrass or guinea grass.

The analysis of supplemental feeds has shown that they are not a significant source of $^{137}$Cs.

One common characteristic of all the farms having high $^{137}$Cs in milk is that the cows are watered by catchment, either by ponds in the field or by systems of roof runoff into cement cisterns. None of the farms visited on which the cows water from rivers had elevated $^{137}$Cs in the milk. Whether these watering procedures are causally related to elevated $^{137}$Cs has not been determined.
SIGNIFICANCE

An understanding of the exact mechanisms responsible for elevated $^{137}$Cs would permit prediction, solely from a knowledge of local conditions, of other areas where high concentrations of $^{137}$Cs in milk might be expected.

PUBLICATIONS


OTHER DATA

Grantee: Dr. Merrill Eisenbud and Dr. McDonald E. Wrenn, New York University Medical Center, New York, New York, and Dr. James Williams and Dr. Michael Heydon, Jamaican Ministry of Health, Kingston, Jamaica

Funded by: Pan American Health Organization, U.S. Public Health Service, and Jamaican Ministry of Health

Timetable: 1964 - continuing
PROBLEM

The Inter-American Investigation of Mortality indicated unusually high mortality from bladder cancer as well as from cancer of the larynx and of the lung in La Plata, Argentina. Further inquiry indicated that high rates were found also in Buenos Aires. One tenable hypothesis was that the nature of the local tobacco was involved. Thus the purpose of this investigation was to collect data regarding a series of cases of malignant neoplasm of the larynx or lung (primary) or of urinary bladder and of a series of controls and to analyze the possible relationships with cigarette smoking, occupation, etc.

METHOD

Cooperating hospitals were selected in Buenos Aires, and hospital residents, social workers, and clerical assistants were employed. Questionnaires were completed on each case and on each control. The subjects were selected on the basis of histological confirmation of the diagnosis, an interview with the patient, and other established criteria. The questionnaire had two parts; Part II, which contained the diagnostic information, was separated from Part I, which was used for the interview. The interviewer was not told whether he was interviewing a case or a control. For each case two controls, matched as closely as possible in terms of age, sex, and residence, were selected – one control with a diagnosis of cancer of some other site and the other control with a diagnosis of a nonmalignant condition. The information collected included residence, occupation, industry, consumption of alcoholic beverages, and detailed data regarding smoking habits and kinds of cigarettes used.

RESULTS TO DATE

This investigation was completed in August 1967. Fifteen hospitals in Buenos Aires cooperated in the study during the first seven months of 1967. The number of cases among females were too few to yield results, and thus data on women are not included. Information was obtained on 48 cases of cancer of the larynx, 180 of the lung, and 97 of the bladder. The study had to be terminated before a sufficient number of suitable controls of both types had been selected and interviewed. However, 249 matched controls with diagnoses or nonmalignant conditions and 76 with diagnoses of cancer of body sites other than the larynx, lung, or bladder were used in the analysis.
Among the patients with cancer of the larynx, lung, or bladder, a higher proportion of men had a history of having been cigarette smokers than among the matching controls. For each cancer site there was an excess of men habitually smoking the distinctive negro tobacco.

Recent data from Uruguay, where a negro tobacco is also used, indicate that mortality of males from cancer of the larynx and bladder is unusually high.

Further study is needed to confirm or refute the suggestion arising from these results that tobaccos may differ in their carcinogenic potential.

SIGNIFICANCE

This research has indicated that further study of tobaccos in regard to their carcinogenic potential is indicated.

PUBLICATIONS


OTHER DATA

Grantee: Dr. G. Wynne Griffith, Pan American Health Organization, Washington, D.C.

Funded by: Anna Fuller Fund

Timetable: August 1966 - 1967
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 (a) 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 (b) 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.

METHOD

A multidisciplinary team, always preceded by an anthropologist thoroughly familiar with the group under study, obtains genealogies; performs medical, dental and anthropometric examinations; and collects blood, saliva, urine, and stool specimens. Each specimen of blood, saliva, and urine is typed in the laboratory with reference to a variety of genetic marker systems, and studies are made of the antibodies, biochemical constituents, and parasites involved.

RESULTS TO DATE

Extended accounts of the field work among the Xavante Indians in Mato Grosso, Brazil, have now been published. In brief, data concerning vital statistics, inbreeding levels, polygamy structure, and other features of this order for several relatively undisturbed groups have been collected and will help to 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 to explain some of the biomedical aspects of modern man, who, in the genetic sense, has only very recently left the primitive state.
PUBLICATIONS


OTHER DATA

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


Timetable: 1962 - continuing
INTENSIVE CARE OF MOTHER, FETUS, AND THE NEWBORN

PROBLEM

The central aim of the project is to study the disturbances occurring in fetal homeostasis during labor, both under "normal conditions" and "fetal distress."

It is hoped to determine the diagnostic and prognostic significance of "latent" fetal distress signs detectable during pregnancy and labor and to explore methods for making early diagnosis of "homeostatic disturbances" and correcting them before irreversible damage has occurred in the fetus.

The ultimate goal of the research is to prevent permanent damage to the child caused by too marked and too prolonged disturbance in fetal homeostasis.

METHOD

High-risk pregnant women (those with severe diabetes, toxemia, arterial hypertension, Rh isoimmunization) are selected.

The following variables are continuously recorded by electronic methods: (1) pressure of amniotic fluid (intrauterine pressure for measuring the intensity and frequency of uterine contractions); (2) maternal arterial pressure, heart rate, and ECG; and (3) fetal ECG, heart rate, and EEG.

During labor, periodic microsampling is made of maternal arterial blood and fetal cutaneous (scalp) capillary blood using the Saling method. The following variables are measured in both maternal and fetal blood: Hb saturation, pO₂, pH, pCO₂, base deficit, and creatinine and uric acid concentration.

At the moment of birth similar measurements are repeated in the blood of the umbilical artery and vein; after birth they are periodically repeated in blood samples obtained from the aorta. In addition, heart rate, ECG, arterial pressure, respiratory movements, temperature, and EEG are continuously recorded in the newborn during the first hours of life.
Several cross-correlations are made between the variables measured, which are also correlated with clinical signs obtained from the mother, the fetus, and the newborn.

The development of the child is followed up with emphasis on neurological and psychological functions.

RESULTS TO DATE

Uterine contractions have been shown to be one important cause of fetal distress. The contraction reduces maternal blood flow to the placenta by the following mechanisms: (1) compression of the intramyometrial part of the vessels supplying blood to the placenta; and (2) compression of the abdominal aorta or the common iliac arteries.

The normal uterine contractions of labor acting on a normal fetus and placenta produce no ill effects as can be judged from records of FHR and measurements of pH, \( pO_2 \), \( pCO_2 \) and Hb saturation in fetal blood, or by the conditions of the newborn (evaluated by the Apgar score). Abnormally strong or frequent contractions cause clear signs of fetal distress in FHR and fetal blood and lead to depression of the newborn and even to the death of the fetus. Contractions of normal intensity and frequency but in too great a number owing to undue prolongation of labor also cause fetal distress.

Fetal blood has been sampled from the fetal scalp (Saling method) throughout labor. The changes associated with "fetal distress" are (1) actual pH lower than 7.20; (2) Hb saturation lower than 32%; (3) \( pO_2 \) lower than 18 mm Hg; and (4) \( pCO_2 \) higher than 45 mm Hg.

These changes in fetal blood were consistently associated with a low Apgar score at birth and with the following changes in FHR: (1) a rise in the baseline (Basal FHR), and (2) type II dips, i.e. transient falls occurring after uterine contractions. These two changes in FHR were also consistently associated with a low Apgar score and are thus considered typical signs of intrapartum fetal distress.

The autonomic nervous system mechanisms involved in FHR changes have been investigated. An adaptive significance has been conferred to these FHR changes, which are considered to be a part of a more complete cardiovascular defensive mechanism of the fetus.

There are changes in FHR that bear no relation to modifications of fetal blood or with the Apgar score — namely, type I dips, transient ascents, rapid small oscillations, and the spikes.
The fetal ECG has been recorded directly with electrodes in the fetal buttock. No important changes occur in fetal ECG until premortem stages of fetal distress.

The consequences of intrapartum fetal distress have been studied in the newborn (low Apgar score) and in the child (higher incidence of respiratory distress syndrome and of neurological damage). The latter results need to be confirmed in a larger number of cases.

In cases of fetal distress, the administration of 100% oxygen to the mother causes a reduction in the amplitude of type II dips and a rise in $pO_2$ in both the fetal blood and muscles of the buttock. The effects on $Hb$-saturation, $pCO_2$, and pH of fetal blood are less consistent and need further study.

SIGNIFICANCE

Disturbances of fetal homeostasis have a great medical and social significance, since they interfere with cell functions and may lead to irreversible structural damage with permanent postnatal sequelae. A substantial proportion of cases with neurological diseases and mental retardation detected in the child are thought to be the result of damage caused to the brain of the fetus or newborn by alterations in the composition of the blood such as hypoxemia, hypercapnia, acidemia and hypoglycemia.

PUBLICATIONS


OTHER DATA

Grantee: Dr. Roberto Caldeyro-Barcia, Hospital de Clínicas, Universidad de la República, Montevideo, Uruguay

Funded by: Pan American Health Organization

Timetable: 1968-1970
PROBLEM

To gain full understanding of the cultural, economic, medical, and biologic factors that influence 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, is carrying 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 findings in the prospective Khanna study show a significant fivefold 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, it is felt that the study of abortions should be part of any broad study of population dynamics, in keeping with the principle that the population must be observed in order to trace the course of events as they happen.

METHOD

It is planned to study 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 are to be near Lima - one on the coast and one in the altiplano. The third community will be in the jungle area. After reasonable experience has been gained in the first town, the method will be extended to the other communities.

Accordingly, a record is kept of each family and of each individual member. At the first interview, retrospective data are collected on all women of child-bearing age (15 to 44 years). As part of the local health program, all but single women are followed at monthly or bimonthly intervals through household visits by trained local interviewers, who are supervised by local health personnel. The interviewers record pregnancies, menstrual periods, dates and terminations of pregnancy, breast feeding, fetal losses, live births, and deaths in each family. Observations on each woman are to be continued for at least three complete years. Time will also be allowed to complete observations on deliveries of women who become pregnant before the end of the third year.
A census of each community is taken at the initiation of the program. Records of arrivals, departures, births, and deaths will be made at one-year intervals. Each birth and death is 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 will be used to promote adequate registration procedures, as well as for the purposes of the present study.

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

RESULTS TO DATE

The first study was started and is well under way in Lurín, a small community in the coastal area with a population of about 3,000 persons. This is being followed by a similar study in Masma, Province of Jauja, Department of Junín, which has an altitude of 3,400 meters.

The census of the population in Lurín was taken on 12 and 13 December 1966. Six interviewers were then selected and prepared for conducting monthly visits to the women of child-bearing age. The monthly observation of women in Lurín began on 12 June 1967 and has continued on a regular basis. In addition to recording monthly information on menstruation, pregnancy, and live births with respect to those women, each home is visited every three months to discover any changes in the family, such as migration, births, or deaths. The forms and procedures for Lurín have been well developed and followed routinely.

One of the goals of the investigation is to estimate the frequency of pregnancy loss. The retrospective data revealed that 450 women had had 2,133 pregnancies, of which 162, or 7.6 per cent, resulted in early, intermediate, or late fetal deaths. Observations from the date of the census onward include 124 terminated pregnancies with 16 fetal deaths (12.9 per cent). Thus, the frequency of pregnancy appears to be higher in the observational data.

The census of Masma, the second community, was scheduled for the week of 4 December 1967.
SIGNIFICANCE

Observational (or longitudinal) data on women of child-bearing age, such as are being collected in this study, include information on the pregnancies that occur and their outcome, on intervals between pregnancies, and on the effects of their frequency on early fetal, late fetal, and infant mortality. Such observational data provide more complete records than do retrospective data. This is especially true for populations with a high rate of provoked abortions. The research program will 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 the relation to subsequent fetal and infant deaths and maternal health is studied best by the longitudinal method. It is expected that knowledge of the health implications of provoked abortions will result from this research.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: World Health Organization

Timetable: 1966-1969
PROSPECTIVE STUDY OF FERTILITY IN SÃO PAULO, BRAZIL

PROBLEM

The objectives of the study are to provide data on the significance of provoked abortions and their relationship to maternal deaths in the city of São Paulo; to detect the possible factors - biological, social and economic - leading to the abortions; and to provide a complete record of the pregnancies that occur within the period of study, including the condition of the child at birth, perinatal mortality and its relation to prenatal care, delivery, number of previous pregnancies, duration of pregnancy, and socioeconomic factors.

METHOD

The study was conducted on a sample basis using a subsample of 1,500 women from the retrospective study of human reproduction completed in 1965 in São Paulo. A record was obtained of each woman of child-bearing age (15-49 years), and the women were followed for a one-year period through interviews by social workers and health workers. Home visits were made in November 1966 and March, July, and November 1967.

RESULTS TO DATE

The prospective data from the four interviews have been collected and plans have been made to enter them on punch cards for processing by computer.

SIGNIFICANCE

This project is a pilot study to collect observational data on women of child-bearing age. The results will be compared with those obtained in the retrospective fertility study conducted in São Paulo. The project, as well as the longitudinal investigation being carried out in communities in Peru, will contribute to understanding the problem of abortion and the relative value of the different methods of data collection among women of child-bearing age.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee:  Dr. Maria Lucila Milanesi, Faculty of Hygiene and Public Health, University of São Paulo, São Paulo, Brazil

Funded by:  Pan American Health Organization/World Health Organization

Timetable:  1966-1967
PARASITIC DISEASE STUDIES ON SERUM SPECIMENS

PROBLEM

The purpose of this project is to study antibodies in a serum bank of approximately 20,000 specimens assembled by INCAP and the Middle America Research Unit during the course of the INCAP-NIH/OIR Nutrition Survey.

Specifically, the project is undertaking to test the sera for Toxoplasma antibodies, to investigate antibodies to Chagas' disease, and to investigate antibodies to the malaria parasites.

METHOD

Sera subsamples are drawn from a serum bank of approximately 20,000 specimens taken from people throughout all of Central America. These sera are screened for Toxoplasma antibodies by the fluorescent antibody method. The complement-fixation test is employed to investigate antibodies to Chagas' disease.

Eventually, antibodies to malaria will be investigated by the fluorescent antibody procedure.

RESULTS

The preliminary results showed prevalence rates of complement-fixing antibodies to Chagas' disease in Guatemala ranging from 0 to 13% in the various localities studied. There was a correlation between higher prevalence rates and lower altitudes, which is probably accounted for by the higher density of vectors in the lowlands.

In El Salvador the prevalence was higher than in Guatemala. Rates ranged from 0 to 20%.

The case of Nicaragua is of particular interest because there is little information available on the epidemiology of Chagas' disease in that country. The rates ranged from 0 to 20%, indicating that the problem is as acute there as in El Salvador. The endemic areas in Nicaragua are concentrated on the Pacific slope.

High prevalence rates of Toxoplasma antibodies have been found in all the countries.
SIGNIFICANCE

No comprehensive seroepidemiologic survey such as this has been conducted before in Central America, or in any other large developing area. The study of a large number of serum samples for antibodies to agents of importance in terms of their contribution to morbidity and mortality has important public health implications. The known interaction between nutrition and infection makes these investigations even more significant for the over all health of the population.

PUBLICATIONS

Preliminary results have been presented at scientific meetings.

OTHER DATA

Grantee: Dr. Bryce C. Walton, Middle America Research Unit and Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala

Funded by: Middle America Research Unit/U.S. Public Health Service

Timetable: 1965-1968
STUDY OF ANTIMALARIAL DRUG THERAPY

PROBLEM

The radical cure of *P. vivax* infections is an essential part of malaria eradication in the late attack and consolidation phases. The standard treatment is chloroquine plus 14 daily doses of primaquine, which should be given under supervision. In rural areas, supervision of the 14-day course is not economically feasible.

Various attempts have been made to shorten the 14-day course. A 5-day treatment has produced fair results in India and Mexico, though not as good as those from the 14-day series.

The Colombia Malaria Eradication Service made a preliminary trial of three antimalarial drugs given in combination over a 3-day period. The adult dose was as follows: 750 mg chloroquine (less than standard amount); 75 mg primaquine (same as that of the 5-day course); and 100 mg pyrimethamine. The pyrimethamine was added to assist the action of the other two drugs. The first results have been promising.

The present project was set up in cooperation with the Colombia Malaria Eradication Service to measure more accurately the field response of *P. vivax* cases to the 3-day-triple-drug treatment as compared with the standard 14-day radical treatment. Malaria transmission continues in the study area and reinfections cannot be excluded.

METHOD

Periodic malaria surveys are made in an area of Colombia that has a fairly high incidence of cases. Positive cases in the same vicinity are paired as nearly as possible by age and sex, one person to receive the 3-day treatment and the other to receive the 14-day treatment. At the same time a noninfected person is matched to the pair to produce a control group, and is given a 14-day treatment with primaquine to assure that he is really free of latent malaria infection.

The trios so constituted are followed monthly for at least a year, new trios being formed until there are enough subjects in the study to yield significant results.
The number of relapses or reinfections in the two groups of treated cases will be compared month by month with each other and with those in the third or control group. The latter group is expected to indicate the probability of reinfections in the other two groups.

RESULTS TO DATE

Admission of new cases to the study was terminated in October 1967; the follow-up is expected to be completed by mid-1968, after which a final analysis will be made, using advanced factor-analysis methods.

Some of the trios were incomplete owing to lack of comparable subjects or to the loss of one member from the study. In such cases pairs are being studied. Preliminary data show more positives (new infections or relapses) in both the treatment groups - the 3-day and the 14-day - than in the control group, and no impressive difference in frequency of positives between the 3-day and the 14-day schedule. However, no firm conclusion is possible at this stage.

SIGNIFICANCE

If it can be shown that relapses are equal or only slightly more frequent under the 3-day-triple-drug schedule than under the standard 14-day schedule, it is reasonable to hope that radical cure of \( P. \) vivax cases can be carried out under the complete supervision of malaria eradication service's personnel, and considerable savings in effort and cost, or improvement in results, may be expected.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Carlos A. Ferro Vargas, Malaria Eradication Service, Bogotá, Colombia

Funded by: Pan American Health Organization and the Colombia Malaria Eradication Service

Timetable: 1966-1968
FIELD INVESTIGATIONS OF MASS ANTIMALARIAL DRUG TREATMENT

PROBLEM

One of the problems encountered in carrying out large-scale mass drug distribution over a long period is the discomfort caused by chloroquine. Dizziness, nausea, stomach distress, and other reactions are experienced by 25% or more of the persons taking the drug. A different combination of drugs that would accomplish the same result (suppression of parasitemia plus radical cure of existing infections) without causing these reactions would be more acceptable to a higher percentage of persons and could be administered over a longer period of time.

An earlier experience in Panama indicated that pyrimethamine 50 mg plus primaquine 40 mg (adult dose) was acceptable to enough people in several Panamanian villages to clear up some of these villages completely. It was administered by local leaders on a weekly basis without close supervision.

Malaria eradication programs have found chloroquine-primaquine, to be effective in 2-week cycles of administration, but medication by paid staff members was necessary.

The purpose of the present study was to determine whether pyrimethamine-primaquine tablets could be used effectively in 2-week cycles and how well they would be accepted over a long period if given by service mediators.

In addition to the therapeutic effectiveness of this drug combination, the frequency of complaints and refusals was also to be ascertained.

METHOD

The Sambú River basin, an area of high malaria incidence, with a comparatively stable population, of a size that could be covered by 4 mediators was selected. The treatments were to be given every 2 weeks by mediators employed by the project. Mass malaria surveys were made twice before the treatment started, and again at the time of the first cycle and every fourth cycle thereafter (fifth, ninth, etc.). Four different dosages were used according to body weight. An incentive was provided in the form of free medical attention once a month for those who cooperated in taking their medication regularly. Slides were taken from fever cases during the nonsurvey cycles.
Slide-positive cases were given 1500 mg of chloroquine (adult dose) at a subsequent visit to eliminate any risk of pyrimethamine resistance becoming established.

A few persons who apparently became positive within a week or two after receiving the pyrimethamine-primaquine combination were taken to Panama city for study in the hospital.

RESULTS TO DATE

The treatment actually began 6 June 1966. At the time of the first cycle over 17% of the population was positive. This proportion dropped to 2.4% after 8 weeks, and to 1.0% after 16 weeks (8 cycles). It has remained near or below this level ever since.

Unfortunately, the area received quite a large influx of people. The population of 1700 at the time of the first cycle rose at times to as much as 2400. The availability of medical care seemed to be one reason for the immigration. This fluctuation made for great difficulties in measuring the percentage of population treated, but it was seldom over 80%; it varied from 61% to 79% and averaged about 70%. The large majority of untreated persons were absentees; refusals were generally limited to persons holding certain religious beliefs.

There were few complaints of reactions to the drug, and these not serious enough to prevent continuance of the treatment.

The malaria that persisted in the area was almost 100% P. falciparum and was usually related to imported cases. There were very few cases that seemed to clear up only temporarily after pyrimethamine or pyrimethamine-primaquine. Almost all the patients received chloroquine at a later cycle, with permanent clearance of the parasitemia. P. vivax has been rare in the area since treatment began. Although several P. falciparum cases appeared in persons supposedly treated two weeks before, the overall incidence became and has remained so low that pyrimethamine resistance is not considered to have been a cause of failure, or a contraindication for use of this combination.

SIGNIFICANCE

This study is mainly operational research, designed to see whether or not 2-week cycles of pyrimethamine-primaquine combined tablets could be used in a mass drug campaign where chloroquine-primaquine is not well accepted because of too uncomfortable reactions.
The drug was generally effective in rendering the patients free of parasites and in preventing relapses. It did not do as well as chloroquine-primaquine in other programs against *P. falciparum*, but it showed no sign of failure against *P. vivax*. Indeed, evidence of transmission and relapses of this species in the population treated (mostly Amerindian) was rapidly halted.

Complaints were low enough to encourage the hope that this drug combination could be offered to persons who genuinely suffer uncomfortable reactions to chloroquine in the standard mass drug distribution program. However, since it appears to be somewhat less effective therapeutically against *P. falciparum*, its full substitution for chloroquine-primaquine would be undesirable. The study will terminate in April 1968 and a report will be published.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Ralph Comer, Gorgas Memorial Laboratory, Panama City, Panama

Funded by: Pan American Health Organization

Timetable: May 1966 – April 1968
PROBLEM

In mass drug distribution campaigns, the population soon becomes tired of taking tablets every two weeks, especially when they cause some discomfort, as in the case of chloroquine. The people begin to refuse treatment, absent themselves, or pretend to swallow the drug and spit it out.

The purpose of this project is to determine the feasibility of using subprofessional medicators to administer intramuscular injections of long-acting cycloguanil pamoate (CI-501 or Camolar) in a large-scale operation, the acceptability of this medication to a population requiring mass drug distribution, and its therapeutic effectiveness. In addition, methods and norms for operation need to be established, and recording systems, comparative personnel requirements, costs, and the like need to be studied.

The drug had been given in 15 previous field trials in other parts of the world, and revised dosages had been worked out that were demonstrated to be safe and effective.

The Government of Guatemala agreed to furnish a physician and a subprofessional staff. Fewer subprofessional staff members are needed for an injectable antimalarial drug program such as this than for projects in which the drug is given by mouth.

METHOD

Personnel to administer the drug - nurses or nurse's aides - were given training in intramuscular injections, and special training in the handling and injection of Camolar, which is a thick suspension. Seven men and eight women were selected to perform house-to-house injections, working in pairs whenever possible.

Discardable 6 ml plastic syringes and discardable sterile needles, 1 1/2 inch, 18-gauge, were supplied. To reduce local reactions, 20-gauge needles were substituted, and they caused much less apprehension and trauma.

Attempts were made to induce people to come to injection centers, but at best not more than 50% did so, and those who did not come were sometimes more difficult to treat in the clean-up house-to-house visits owing to the local reactions current among some of their relatives. After the first week, all injections were made in visits to houses, with revisits in the late afternoon or next morning to meet absentees; a second sweep of the area was made after the first coverage.
Two districts with a population of about 6,000 each were selected. Each district was covered in about 6 weeks by 15 medicators.

Drug sensitivity tests were performed before the area was selected, and preliminary treatment with proguanil was tried in 26 heavily infected persons. All cleared up eventually, though some *P. vivax* cases took more than 6 days to do so.

Malaria surveys were conducted at the time of the first round of injections and again at 4 and 5 1/2 months. In addition, passive case detection and search for cases of known contact were carried out continuously.

All moderate and severe reactions were examined by physicians, and several treatments were tried.

**RESULTS TO DATE**

Two rounds of injections have been completed in the trial area, and acceptance was better (average 80%) in the second round than in the first (average 77%). Both were better than acceptance of oral treatments. The chief obstacle to full acceptance was local reaction caused by the depot drug. More than half of the subjects had some local feeling of pain on walking or tenderness to pressure. Most cases were mild and transient, but 2% or more were severe enough to prevent work for one or more days. Reactions were of two types - early, which appeared in a few hours or a day or two and were usually mild and short-lived, and delayed, which appeared after 8 to 30 days or more of latency, lasted a longer time, showed more induration, and caused more pain and interference with activity. Fortunately, the latter were quite rare.

In spite of the reactions, most persons willingly accepted the second injection, and as a rule complaints of reactions were fewer the second time. This was felt to be the result of a number of factors: better technique on the part of now experienced injectors, use of smaller needles, provision of aspirin to each household at the time of injection, and the use of antihistamines and corticosteroids in treating the severe cases. The response to the latter drug was rapid and gratifying.

There was no evidence of generalized sensitivity in either the first or second round.

The malaria incidence was kept very low in the injected group during 6 months, but a few breakthroughs occurred from 3 months onward, mainly in persons with moderate and severe reactions that cause more rapid absorption of the deposit of drug. There has been no evidence as yet of resistance to the drug. No infected reactions have occurred, but in the
first round 1 out of 1,000 persons injected developed a draining sinus from which drug and sterile pus were extruded. These were considered to be due to deposits in subcutaneous tissue. They were almost painless and healed rapidly.

SIGNIFICANCE

This is operational research. It has developed a body of experience essential for planning the future use of this arm of attack on malaria in any eradication program. Various techniques of administering the program have been considered, including health education projects. In addition, the type of training and the amount and kind of manpower needed for carrying out a large-scale injection campaign have been determined. The program has developed and tested techniques of injection designed to reduce local reactions, and it has tested various methods of treating disabling local reactions.

The need for high-quality training and supervision is obvious, and the method should not be attempted unless these are available. Third and fourth rounds are planned for the same population group (about 13,500 persons, 10,500 injected in each round) to watch for resistance of the parasite or of the subjects themselves.

The cost, in terms of both personnel and supplies, is lower with this injectable long-acting drug, and so far the acceptance is better than with oral mass drug distribution. However, it does not produce radical cures of \( P. \) \textit{vivax}, and it may therefore have to be continued over a longer period, thus incurring a greater risk of drug resistance.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. L. J. Uribe, Guatemala Malaria Eradication Program, Guatemala City, Guatemala

Funded by: Pan American Health Organization

Timetable: 1967-1969
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 situation 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 aim of the project is 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.

METHOD

Initially, the use of new insecticides and of DDT on different types of surfaces 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 were performed using standard WHO methods. For measuring the impact of irritability on lethality of residues, the mosquitos 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 under the WHO integrated study has been concentrated on a new carbamate, OMS-33. It includes Stage IV, Experimental Hut Trials, in which seven huts have been built and under test since July, and Stage V, Village-Scale Trials, in which three villages have received two rounds of spraying. Stage VI of the WHO Scheme, a large-scale operational field trial was begun on 3200 houses in April 1966. The entomological effectiveness was studied by all standard applicable methods, and, in addition, wall traps were developed, and the ratio of live to dead mosquitoes captured in houses in the morning was found very useful. Extensive toxicological observations were made on spraymen and on the residents of the sprayed houses.

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 three 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, owing to protection afforded the 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 on the use of the E-R test box for evaluating new insecticides. The probable effect of a new insecticide placed over old residual DDT deposits was measured. Carbamates were especially good at "neutralizing" the irritation caused by DDT.

The 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). The large-scale operational field trial brought out several new aspects of the action of OMS-33, namely its marked air-borne effects for four to five weeks and its sterilizing effects in compact villages in contrast to its short-term effects (six weeks sometimes) in isolated, well-ventilated, houses. It was shown that clinical signs of cholinesterase reduction (nausea, vomiting,
weaknesses, etc.) came on so early and were so rapidly reversible (1/2 to 2 hours) that these served to prevent any serious overexposure of spraymen or residents. The population liked the insecticide because it killed all household pests, and they gladly accepted spraying every three months.

The preliminary measurements of malaria incidence show a marked superiority to DDT but the definition of epidemiological conditions under which it will succeed or fail to halt all transmission remains to be made.

SIGNIFICANCE

All these studies are fundamental to finding an effective and economic 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 causes for failure to halt transmission in the Americas.

PUBLICATIONS


3. Whittemore, F. W. "The Selection of Insecticides for Full Scale Evaluation in Malaria Problem Areas." In preparation


OTHER DATA

Grantee: Mr. J. R. Austin, Pan American Health Organization, El Salvador, C. A.

Funded by: Pan American Health Organization/World Health Organization

Timetable: 1959-1967
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 the Americas in point of population, though in degree the level of residual persistence is only about 2 to 8 cases annually per 1000 population. The worst of the problem area has received a 6 1/2-month course of mass drug treatment. This has proved to be an effective but very expensive method, since, because of migration, permanent success cannot be assured until the entire problem area is brought under control. It has been followed by attempts to halt transmission through increasing the frequency of spraying, but with only slight benefits.

The present effort is to determine, as an additional method of attack, 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.

**METHOD**

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

PAHO personnel were withdrawn in June 1966 owing to loss of key staff. Striking findings came from the study of persistence in four localities where agricultural workers slept in unsprayed temporary shacks or in the open. The Mexican Government carried on the project until December 1967. The year 1967 saw an increase in malaria in the area under study, owing partly to more favorable transmission conditions. Because of the cost of the operation and a shortage of funds, the project was terminated in December 1967.

SIGNIFICANCE

Malaria eradication by use of residual DDT has progressed considerably in Mexico. Some small additional attack measures beyond residual spraying of houses will be required among about 2 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 being explored. This is far more acceptable to the public than mass drug treatment. If it is shown to have the scope anticipated, 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 funds.

PUBLICATIONS

OTHER DATA

Grantee: Dr. Pérez Yekutiel, Pan American Health Organization, Mexico, D.F., Mexico

Funded by: World Health Organization and the Government of Mexico

Timetable: 1964-1967
PLAN OF INDIVIDUAL RESPONSIBILITY FOR MALARIA CONTROL
IN LIMITED AREAS

PROBLEM

In some problem areas, the building of new or temporary houses, the alteration of existing houses, and the washing, painting, or other modification of sprayed surfaces between spray cycles impair the success of DDT spraying programs.

It has been found in such areas that the unaltered sprayed surfaces often appear white from the accumulation of deposits from previous sprayings. Tests of residues have shown that wherever such deposits are visible they are biologically active against the vectors.

It was desired to test the feasibility and economy of having one man perform the dual function of searching for malaria cases in monthly rounds of a limited and permanently assigned area, and at the same time of examining visually the condition of spray deposits in each house, spraying all new surfaces and any old surfaces on which a spray deposit was not visible.

METHOD

In the states of Morelos and Puebla, where malaria had persisted because of the foregoing factors, and where the vector was susceptible to DDT, three districts were chosen, having 55 localities, 75,566 houses, and an estimated population of 267,000. Because of the high population density and good communications, a high output per worker was planned. An average of 690 houses and 2,600 inhabitants were to be visited once a month by each worker.

Each worker was trained and expected to visit each house, take blood slides from fever cases, look for and respray all wall surfaces that did not have visible deposits, maintain the supplies for the voluntary collaborator posts in his area (average of four per worker), assure that the existence of the posts was known to the residents.

All cases discovered were to be investigated and given radical treatment by the chief of the group or extra assigned personnel, if needed.
RESULTS TO DATE

Migration into the area was much larger than expected. At one time the population reached 379,000. Temporary shelters became a very large problem in the rice-growing area. The addition of 11% new houses and the alteration of 11% of the surfaces during the first six months of operation placed a heavy demand on respraying.

The results were very encouraging in two districts but disappointing in the third. In the beginning, the enthusiasm of each worker was high because of his individual responsibility for his area. The work load was probably too heavy in some areas, and the spraying was not always well done. There was too little flexibility to adjust the size of work districts to the changing volume of new houses.

At the end of two years, it was felt that the method had proved to be a success in two districts but a failure in the third, where a full-scale respraying had to be done. Because of the costs of the operation, which were now in addition to those for regular spraying, and because of lack of finances, it was not possible to continue.

SIGNIFICANCE

This was purely operational research to determine the effectiveness and economy of another possible method for spraying new houses and altering spray deposits in old houses between spray cycles. It was hoped that assigning to one worker complete responsibility for maintaining a limited area protected with DDT while at the same time looking for malaria cases might be more effective than routine spraying every six months - and more economical, owing to the heavy DDT deposits already found inside many houses.

The capacity of individual workers to carry out such an operation under various conditions of population density, migration, and alteration of houses, has been determined. The limits of effectiveness of the method and its comparative costs have been determined for use in future planning, should funds become available. The necessity for operational flexibility was demonstrated.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: National Commission for the Eradication of Malaria, Mexico City, Mexico

Funded by: Pan American Health Organization and the Mexican National Commission for the Eradication of Malaria

Timetable: 1966-1967
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, made of six individuals - four from Brazil, one from Chile, and one from the United States - representing both clinical and research interests and coming from various types of organizations, such as medical schools, national laboratories, and pharmaceutical laboratories. These individuals have been consulted separately by the principal investigator; they met together during the 7th International Congresses of Tropical Medicine and Malaria to exchange ideas and agree on procedures; and they have been kept informed of each other's work through correspondence.

METHOD

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 at the same time damaging the host. Workers on both these approaches are represented in the Chagas' Disease Chemotherapy Research Group, who have not only performed their own research 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 on the criteria of cure have also been included in the Group's endeavours.

RESULTS

In addition to empirical screening of new compounds, a number of more basic studies have continued, including comparisons of selected chemotherapeutic substances against a variety of strains of *T. cruzi*, reevaluation of older compounds (the action of which was uncertain) in experimental models, further development of tissue culture techniques that allow for better understanding of the mode and site of action of various drugs, metabolic studies for pathways of nucleic acid synthesis, and a search for factors that may modify the functioning of these pathways.

The Group met for a second time three years after its inception and reviewed its progress. After a discussion of the Group's limitations and a critical evaluation of its functions and potentialities, it was decided that this association of experts had value and should be maintained. The active members of the Group now consist of two individuals each from three major representative fields - experimental chemotherapy, biochemistry-physiology, and clinical medicine.

New types of compounds with activity against *T. cruzi* have been found, and several substances are now undergoing laboratory testing in anticipation of clinical trials by the Group if preliminary observations on high efficacy with satisfactory tolerance are borne out by extended pre-clinical investigations.

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* is desirable. 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.

PUBLICATIONS


OTHER DATA

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

Funded by: Pan American Health Organization

Timetable: 1963-continuing
A COOPERATIVE STUDY OF COMPLEMENT-FIXATION ANTIGENS
FOR THE DIAGNOSIS OF CHAGAS' DISEASE

PROBLEM

The aim of the project is to select the best complement-fixation antigen for routine diagnosis of Chagas' disease; to adopt one antigen as a standard; and to develop a standard method for the Chagas' disease CF test.

METHOD

Seven laboratories in six countries will test eleven antigens against sera collected in various endemic zones. Prior to the tests, the sera will be collected and distributed to the cooperating laboratories for a "blind" study. All laboratories will use a standard method adopted by consensus. The results will be collected and analyzed at PAHO headquarters.

RESULTS TO DATE

As of the end of 1967, the eleven antigens had all undergone a specificity test against approximately 100 negative sera. In addition, standard procedures for the test had been agreed upon.

SIGNIFICANCE

A standard antigen and a standard procedure are needed to stabilize and improve the existing data on the prevalence and distribution of Chagas' disease. When these are adopted it will be possible to compare data from different laboratories. It will also be possible to proceed with a program to determine the prevalence and distribution of the disease throughout the Americas. This will lead to a more rational attack on the disease in the various endemic areas.

The project will also permit evaluation of the quality of the work in the cooperating laboratories and will serve as a training program in CF serology.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Seven cooperating laboratories (incidental costs). The coordinator of the study (travel)

Funded by: Pan American Health Organization

Timetable: 1967-1969
A RETROSPECTIVE STUDY ON THE DEVELOPMENT OF CHAGAS' DISEASE SYMPTOMS IN PATIENTS INFECTED FOR UP TO SEVENTEEN YEARS

PROBLEM

The progress of Chagas' disease in persons diagnosed at various times in the past is being studied by reviewing their clinical histories and by re-examining them in 1967.

The aim is to measure the extent to which infected persons acquire clinical signs of damage due to the infection as the years pass.

METHOD

Several hundred persons with a confirmed diagnosis of Chagas' disease in the past are being examined. In some cases the acute attack was recorded as long as 17 years before the initiation of the current study.

Four clinicians from four endemic zones of Brazil are cooperating in the investigation.

All clinical records will be searched for changes in symptoms. The 1967 tests and physical findings will be checked against the older records.

RESULTS TO DATE

None are reported.

SIGNIFICANCE

Longitudinal studies of Chagas' disease are needed to determine the clinical significance of infection and the probability of serious consequences over time. There is essentially no information on the subject.

This is a retrospective study. Though it is less useful than a true longitudinal study, it has the merit of being less expensive.

The availability of excellent records and the cooperation of strongly interested physicians has made this investigation possible.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Aluizio Prata, Hospital das Clínicas, Salvador, Bahia, Brazil

Funded by: Pan American Health Organization

Timetable: 1967
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 purpose of the present investigations in Mexico and Central America is (a) to study the ecology, geographic distribution, 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.

METHOD

Specimens likely to yield arboviruses, such as mosquitoes, mammals, and birds as well as sera to detect arbovirus antibodies, are being collected in Mexico and Central America. Sentinel animals such as Syrian hamsters are exposed in coarse wire mesh cages, hung under protective roofs in suitable habitats, to detect virus transmission by flying arthropods. Virus isolations are carried out in suckling mice and in various cell culture systems. Antibody tests are performed by neutralization, hemagglutination-inhibition, and complement-fixation techniques.

RESULTS TO DATE

Venezuelan encephalitis virus and a group C arbovirus were found to be active in study areas on the Atlantic coastal lowlands of Mexico between February and May 1966, and Patois virus was active in these same areas in April and May. During the summer of 1966 a horse epizootic occurred in Tamaulipas. Patois virus was isolated for the first time in Belize in April and May 1966 through the use of sentinel hamsters. During the period July to September 1967, Venezuelan encephalitis, a group C virus, and Patois virus were isolated for the first time in Honduras; Venezuelan encephalitis was isolated for the first time in Belize; and Patois was again isolated in the latter county. Additional agents from mosquitoes, birds, wild mammals, and sentinel hamsters are under further study.
SIGNIFICANCE

The research provides information on arboviruses, especially Venezuelan encephalitis virus, in Mexico and Central America, and it evaluates the role of birds and domestic animals in the ecology of arboviruses. Complete understanding of the natural cycles of arboviruses will make way for intelligent approaches to the prevention and control of epidemics caused by these viruses.

PUBLICATIONS


**OTHER DATA**

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

Funded by: United States Army Medical Research and Development Command

Timetable: 1961-1967
HERON ECOLOGY AND NORTH AMERICAN ENCEPHALITIS VIRUSES

PROBLEM

The effectiveness of wild birds in the natural history and distribution of arthropod-borne animal viruses is related to the rapidity of their population turnover, their ability to move quickly over long distances, the regularity of their migrations, and their temporal association and mixing with other animals and with human populations. However, there is still a lack of critical information concerning life histories of many avian species, even among the better-known groups. The purpose of this research program is to quantitate the involvement of birds, especially herons (order Ciconiiformes), and associated colonial nesting species in natural cycles of arboviruses.

METHOD

Research has centered along four lines during the past year. 1) Field work was continued through the collection of blood and plasma from nestling herons and associated nesting birds at two colonies in Mexico, one of which had not been previously sampled. Sentinel hamsters were exposed at three study areas in the vicinity of Minatitlán, Veracruz, to determine the level of virus activity in the vicinity of nesting colonies. 2) Laboratory work was continued. Virus isolations were attempted in suckling mice and tissue culture systems, and antibody studies were made on previously collected materials. 3) Aviary studies were carried out on four species of herons that range widely throughout Mexico and Central America to determine the reactions of each to inoculation with "mosquito-size doses" of Venezuelan encephalitis virus. 4) Manuscript work was continued.

RESULTS TO DATE

Laboratory analysis of field materials is in progress. Venezuelan encephalitis and a group C virus have been isolated from hamsters exposed near Minatitlán in 1967. One or possibly more agents from the blood of nestling herons are under study. Three of the four species of herons inoculated with Venezuelan encephalitis virus developed viremia lasting one or more days. The level of viremia is now under study. One major manuscript has been submitted and others are under preparation on life history studies conducted at San Blas, Nayarit.
SIGNIFICANCE

The serological studies, as yet incomplete, indicate major differences in frequency of antibodies to a given virus among heron species nesting in the same colony. Aviary studies that eliminate behavioral and microhabitat differences may determine whether these differences are under genetic control. Thus they may reveal differential adaptation to arboviruses and help to explain the varying degrees of involvement in arbovirus cycles by various species of the order Ciconiiformes. Studies of the nesting biology of the Green Heron in Mexico constitute the first major research on a tropical nesting heron and provide comparison with the well-documented behavior of this same species from temperate North America. The detailed studies being conducted on the nesting biology of Boat-Billed Herons are the first investigations made of this unique species.

PUBLICATIONS


OTHER DATA

Grantee: Dr. Robert W. Dickerman, Cornell University Medical College, New York, N.Y.

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

Timetable: 1967-1971
IDENTIFICATION OF TYPES AND SUBTYPES OF FOOT-AND-MOUTH DISEASE VIRUS

PROBLEM

The plurality of strains of foot-and-mouth disease virus (FMDV) 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 the increasing use of vaccination in various countries.

METHOD

The following methods are used for identifying the strains:

Complement-fixation (50% hemolysis)
Passages in guinea pigs, suckling mice, and/or cattle
Obtainment of homologous sera in guinea pigs
Serum protection of suckling mice
Cross-immunity in guinea pigs and cattle

RESULTS TO DATE

During the period from 1962 to 1967, 3,124 specimens received from 18 countries and 20,272 specimens obtained during the course of research at the Pan American Foot-and-Mouth Disease Center were typed.

To date, 12 subtypes of foot-and-mouth disease virus have been identified and have been recognized by the World Reference Laboratory in Foot-and-Mouth at Pirbright, England. The following table shows the designations they have been given:
<table>
<thead>
<tr>
<th>Number and Name of Subtype</th>
<th>Year of Isolation</th>
<th>Countries Where It Has Been Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>O8 - Brazil - O Bahia</td>
<td>1959</td>
<td>Brazil (1959), Argentina (1962), Uruguay (1962)</td>
</tr>
<tr>
<td>A13 - Brazil - A Santos</td>
<td>1958</td>
<td>Brazil (1958), Uruguay (1960)</td>
</tr>
<tr>
<td>A16 - Brazil - A Belém</td>
<td>1959</td>
<td>Brazil (1959)</td>
</tr>
<tr>
<td>A17 - Brazil - A Guarulhos</td>
<td>1959</td>
<td>Brazil (1959), Argentina (1961),</td>
</tr>
<tr>
<td>A19 - Argentina - Suipacha</td>
<td>1962</td>
<td>Argentina (1962), Brazil (1964), Uruguay (1964)</td>
</tr>
<tr>
<td>A24 - Brazil - A Cruzeiro</td>
<td>1955</td>
<td>Brazil (1955)</td>
</tr>
<tr>
<td>A25 - (Argentina 59)</td>
<td>1959</td>
<td>Argentina (1959)</td>
</tr>
<tr>
<td>A26 - (Argentina 66)</td>
<td>1966</td>
<td>Argentina (1966), Peru (1967)</td>
</tr>
<tr>
<td>A27 - (Colombia 67)</td>
<td>1967</td>
<td>Colombia (1967), Peru (1968)</td>
</tr>
<tr>
<td>C - Brazil - C Resende</td>
<td>1955</td>
<td>Brazil (1955)</td>
</tr>
<tr>
<td>C - Argentina - Tierra del Fuego C</td>
<td>1967</td>
<td>Argentina (1967)</td>
</tr>
</tbody>
</table>

**SIGNIFICANCE**

The results of this research point to the need for continuous screening of 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.

PUBLICATIONS


OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

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 (FMDV) 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.

METHOD

Specimens are collected using the apparatus described by Van Bekkum which, upon being inserted into the esophagus, permits the collection of a mucosal-saliva mixture 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 IU 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

By inoculation in suckling mice and tissue culture, foot-and-mouth disease virus can be demonstrated in esophageal/pharyngeal material. 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 six months after the outbreak was low for cattle and high for pigs.
Studies performed with esophageal/pharyngeal material obtained from buffaloes naturally infected with FMDV type O seem to demonstrate that the carrier state is more difficult to be established in these animals than in cattle. This correlates with the mild symptoms observed in the infected animals, followed by a quick recovery, although the buffaloes had never been vaccinated or previously exposed to FMDV.

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.

PUBLICATIONS


OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American

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 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.

METHOD

The following methods of identification are used:

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

During 1967 two outbreaks of vesicular stomatitis virus in South America, one among horses and mules in the State of São Paulo, Brazil, and the other among horses in the Province of Misiones, Argentina, proved to be caused by a virus belonging to the Argentine subtype of the Indiana serotype.
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.

PUBLICATIONS


OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

Timetable: 1957 - continuing
FOOT-AND-MOUTH DISEASE ANTIBODIES IN SERUM DRIED ON FILTER PAPER

PROBLEM

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

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

METHOD

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 is determined, as is the maximum adsorption capacity of a known area. The sera of animals with known backgrounds are broken down into their constituent protein fractions, and elution tests are performed comparing the sera and their proteins adsorbed separately on paper.

Once these results are obtained, the efficiency of sera adsorbed on paper is 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

Bovine serum can be dried on blotting paper and stored without refrigeration for at least 60 days without interfering with the detection of antibodies against foot-and-mouth disease virus (FMDV). The same technique has been applied successfully to blood, but without determination of the storage time.

The virus-neutralizing activity of the dried specimens was, on the average, lower than that of the control frozen serum samples, but the difference was of no practical importance in the interpretation of individual results.
It was found that an area $25 \text{ cm}^2$ of saturated Whatman paper absorbs 0.925 ml of serum and that $1 \text{ cm}^2$ eluates 1.75 mg of total protein (1.06 mg of globulins and 0.664 mg of albumin).

The efficiency of the elution of the adsorbed proteins is 69 per cent. An area of $50 \text{ cm}^2$ of Whatman paper absorbs 1.85 ml of serum, which corresponds to 1 ml of serum.

**SIGNIFICANCE**

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

Preliminary results point to its possible use in serological studies and in epizootiological surveys of foot-and-mouth disease in zones where access is difficult, where working conditions are not suitable for the usual techniques, and where slow and defective transportation of specimens affects the results.

**PUBLICATIONS**


**OTHER DATA**

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

**Funded by:** Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

**Timetable:** 1963 - continuing
APPLICATION OF THE AGAR GEL PRECIPITIN TEST IN THE DETECTION OF FOOT-AND-MOUTH DISEASE ANTIBODIES

PROBLEM

Although several methods (serum neutralization in tissue culture, serum-protection in baby mice, and complement-fixation) are already available for the detection of foot-and-mouth antibodies in sera from convalescent or immunized animals, it is of interest to study the susceptibility and accuracy of the gel precipitin test for the same purpose, in view of its simplicity and rapidity and the fact that only a small amount of serum is needed to perform it.

METHOD

The reactions were carried out in 85 mm plastic Petri dishes containing 15 ml of 1% washed agar. The diluent used provided a final concentration of 1.0 M glycine, 0.0125 M sodium barbital, and 0.5% sodium azide. The pH was adjusted to 7.9 by the addition of 1 N HCl. Precipitin reactions were developed at 37° C.

In view of the limited availability of footpad vesicular fluids from infected guinea pigs, infected baby hamster kidney cell (BHK-21) cultures are being used. The fluids are concentrated 10- to 50-fold by precipitation with half saturated (NH₄)₂SO₄ and then dialyzed. The concentrated antigen is stored at -20° C until it is processed.

Antisera are obtained from cattle, sheep, and pigs infected experimentally or from field outbreaks.

RESULTS TO DATE

The specificity of the agar gel precipitin reaction could be demonstrated for convalescent sera from cattle, sheep, and guinea pigs. The research is now concerned with how long after infection the sera of different species of animals give a positive and clear reaction. The behavior of different subtypes will also be studied.

In a comparative study, 324 samples of sera from sheep were controlled. Two sera were positive for Waldmann type C virus (subtype Tierra del Fuego), which confirmed the results obtained with the serum-neutralization test in tissue culture and the serum-protection test in mice.
SIGNIFICANCE

Although the serum-neutralization in tissue culture, the serum-protection in mice, and the complement-fixation tests seem to be more sensitive than the agar gel precipitin test, the latter can be very useful when only a small amount of serum is available.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

Timetable: 1967
IN VITRO STUDIES OF THE KINETICS OF FOOT-AND-MOUTH DISEASE VIRUS

PROBLEM

The production of foot-and-mouth disease virus (FMDV) for the preparation of inactivated and modified live vaccines still presents many financial, technical, and scientific difficulties. Tissue culture techniques have proved very useful, especially with respect to isolation of the virus, the establishment of clones, serum-neutralization tests, and, recently, the use of the BHK-21 C-13 cell line as a source of the virus. There are serious objections, however, to the use of heteroploid cell lines for modification of the virus and for the production of vaccines for use in man and in animals used as food sources. Although 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. It is important, therefore, to study the relationships between the FMDV and the cell, using diploid cultures and strains of the virus that are antigenically potent.

METHOD

Because of the need to control the viral antigens in some of the tissue systems in which there is 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 orange and Jacobson strains and phase-contrast microscopy, is also used in studying the virus-cell binomium. In order to increase the probabilities of isolating the 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, cultures under different environmental conditions and from various tissues and organs such as brain, pancreas, heart, and muscle are being used.

RESULTS TO DATE

From pig, rabbit, and calf embryo organs and tissues, 41 diploid cell strains have been established with the following designations
RFB-I to RFB-XII (calf embryo kidneys)
RS-I to RS-XV (pig kidneys)
TFB-I to TFB-III (calf embryo thyroids)
PFB-I and PFB-II (calf embryo pancreas)
RC-I to RC-III (rabbit kidneys)
PC-I and PC-II (rabbit lungs)
TS-I (pig thyroid)
PS-I and PS-II (pig pancreas)

With regard to the susceptibility of these various diploid cells, it has been found that strain RS-I (derived from pig kidney) is as sensitive to FMDV as the cell line BHK-21 C-13. The others are less susceptible than the corresponding primary cultures. Adaptation and modification of several FMD virus strains to some of these diploid cells is under way, and some systems have already reached the 45th passage level.

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.

Serial subcultivations of diploid cell strains inoculated with FMDV in which the virus multiplies without any cytopathic effect were performed. Preliminary observations indicate that these "carrier" cultures, unlike parallel noninoculated cultures, undergo a series of morphological changes and do not enter into "phase III", whereas the control cultures cease to multiply. Some of the morphological changes correspond to those described by Stenkvis et al. when hyperimmune serum was used in tissue cultures previously inoculated with FMDV.

SIGNIFICANCE

A better knowledge of the basic properties of foot-and-mouth disease virus could lead to the preparation of vaccines that are more antigenic, easier to test, and more rationally and inexpensively 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. In view of the objections to the use of heteroploid strains, it is evident that diploid strains must be used in modifying the virus and producing the vaccines. 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 between "complete" transformations induced by oncogenic viruses and simple morphological transformation.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

Timetable: 1965 - continuing
DEVELOPMENT OF MODIFIED LIVE VIRUS VACCINES AGAINST FOOT-AND-MOUTH DISEASE IN EMBRYONATED CHICKEN EGGS

PROBLEM

Several investigators have demonstrated the possibility of immunizing cattle against foot-and-mouth disease with modified live virus vaccines. Virus multiplication in the organisms generally produces a solid and durable immunity, and its use as a modified live virus vaccine will depend on the possibility of lowering pathogenicity to an acceptable level and on good antigenicity.

METHOD

The three types of foot-and-mouth disease virus (FMDV) present in South America (A, O, and C) were adapted to 14 days embryonated eggs by intravenous inoculation and kept at 32 to 35°C, alternating, when necessary, with passages in suckling mice, tissue cultures, and one-day old chicks. In some cases, inoculated embryonated eggs were incubated at temperatures of from 26 to 28°C.

Samples are studied in susceptible animals from the standpoint of pathogenicity, infectivity, and immunogenicity, using the following methods:

a) In guinea pigs: intramuscular or intraplantar inoculation, to observe loss of infectivity, postvaccinal lesions (especially myocardial lesions), and immunity against homologous and heterologous subtypes.

b) In cattle: intradermal inoculation of the tongue (IDT), intramuscular inoculation (IM), and cohabitation, to assess possible post-vaccinal lesions, viremia, immunity response and contagiousness.

c) In pigs: oral, subcutaneous, intramuscular or intraplantar inoculation, and cohabitation, to study pathogenicity, immunity response, and contagiousness.

The vaccines are prepared using hearts and livers of chicken embryos, total chicken embryos, muscles of 4- to 6-day-old suckling mice, or cell cultures.
Challenge is made by intradermal tongue inoculation in cattle, intraplantar inoculation of 10,000 suckling mice LD<sub>50</sub> in pigs (one foot), or cohabitation with infected animals.

RESULTS TO DATE

The results of the studies performed with the Campos strain O (Vallee type O, subtype O,) modified by serial passage in 14 days embryonated eggs showed that its pathogenicity decreases progressively, maintaining a good immunity capacity up to the 99th passage level. Experiments performed with the 128th passage showed a marked decrease in immunity.

Data obtained with the 67th, 80th, and 92nd passages of Cruzeiro strain A (Vallee type A, subtype A<sub>24</sub>) in 14 days embryonated eggs showed that the pathogenicity of this virus for highly susceptible cattle (from FMD-free zones in Venezuela, Colombia, Ecuador, and Chile), was 16/32 for virus at the 67th passage, 13/32 at the 80th passage, and 16/104 at the 92nd. At this level the pathogenicity was very mild. In susceptible cattle from an enzootic zone (Brazil), the pathogenicity was milder: 4/18 and 0/66 for the 67th and the 92nd passage levels, respectively. A good immunity was attained in all the cattle vaccinated, even when the challenge was made using an antigenically different virus (subtypes A<sub>18</sub> and A<sub>19</sub>). There was no evidence of spread of infection from the vaccinated cattle to susceptible contact animals. This virus retains a high degree of pathogenicity when inoculated in pigs even at the 113th passage level.

The results obtained from preliminary experiments with the Waldmann type C virus (Resende subtype C, Brazil), modified at low temperatures, indicated a low pathogenicity for cattle and swine. Immunity was good for cattle and low for swine.

SIGNIFICANCE

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


OTHER DATA

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

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

Timetable: 1957 - continuing
BIOLOGICAL PROPERTIES OF MODIFIED LIVE FOOT-AND-MOUTH DISEASE VIRUSES

PROBLEM

The study of the biological properties of the modified live foot-and-mouth disease virus (FMDV) used in vaccines is of great importance for the differentiation of these viruses from the respective field strains. This is especially true since the physical-chemical markers, which are also being investigated, are not stable enough to serve as secure indicators for differentiation between modified and field viruses.

These studies are also important to determine the degree of attenuation (virulence) of the viruses under modification.

METHOD

The following tests are being performed:

Titration in cattle (intradermal tongue inoculation) and contagiousness for cattle and swine.

Pathogenicity for swine (intradermal heel inoculation) and contagiousness for cattle.

Comparative titration in embryonated eggs, suckling mice, and guinea pigs.

Titration in suckling mice of the virus obtained from the inoculated embryonated eggs.

RESULTS TO DATE

Vallée type O virus: Studies were carried out to compare the biological properties of a field virus from Colombia (O 4785, subtype O1) with a modified live virus strain (Campos type O E99, subtype O1).

The results showed a marked difference in the titers obtained in cattle: \(10^{-4.5}/0.1 \text{ ml}\) for the field virus and \(<10^{-1}/0.1 \text{ ml}\) for the modified virus. Severe lesions were observed in the tongue and in the four feet of the two cattle inoculated with the field strain. One of the two animals inoculated with the modified virus presented three local...
lesions, not confluent, at the points of inoculation with the $10^{-1}$ virus dilution, and benign lesions in the four feet. Virus isolated from this animal was titrated in cattle with negative results ($<10^{-1}$), and no generalization. Contagiousness for cattle was positive for the field virus and negative for the modified. Contagiousness for pigs was positive, with severe lesions for the field virus; one of the two contact pigs for the modified virus showed benign lesions. Both viruses were shown to be pathogenic for pigs, the lesions from the field virus being more severe. Contagiousness for cattle was positive in the case of field virus and negative in the modified.

The comparative titration in embryonated eggs, suckling mice, and guinea pigs showed a marked difference in the titers obtained in embryonated eggs and guinea pigs. In all cases the titers obtained in new-born mice were high for both viruses. The modified live virus determined the death of all embryos in which virus multiplication was present with a high titer. The field virus when used in high concentration, is capable of multiplying without determining the death of the embryo.

Vallée type A virus: Titrations were made in embryonated eggs of field virus (Cruzeiro subtype A24, Brazil), modified live virus (Cruzeiro type A, EP92), and virus recovered from esophageal/pharyngeal material of cattle vaccinated with the previously mentioned modified live virus. Although in all cases the titers in new-born mice were high, there was a marked difference in the titers obtained in embryonate eggs between the field and the modified viruses.

The results of the preliminary experiments showed that cattle inoculated with the modified live virus (Cruzeiro type A, EP92), with virus from postvaccinal reactions, and with virus recovered from esophageal/pharyngeal material of cattle vaccinated with modified live virus did not present lesions, and no contagiousness was detected for contact pigs. Pigs inoculated with the same virus presented benign lesions, and no contagiousness was found among the contact cattle.

Waldmann type C virus: Preliminary results obtained with lapinized virus (Resende type C, Brazil) showed that cattle inoculated with the vaccine virus and with virus isolated from postvaccinal reactions could transmit and produce severe FMD vesicular lesions in contact swine; however, swine inoculated with several virus samples and having severe lesions could not infect the two contact bovines.

Virus isolated from carriers (passed three times serially in suckling mice) had relatively high titers for cattle with generalization and vesicular lesions (5/8).
SIGNIFICANCE

Studies on the biological properties of modified live FMD viruses are of great importance for the differentiation of the virus used in live vaccines and the corresponding field strains and for the determination of the degree of virulence.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

Timetable: 1967 - continuing
USE OF FOOT-AND-MOUTH DISEASE MODIFIED VIRUS VACCINES IN SOUTH AMERICA

PROBLEM

Foot-and-mouth disease is enzootic in all the South American countries, where it mainly affects cattle. Susceptible cattle are protected through systematic and periodic vaccination on a relatively large scale. Inactivated vaccines, given in three doses a year, are the most commonly used. Despite their limitations, these are the only vaccines employed in the meat-exporting American countries.

Modified live virus vaccines are preferable to the inactivated type. 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 non-meat-exporting countries and for those that maintain large stockraising areas whose products are used for domestic consumption.

METHOD

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 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. 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 $4 \times 10,000$ 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 to approximately 5,000 cattle. These animals are subject to over-all observations and, in addition, to individual observations in a representative sample. Immunity is measured by means of periodic examinations, usually on a monthly basis, of the blood antibodies in selected groups, by virus elimination, and by recording morbidity in natural outbreaks of the disease. Subsequently, vaccination may be extended throughout the country according to its particular requirements.
RESULTS TO DATE

A total of 15,000,000 doses of vaccine prepared with Vallée type O virus (subtypes O₁ and O₃), modified in chicken embryos and one-day-old chicks, were administered to cattle in the field in Ecuador and Venezuela. The index of pathogenicity was low, and there has been no significant fall in immunity during the course of the observations, which have lasted from five to ten months.

The Vallée type A virus (subtype A₂₄, Cruzeiro), modified in chicken embryos, is the most widely used in the field. In Venezuela about 24,000,000 doses have been given since 1962. Over a period of four years, Ecuador has used about 700,000 doses, Brazil 70,000, Colombia 25,000, Chile 5,000, and Guyana 5,000. In all these countries the pathogenicity observed was low and the immunity good. Protection was achieved against different subtypes.

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; bivalent AC and trivalent OAC in Brazil, in small and limited field experiments. It appears that the pathogenicity of the individual strains is not being modified. This is also true of the antigenicity observed in the laboratory and in the field.

In further investigations with the lapinized type C virus (used in a bivalent vaccine with an avianized Vallée type A virus), Waldmann type C virus was recovered in almost all the vaccinated and contact cattle in different organs and tissues. These results prompted further studies of the biologic characteristics of this virus, which have shown that it is not indicated for use in field vaccination.

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 (1965 and 1967) has shown them to be highly effective in halting and controlling the disease. The outbreak in British Guiana was quickly controlled, and no new cases have appeared since. From the time that intensive campaigns with the vaccines have been undertaken a pronounced fall in the number of typings of the virus concerned has been observed in Ecuador and Venezuela. The 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. The results, in view of the difficult environmental conditions in most of South America (socioeconomic conditions, vastness of areas to be covered on the ranches, poor communications, adverse topography and climate, low degree of development of official health services, etc.), indicate the value of these vaccines in controlling foot-and-mouth disease.

In order to promote confidence in modified live virus vaccines and encourage their general use, further specific studies must be made of their behavior in the field, in various cattle populations, and under variable environmental conditions; the genetical stability of the modified viruses; markers; pathogenic effects on different breeds, ages, and situations; age at the first dose; need for revaccination in order to achieve an adequate level of protection; spread of the virus in the species and in other animal species; virus carriers; survival of the virus in meat and other subproducts; duration of immunity; and vaccination of pigs and sheep.

PUBLICATIONS


OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

Timetable: 1962 - continuing
DURATION OF IMMUNITY IN CATTLE VACCINATED WITH MODIFIED LIVE FOOT-AND-MOUTH DISEASE VIRUS

PROBLEM

It has been shown that modified live virus vaccines are capable of immunizing cattle against foot-and-mouth disease virus (FMDV). A knowledge of the duration of this immunity after revaccination is of great importance in determining the number of revaccinations needed and their proper sequence.

Laboratory experiments and epizootiologic observations have also shown that it is difficult to immunize young cattle. This has led to studies on the immune response and the duration of immunity in these animals.

METHOD

Calves

The experiment was carried out on 187 calves between 3 and 6 months of age. They were submitted to two different treatments. In the first group, 83 calves were vaccinated 3 times, intramuscularly, at 30-day intervals. In the second group, 79 calves were vaccinated 4 times. The first 3 doses were given at 7-day intervals and the 4th dose was given 90 days after the first dose. A third group of 16 calves were kept as contact controls without vaccination. The immunity tests were performed by the inoculation of homologous virus and by studying the seroprotection indexes in suckling mice.

One- to two-year-old cattle

Eighty cattle were used in this study. They were divided into four groups and given the following treatment: group I (30 cattle) received one dose of vaccine; group II (22 cattle) received two doses of vaccine 30 days apart; group III (16 cattle) received two doses of vaccine 240 days apart; and group IV (12 cattle) were not vaccinated and were placed in contact.

The vaccine used was monovalent Vallée type A virus (subtype A24, Cruzeiro) prepared in chicken embryos with the 92nd passage virus.
RESULTS TO DATE

Calves

Postvaccinal reactions took the form of benign lesions among 3% of the vaccinated calves. The results of the immunity studies for group I showed that in the challenge performed at the 6th month after the first inoculation, protection existed in 7 out of 8 calves, at the 8th month in 6 out of 8, and at the 12th month in 1 out of 8. For group II, 7 out of 8 were protected at the 6th month, 3 out of 4 at the 8th month, and 4 out of 7 at the 12th month.

With regard to immunity measured in terms of serum antibodies, there was a pronounced increase in the arithmetic mean in the two groups of vaccinated calves.

In both groups the arithmetic mean of the sera remained the same up to the 12th month after the beginning of treatment, which is very near the line established as indicative of protection.

In group I the antibody levels were at or above the 1.7 line, thus coinciding with the results of the challenge tests. In the test performed at the 12th month, 7 out of 8 calves showed antibody indexes below 1.5, which could account for the discrepancy between the challenge test and the arithmetic mean of the antibody levels among the remaining animals.

In the second group the antibody-level and the challenge-test curves were closely related.

In the third group (nonvaccinated calves) the antibody levels were never above 1.7 and in the challenge tests all the calves were unprotected.

One- to two-year-old cattle

Of all animals vaccinated for the first time, only one presented a benign postvaccinal reaction. After revaccination no signs of pathogenicity were detected.

In group I, which received only one dose of vaccine, the following results were observed: 10 cattle showed complete immunity at the 30th day; 6 out of 10 showed immunity at the 120th day, and 5 out of 10 showed immunity at the 180th day. In the last test 4 unvaccinated contact animals presented generalization. All 4 control animals in each experiment had generalized reaction.
The curve of antibody levels is represented by the arithmetic mean of the indexes obtained from the animals used in the challenge test, which decreases after 30 days, staying close to the level of acceptable immunity up to the 6th month.

The cattle in group II tested 7 months after revaccination showed good immunity (8/8), whereas the 4 controls presented generalization. In the test performed one year after revaccination, 7 out of 9 vaccinated animals remained immune, whereas the 4 controls and the nonvaccinated contact animals contracted the disease.

In group III, all the cattle were shown to be immune (7/7) 210 days after revaccination, whereas the nonvaccinated contact animals and the 4 controls presented generalized lesions. An identical result was obtained one year after revaccination in a test performed on 8 vaccinated animals.

In group II the antibody level stayed above the indicative limit of protection for 7 months after revaccination, diminishing slowly thereafter until the 12th month of the test.

SIGNIFICANCE

It is of great importance to know the exact duration of immunity conferred on cattle (young and adult) by vaccination and revaccination in order to assure continuous protection against foot-and-mouth disease.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

Timetable: 1966-continuing
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.

One of the possible obstacles to the use of live vaccines is the lack of knowledge about the duration of survival of the modified viruses in vaccinated cattle. It is therefore necessary to know the exact duration of the survival of the virus in the different organs and tissues and in the esophageal/pharyngeal material of vaccinated animals.

METHOD

Cattle vaccinated with bivalent modified live virus vaccine (lapinized Waldmann type C and chicken embryo Vallée type A) have been slaughtered at intervals and their organs and tissues examined for the presence of virus.

In the studies dealing with the esophageal/pharyngeal material, the cattle have been given the same vaccine and then revaccinated either on the 30th day or on the 180th day after the primary inoculation. Samples are collected monthly. The esophageal/pharyngeal material is collected using the cup probang of Grea and Tallgreen, which, upon being inserted into the esophagus, permits the collection of a mucosal-saliva mixture from the esophagus, pharynx, larynx, and mouth. These specimens are treated with chloroform, shaken 30 minutes in a cold chamber, and centrifuged. The supernatant liquid is treated with 2,000 IU of penicillin and 20 mg of streptomycin per ml.

Suckling mice, pigs, and monolayers of BHK-21 C-13 and calf kidney (RFFB-II) cells are used for isolation of the virus, and the complement-fixation test is employed for identifying its type.

RESULTS TO DATE

In the studies of organs and tissues it has been possible to isolate type C virus, but never type A virus, in cattle slaughtered between 18 and 64 days after vaccination with bivalent modified live virus vaccine.
Waldmann type C virus has been isolated from specimens of skin (20 days after vaccination), pancreas (20 days), kidneys (20 days), coagulated blood (34 days), tonsils (36 days), and bone marrow (62 days).

In the studies of esophageal/pharyngeal material, it has been possible to detect lapinized virus in samples of esophageal/pharyngeal material from vaccinated cattle and nonvaccinated contacts up to 270 days after vaccination. In two cases, avianized Vallée type A virus was detected at the 90th and 240th days after vaccination. Indeed, virus has been isolated up to 270 days after vaccination and, apparently, revaccination at 30 or 180 days did not increase the number of carriers. At the 210th day after vaccination, samples of esophageal/pharyngeal material were inoculated in pigs, suckling mice, and tissue cultures (strain RFB-II cells). Lapinized type C virus was detected in suckling mice and RFB-II cells but not in pigs. The data show that suckling mice and RFB-II cells seem to be more susceptible than swine. Apparently it was necessary to use these two methods on every material, since otherwise it would have been impossible to isolate virus from 3 or 4 out of the 9 known positive cases.

The data also show that there is no relation between the absence or presence of antibodies to foot-and-mouth disease and recovery of virus.

SIGNIFICANCE

It is of great importance to ascertain how long live modified viruses survive in cattle, since the 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.

PUBLICATIONS


OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

Timetable: 1966 - continuing
HEAT INACTIVATION OF NATURAL AND MODIFIED STRAINS OF FOOT-AND-MOUTH DISEASE VIRUS

PROBLEM

The objective 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 (FMDV) and how to inactivate it in meat so as to make exportation to non-enzootic areas possible. The immediate goal 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 strains of virus under identical environmental conditions and then to study the effects of various environmental factors on the characteristics of thermal inactivation.

METHOD

The effect of temperature on the antigenicity and infectivity of the strains of the three types of FMDV, natural and modified, in a buffered phosphate solution is being determined. Subsequently, the thermal effects on the virus of suspension, pH, concentration, ionizing irradiation, and other factors will be studied.

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

RESULTS TO DATE

In the case of Vallée type A virus, the rate of thermal inactivation of viral infectivity per unit time is decimal. The type A modified strain shows decimal reduction times significantly higher than those of the natural strain of the same subtype (Cruzeiro A24, Brazil) with identical temperature, suspension medium, and pH. The suckling mouse appears to be the most sensitive test medium for modified and natural strains of type A 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.

The rate of thermal inactivation at 550°C did not change when suspensions with different virus concentrations were used.

Variations between pH 7.0 and 7.5 did not interfere with the rate of thermal reduction time, at pH 6.5 or 8 an increase was noticed, at pH 6 the inactivation was noticed and very rapid at pH 8.

Accidental bacterial contaminations of virus suspensions did not seem to affect the rate of thermal inactivation.

In the case of Waldmann type C virus, studies were performed comparing the thermal inactivation characteristics of three strains of Resende sub-type C, (Brazil) - one from cattle tongue epithelium, another modified in rabbits, and a third modified in chicken embryos - all with a final passage in suckling mice.

The results showed that there is no significant difference in the rate of thermal inactivation between the natural virus and the strain modified in rabbits, whereas, as demonstrated with type A, it is considerably higher in the virus modified in chicken embryos.

Thermal reduction times of concentrated and diluted suspensions were significantly different at 550°C.

**SIGNIFICANCE**

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 for trade purposes both to the countries in which the disease is enzootic and to countries free of the disease.

**PUBLICATIONS**

None thus far.
Grantee: Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil


Timetable: 1964-1967
APPEARANCE OF SUBTYPES OF FOOT-AND-MOUTH DISEASE VIRUS IN PARTIALLY IMMUNE CATTLE

PROBLEM

The large number of subtypes of foot-and-mouth disease virus (FMDV) found in South America seems to be due to continuous passages of viruses in partially immune animals in the field. The results of tests made by Hyslop et al., based on repeated field observations, have experimentally confirmed this theory.

A report from the São Paulo Biological Institute to the effect that it was not possible to diagnose a type O virus by the complement-fixation test with the sera usually used for routine tests has prompted a more detailed investigation of this problem.

Through a series of passages of this Vallee type O virus in possible partially immune cattle with a known background of previous vaccination, it is possible to determine serological and immunological variations. This study has a great practical value due to the fact that it was not planned but resulted rather from a problem that occurred during the production of virus in cattle at the slaughterhouse for the preparation of vaccines.

METHOD

Studies of type O virus (São Paulo type O, strain 66), which was first isolated in 1966 during an outbreak of foot-and-mouth disease in the State of São Paulo, Brazil, have been made from cattle tongue epithelium. This virus, when diluted at 1:5 in buffered phosphate pH 7.6, clarified by centrifugation, has been inoculated intradermally in the tongue of 5 susceptible bovines. In the subsequent passages, a virus suspension (1:10 in buffered phosphate m/25) has been prepared from a pool of tongue epithelium collected during the previous passage. During the interval between passages, the tongue epithelium was stored at -20°C. The virus suspension (1:10) was inoculated intradermally in doses of 5 ml per bovine.

The subjects chosen for the studies in animals were crossbred cattle, predominantly zebú, that had been slaughtered the day after arrival at Santos and were known to have been vaccinated one to three months previously.

In the complement-fixation test the antigen was cattle tongue epithelium, diluted at 1:5 in physiological solution pH 7.6, and the sera were of a specific subtype produced at our laboratory, the dilutions corresponding to two 50% complement-fixation units in relation to the homologous antigen.
RESULTS

The serological components of São Paulo type O virus, strain 66, were determined in different passages, using the following hyperimmune sera of guinea pigs:

<table>
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<tr>
<th>Sera</th>
<th>Passage</th>
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<tbody>
<tr>
<td>O, São Paulo, strain 66</td>
<td>São Paulo, strain 66</td>
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<tr>
<td>O Lombardia</td>
<td>Lombardia</td>
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<tr>
<td>O Brescia</td>
<td>Brescia</td>
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<tr>
<td>O Venezuela</td>
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<tr>
<td>O Pirbright</td>
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<tr>
<td>O Bahia, Brazil, strain 59</td>
<td>Bahia, Brazil, strain 59</td>
</tr>
<tr>
<td>O Campos, Brazil, strain 58</td>
<td>Campos, Brazil, strain 58</td>
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<td>O Argentina, strain 64</td>
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<td>O Uruguay, strain 65</td>
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<td>O Peru, strain 63</td>
<td>Peru, strain 63</td>
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<tr>
<td>O Lausanne</td>
<td>Lausanne</td>
</tr>
<tr>
<td>O Matadero, strain 66, CPFA 7631</td>
<td>Matadero, strain 66, CPFA 7631</td>
</tr>
</tbody>
</table>

A progressive weakening of the reaction to the different sera was noted from the original virus up to its 19th passage in cattle (Matadero type O, strain 66), while beginning with the 7th passage a new component appeared and became the only one present in the 19th passage. This was confirmed when bovines vaccinated with the latter virus were confronted against the homologous (original) virus and no protection with respect to the original virus was observed. Similar confirmation was obtained with the serum-protection tests.

In the studies of cross-immunity in cattle, Schmidt-Waldmann type vaccines in doses of 5 ml with a 10^7.3 suckling mide LD_{50} per ml were applied subcutaneously to 16 selected cattle without significant antibody levels against virus types A, O, and C. The vaccine was prepared with virus of the 19th passage (Matadero type O, strain 66). Three weeks after vaccination 8 cattle were challenged (intradermal inoculation of the tongue) with 10,000 suckling mide LD_{50} of the homologous virus (Matadero type O, strain 66) and the remaining 8 with the heterologous virus (São Paulo, type O, strain 66). Tests performed with the homologous virus showed protection to 7 out of 8 cattle. When the challenge was performed with the heterologous virus none of the animals showed protection.
During the passages of São Paulo type O virus, strain 66, at the slaughterhouse, a gradual increase in the number of reacting bovines and in the weight of the epithelium was observed, which may be explained by the progressive change in the virus strain.

In the suckling mice serum-protection tests, sera from recovering bovines were employed against the different subtypes 21 days after inoculation with the experimental viruses.

The results show that the spectrum of the sera from cattle recovering from the original virus strain (São Paulo type O, strain 66) is wider than that obtained from Matadero type O virus, strain 66.

SIGNIFICANCE

The continuous appearance of new subtypes of FMDV in South America constitutes a serious problem for campaigns against the disease, since the vaccines to be used must be prepared with virus of the same subtype as the one present in the field area concerned. A better knowledge of how the new subtypes are formed is therefore much needed.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization, Program of Technical Cooperation of the Organization of American States

Timetable: 1967 - continuing
RICKETTSIAL ZOONOSES 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 the blood of these two hosts. More recently, isolates have also been reported from donkey blood samples in focal villages in Egypt (see Philip and Imam, 1967). Some of these isolates have been confirmed at the Rocky Mountain Laboratory in Montana. Experimental typhus infection of lambs and of ticks in Ethiopia and of a donkey, calves, and young goats in Montana 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 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.

METHOD

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

RESULTS TO DATE

In collaboration with the respective health and scientific authorities, animal and human blood samples were collected by members of the Rocky Mountain Laboratory from the areas in which the presence
of human typhus was confirmed serologically - Puno, Peru, September 1963; the high Ecuadorean plateau, July 1964; Cautin, Chile, November 1965; and La Quiaca area, Argentina-Bolivian border, December 1965. By a new technique, a few suspect burros, cattle and sheep CF reactors for typhus were proved nonspecific, so that on the collection dates none of 683 serum samples - mainly from cattle, sheep, goats, donkeys, llamas, a few horses, pigs, alpacas, and pet guinea pigs - was found to be positive for typhus-group antibodies.

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

In a nonendemic area (at the Rocky Mountain Laboratory), the susceptibility of a young donkey and two 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 TN) 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. They persisted in detectible titers in the donkey, however, for at least 801 days.

SIGNIFICANCE

Observations of extrahuman cycles of epidemic typhus in northeast Africa have focused attention on possible involvement of domestic animals in rickettsial zoonoses, including 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.

PUBLICATIONS


**OTHER DATA**

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

**Funded by:** Pan American Health Organization and the Rocky Mountain Laboratory, Hamilton, Montana

**Timetable:** 1963 - continuing
BRUCELLOSIS IN WILDLIFE

PROBLEM

Studies performed by the Center have shown a high prevalence of brucellosis in wild foxes (Dusicyon gymnocaerus antiquus and D. griseus griseus) in the Provinces of Buenos Aires and Rio Negro, Argentina. The natural occurrence of this infection has also been recognized in the European hare (Lepus europeus). Brucella infection in other animal species is being studied in order to assess the role of wild fauna as a reservoir of brucellosis for livestock and humans.

METHOD

Bacteriological and serological examinations were performed on 550 "vizcachas" (Lagostomus maximus), 40 "mulitas" (Dasypus septemcinctus), 131 "peludos" (Chaetophractus villosus), and one "hurón" (Galictis furax huronax). All of these animals were captured in the central part of the Province of Buenos Aires.

RESULTS TO DATE

All the Lagostomus specimens were negative bacteriologically and serologically. This has also been true of the specimens of the armadillos (Dasypus and Chaetophractus) studied so far.

A strain of Brucella abortus, biotype 1, was isolated from the musteline animal Galictis furax huronax. This was a pregnant female. Brucella was also isolated from four fetuses and from the amniotic fluid of the fifth fetus.

SIGNIFICANCE

The fact that Brucella was isolated from the organs of fetuses and from the amniotic fluid tends to indicate that during parturition or in event of abortion wild animals might contribute to pasture contamination with Brucella organisms.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejia (Buenos Aires), Argentina

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

Timetable: 1966-1968
SEARCH FOR PHAGES IN BRUCELLA CULTURES
ISOLATED IN LATIN AMERICA

PROBLEM

To date, all known Brucella phages have been isolated in the USSR and in Poland. The availability of stable laboratory strains isolated in Latin America would be important. The isolation, reinforcement, and stabilization of specific Brucella phages can lead to improved methods for the typing of Brucella isolates from man and animal origin. It may be found that specific type phages will be isolated that can characterize the genus Brucella into types or variants. Such phages would have great epidemiological importance.

METHOD

At present isolations are being attempted from over 400 cultures of B. abortus, B. melitensis, and B. suis submitted to the Center by several Latin American countries. Additional cultures as they are received will be included in the study. Later, attempts to isolate Brucella phages will include material from confirmed cases involving man and animal. Four techniques are concurrently being employed: the method described by M. S. Drosevkina which uses one-month-old cultures to prepare filtrates; the method of J. Parnas, which consists of using cultures of eight months of age when they are in the decline or death growth phase; and two techniques described by Adams involving the broth and semi-solid overlay plate methods.

RESULTS TO DATE

The four-tube method of M. S. Drosevkina has been used to examine 379 strains; the method of Parnas has been applied in the study of some 260. In this latter procedure, the strains were grown on two different culture media (510 cultures), divided into pools of ten, and tested. A total of 51 pools were examined for the presence of phages. Using the filtrates obtained from these two techniques, 471 filtrates were tested using the broth technique and 234 filtrates were tested by the semi-solid overlay plate technique described by Adams. All filtrates were tested against the three Brucella species - B. abortus 544-1, B. melitensis 16 M, and B. suis 1330. With the exception of two cultures, all filtrates produced negative results. The two exceptions, B. melitensis and B. suis, demonstrated positive reactions in all four techniques. Attempts are presently in progress to reinforce these two possible phages.
SIGNIFICANCE

In addition to the broad problem stated in the beginning, it should also be pointed out that at present there are no available Brucella phages that are stable and useful for phage identification of \textit{B. melitensis}. Since the two positive reacting filtrates demonstrated plaques to \textit{B. melitensis}, if they can be reinforced and stabilized they could be of extreme value in the identification of Brucella isolates using the plaque technique.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejia (Buenos Aires), Argentina


Timetable: 1967-1968
DEVELOPMENT OF A RAPID DIAGNOSTIC TEST FOR RAM EPIDIDYMITIS

PROBLEM

The only serological methods now available for the diagnosis of ram epididymitis are the complement-fixation and the hemagglutination tests. These tests can be performed only in a few laboratories in Latin America. A rapid plate test for Brucella ovis could be used in the field and performed by personnel having a minimal amount of training.

METHOD

Preliminary trial testing was done by sensitizing latex particles with whole intact inactivated refrigerated and heated suspensions, disrupted cellular debris, and filtrates of disrupted and whole suspensions. An antigen that consisted of a 25 times slow freeze-thawed heavy cell suspension followed by centrifugation at 15,000xg and the resulting debris tagged to latex proved to be the most promising.

RESULTS TO DATE

A randomized test using sera from 13 serologically proven naturally infected animals and 14 normal control animals has indicated a high accuracy of this plate test method developed at the Center. Ram sera from ranches known to be naturally infected with ram epididymitis are being now collected for a more extensive evaluation of this test.

SIGNIFICANCE

The serologic diagnosis of ram epididymitis is now limited to the complement-fixation and hemagglutination tests, both of which require well-equipped laboratories and trained personnel. The development of a rapid diagnostic test will be of great value as a field tool for epidemiological and control purposes.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1967-1968
SAFETY AND PRELIMINARY SEROLOGICAL TESTING OF B. ABORTUS STRAIN 45/20 VACCINE

PROBLEM

It is widely accepted that B. abortus strain 19 vaccine confers good immunity in cattle but produces seroagglutination titers in vaccinated animals similar to those found in infected cattle.

A non-agglutinogenic vaccine such as strain 45/20 would be of great value if safety and immunogenic properties were comparable to strain 19 vaccine.

The purpose of this study is to determine the safety for cattle and to measure the serological response of this type of vaccine as a first step in evaluating the product for bovine brucellosis control.

METHOD

Two trials, the first in 1964 and the second in 1965, were carried out with a commercial killed vaccine using "Duphavac" adjuvants from Philips Duphar Co., The Netherlands.

In the first a total of 34 head of cattle (29 females and 5 steers), between seven months and seven years of age, were vaccinated with 3 ml of the bacterin by intramuscular route in the neck, just in front of the scapula (left side). Seventy days later, 31 of these animals were inoculated with 2 ml and 2 animals with a triple dose of 6 ml by the same route and region but in the right side. One animal died after the first inoculation, of intercurrent disease.

In the second trial, 30 animals, 14 of which had been vaccinated with this bacterin the year before, were used. Two vaccinations were performed—the first, an inoculation of 2 ml by intramuscular route in the neck just in front of the scapula (right side), and the second, 72 days later, an inoculation of the same dose by the same route but on the left side.

The animals were bled at the time of vaccination, 14 days thereafter, and then monthly for seven months. The standard agglutination test was used in the first trial. In the second trial, Rivanol and complement-fixation tests were also included.
RESULTS TO DATE

Local reactions:

In the first trial, no local reactions were observed after the first dose. However, after the second dose, 22 animals showed reactions ranging from small nodules to indurations of 10 x 17 cm. None of the animals showed abscesses. It was remarkable that there were no local reactions in the two animals that received a triple dose of vaccine (6 ml).

In the second trial, between the third and fifth days after the second dose 20 animals were found with local reactions ranging from small nodules to indurations of 27 x 18 cm. Of these animals, 12 were vaccinated twice during the first trial. Twenty days after vaccination, the local reactions had disappeared in all animals.

Serologic reactions:

In the first trial, two months after vaccination, 5 animals showed agglutination titers in the suspect range, whereas the rest of the group was negative.

In the second trial 14 days after the second vaccination, 3 animals were classified as reactors and 11 as suspicious, by the standard agglutination test. By the Rivanol plate test there were 11 reactors; by the complement-fixation test 13 animals were highly positive and 5 suspicious. Seven months after the second vaccination, all the animals were negative by the Rivanol test and only one suspicious by the tube-agglutination test. Five animals, however, remained positive by the complement-fixation test.

SIGNIFICANCE

The findings show that this type of vaccine may frequently produce marked but not permanent local reactions (swellings).

The vaccine produces a low response to the serum-agglutination test in a reduced number of animals for a short time. The response to the complement-fixation test, however, is more prolonged.

Further studies to evaluate immunity conferred by the vaccine to cattle are indicated.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1964-1967
SCREENING OF DRUGS FOR THE TREATMENT OF CANINE ECHINOCOECCOSIS

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 is also being investigated.

METHOD

Dogs are infected by oral administration of specified numbers of Echinococcus granulosus scoles in sheep hydatid cysts, obtained from local abattoirs. Various drugs are tested against these infections, with approximately half the dogs in any one trial serving as controls. Observations of post-dosing effects are made along with an 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. In the case of field trials, fecal samples from arecoline-purged treated and control dogs are examined and the results compared.

RESULTS TO DATE

The most promising and efficacious drug tested thus far is bunamidine (N:N-di-butyl-4-hexyloxy-1-naphthamidine hydrochloride).

This drug has been used in 13 separate experimental trials and in a field trial involving 613 treated dogs on 479 farms in the Partido (county) of Azul, Province of Buenos Aires, Argentina, with 654 dogs being kept as controls on the same farms. The experimental and field trials have yielded the following results to date: a single dose of 50 mg/kg showed high efficiency in completely clearing dogs of mature infections. However, the drug showed poor performance against immature infection. When given twice at a six-week interval in an average dose of 50 mg/kg, its ability to completely clear dogs of the taenia was
demonstrated in both experimental and field trials. Worm reduction was observed in all the groups of treated dogs.

New experimental trials with this and other drugs 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.

PUBLICATIONS


OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1960-1969
LEPTOSPIROSIS IN ARMADILLOS

PROBLEM

Wild animals are an important reservoir of leptospirosis and responsible for infection of human beings and livestock. Different serotypes of Leptospira were isolated by the Center from wild foxes (*Dusicyon gymnogercus*). From opossums (*Didelphis azarae*) a serotype of the *bataviae* group was cultured that was later identified as *L. paidjan*. In this phase of the project bacteriological and serological investigations have been made in two armadillo species, *Chaetophractus villosus*, commonly named "peludos," and *Dasypus septemcinctus*, commonly called "mulitas."

METHOD

Armadillos of both species were collected in the central area of the Province of Buenos Aires, bled by intracardial puncture, euthanized, and autopsied. Kidney tissue and urine were processed for Leptospira isolation. Cross agglutination-absorption tests were used to identify the isolated strains.

RESULTS TO DATE

Fifteen Leptospira strains were isolated. Five of them were identified as *L. paidjan*, one was a new serotype of the *bataviae* group, for which the name of *argentintensia* was proposed; nine strains are being classified.

The microscopic agglutination test showed the highest titers for the *bataviae* and *sejroe* serotypes.

SIGNIFICANCE

The first isolation of *L. paidjan*, and, in fact, the first *bataviae* serotype to be recognized in Argentina, was made from opossums in the northern part of the country. The new results show that this serotype is widespread and has since been isolated from armadillos and from a cow in the Province of Buenos Aires. A new serotype of the *bataviae* group has also been isolated. This research contributes to a better knowledge of the epidemiology of leptospirosis.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina

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

Timetable: 1966-1968
PROBLEM

It is necessary to determine the period during which rabies neutralizing antibodies are present in the milk of vaccinated cows and to establish the duration and level of such antibodies transferred to the offspring.

The main purpose of the study is to assess whether the transferred antibodies interfere with vaccination.

METHOD

The following samples were taken:

(a) Blood samples from the dam and her offspring prior to the ingestion of colostrum by the calf.

(b) Blood samples from the offspring at 7-day intervals after birth during the first month and every 15 days thereafter until definite disappearance of antibodies.

(c) Colostrum samples and milk samples once a week during the first month after birth and every 15 days thereafter until complete disappearance of antibodies.

The transfer phenomenon is being studied in females immunized both with live and with inactivated vaccines.

Antirabies antibodies are being studied in sera from dams and their offspring by the serum-neutralization test in mice. This study is being carried out qualitatively by screening in all cases and quantitatively in those cases where persistence is longer.

Antibody levels in milk are being determined by the "lactoneutralization" test, qualitatively in all samples and quantitatively in those cases where antibody persistence is longer.
RESULTS TO DATE

All cows with detectable neutralizing antibodies in their sera were found to contain detectable antibodies in their colostrum and also in their milk. The duration of these antibodies in the milk appears to be related to duration of the antibodies in the mother's serum.

The serum of offspring bled on the first day after birth was found to be negative before ingestion of colostrum; antibodies appeared during the succeeding days.

The persistence of neutralizing antibodies in the offspring sera was longer when the dam had a high antibody titer.

These preliminary results lead to the belief that antibodies, in addition to being transferred through the colostrum, may also be passed by milk.

SIGNIFICANCE

It is hoped that this study when completed may indicate at what age calves should be vaccinated against rabies.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1967-1968
POTENCY OF RABIES VACCINES USED IN THE AMERICAS

PROBLEM

The purpose of this study is to ascertain the potency of rabies vaccines used in the American countries.

METHOD

Over a period of 18 months, 62 lots of vaccines were received under refrigeration from 11 different countries - Argentina, Bolivia, Brazil, Canada, Chile, Colombia, Mexico, Peru, the United States, Uruguay, and Venezuela. Some were inactivated vaccines of nervous tissue origin and others were live modified virus type vaccines.

The inactivated vaccines of nervous tissue origin were examined by the Habel test in mice and the live modified virus type vaccines were examined by the Koprowski test in guinea pigs.

RESULTS TO DATE

Most of the inactivated vaccines passed the corresponding potency test; some of them were of excellent immunogenic capacity. Three out of 49 nervous tissue type vaccines did not meet the requirements of the potency test.

Of the live virus modified vaccines, 7 out of 13 failed to meet the potency test requirements.

SIGNIFICANCE

This study has proven that there is not an important problem in the preparation of inactivated nervous tissue origin vaccines in the American countries.

On the other hand, there is need for improvement of production methods in the preparation of live virus modified vaccines, especially in Latin America.
PUBLICATIONS

"Resultados de las Pruebas Realizadas en Vacunas Antirrábicas."
Zoonosis Bull (Bs.As.) 8 (1): 16-17, 1966.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires),
Argentina

Funded by: Pan American Health Organization/World Health Organization,
United Nations Development Program, and the Government of Argentina

Timetable: 1967
COMPARISON OF SAFETY AND POTENCY OF THE SUCKLING MOUSE BRAIN
AND THE PURIFIED RABIES VACCINES

PROBLEM

Rabies vaccines are known to produce a wide range of reactions - from local to fatal - in vaccinated persons.

A purified rabies vaccine (PRV) has been prepared by centrifugation and column chromatography in Ecteola. When this vaccine was compared with two human vaccines produced commercially in the United States (duck embryo and Semple), it was shown to be safer and more potent than the latter preparations.

It is of interest to compare the PRV with the suckling mouse brain (SMB) vaccine, which is widely used in Latin America.

METHOD

The comparison between the purified rabies and the suckling mouse brain vaccines is being based on potency by the Habel and NIH tests, antibody response and protection determined in guinea pigs, and on lipid and nitrogen content.

RESULTS TO DATE

The potency tests have given comparable results for both vaccines.

No statistically significant differences have been noted in the two groups of inoculated guinea pigs when they were challenged at 15, 60, and 90 days after the first vaccine dose, although there is a slight difference in protection in favor of the SMB vaccine. This would indicate the need to study a larger number of inoculated animals.

Similarly, the median of the serum-neutralizing antibodies produced in the two groups of guinea pigs from 1 to 60 days after the beginning of vaccination is comparable.
SIGNIFICANCE

The production of a rabies vaccine that contains a low concentration of antigens other than the viral antigen while still maintaining its potency is very much needed for the treatment and prophylaxis of rabies in human beings.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1967-1968
SEROLOGICAL EVALUATION OF DIFFERENT SHORT SCHEDULES FOR HUMAN RABIES PRE-EXPOSURE VACCINATION

PROBLEM

During recent years, importance has been given to rabies prophylaxis before exposure; this is justified because the number of persons exposed to infection during their work (veterinarians, ecologists, physiologists, pharmacologists, medical students, dog catchers, and others) is becoming considerably larger.

Several experiments have been made using reduced schedules in order to (a) determine whether it is possible to obtain a rapid and good neutralizing antibody response with a few doses of vaccine, and (b) evaluate the antibody response of such vaccinated persons when they receive a new injection of vaccine a year or more after the primary immunization.

METHOD

Persons working with rabies virus or with experimental animals or animals exposed to natural infection have been the subjects of this study. Suckling mouse brain vaccine (SMB) is being used according to the following schedules:

1) One group is receiving 1 dose of vaccine and a booster 25 days later.

2) One group is receiving 2 doses of vaccine (spaced one day apart) and a booster 25 days after the second dose.

3) One group is receiving 3 doses of vaccine (spaced one day apart) and a booster 25 days after the third dose.

4) One group is receiving 5 doses of vaccine (spaced one day apart) and a booster 25 days after the fifth dose.

Determination of the level and persistence of the antibodies produced will indicate the most convenient schedule. The effect of a booster one year after vaccination on the antibody level will also be determined.
On the basis of preliminary results, the third dose group was considered the most convenient one for this study. To date, 33 persons have been submitted to this schedule.

RESULTS TO DATE

All persons vaccinated with the 2, 3, and 5 doses showed a clear neutralizing antibody response after 21 days.

Twelve out of 21 persons who received 2, 3, or 5 injections had detectable antibodies a year after completing the schedule.

Seven persons who received 2, 3, or 5 vaccinations produced a very high immune response when they received a booster dose after one year.

SIGNIFICANCE

So far, the 3-dose schedule appears to be very convenient for primary pre-exposure treatment when suckling mouse brain vaccine is used.

These results would indicate that it is possible to obtain a good state of immunity in humans with three SMB vaccine doses given over periods as short as 5 days.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1966-1968
EVALUATION OF RABIES VACCINES FOR BOVINE USE

PROBLEM

Hundreds of thousands of cattle die annually in the Americas from bat-, fox-, or canine-transmitted rabies. Various types of vaccines are currently being used to protect cattle in enzootic areas. The degree and duration of protection conferred by these vaccines, however, is generally unknown. Vaccine safety is also a problem in some cases.

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

METHOD

Controlled studies of rabies vaccines are being conducted in cattle at the Center's farm annex. Seven vaccines are currently under study: (1) the suckling mouse brain UV inactivated 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 sheep brain vaccines inactivated with betapropiolactone alone or in combination with phenol (CEPANZO); (6) the suckling mouse brain UV inactivated vaccine with aluminum hydroxide adjuvant; and (7) a modified live dog kidney tissue culture vaccine.

Groups of eight to twenty 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 were collected periodically for serum neutralization tests at 30 days, 100 days, 200 days, and one year after the first vaccine inoculation. Direct challenge with live virus strains was carried out using the new isolation facilities of the Center. Animals that were not challenged received a booster inoculation after a year.

RESULTS TO DATE

All vaccines except number 7 provoked detectable rabies neutralizing antibodies one month after vaccination. Three vaccines (numbers 2, 5, and 6) demonstrated antibodies for a year after the primary
vaccination. With vaccines 1, 2, 3, 4, 5, and 6 revaccination after one year (booster inoculation) resulted in a rapid increase in antibody titer.

Direct challenge showed good immunity in vaccines 1, 2, 3, 4, and 6. Vaccine number 7 failed to protect the animals. Surviving animals had good antibody titers 60 days after virus inoculation. Three out of four control animals died with rabies.

SIGNIFICANCE

Several types of rabies vaccines have been shown, by the serum neutralization test and by exposure to artificial infection, to confer protection for at least one year. Though confirmation by field trials is pending, there is a good indication that lasting immunity can be established in cattle by several types of vaccines.

PUBLICATIONS


OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program and the Government of Argentina

Timetable: 1965-1968
COMPARISON OF INOCULATION ROUTES FOR ANTI-RABIES VACCINATION IN DOGS

PROBLEM

The principal aim in this study is to ascertain the best route in dogs for antirabies vaccination with suckling mouse brain vaccine (SMB).

The second purpose is to ascertain how soon antibodies appear in vaccinated dogs.

METHOD

A study of the level of neutralizing antibodies developed in dogs vaccinated by the subcutaneous or intramuscular routes was carried out with a single batch of SMB vaccine.

Thirty-one dogs obtained from a rabies-free area were injected by the subcutaneous (16) or intramuscular (15) routes.

Blood samples were collected from the dogs before vaccination and on the 15th, 30th, 106th, and 175th days after vaccination.

Subsequently, ten dogs obtained in the same rabies-free area were injected by intramuscular route with another batch of SMB vaccine. Blood samples were taken on the 7th and 15th days.

Sera obtained were tested for the presence of antibodies by the serum-neutralization test in mice.

RESULTS TO DATE

None of the dogs contained antirabies antibodies in their blood prior to vaccination.

At the 15th and 30th days all the 31 vaccinated animals showed antibodies in their sera. At the 106th day, 8 out of the 16 dogs injected subcutaneously and 14 out of the 15 animals vaccinated intramuscularly showed antibodies in the blood. At the 175th day, 4 out of 10 animals vaccinated by the subcutaneous route and 8 out of 10 dogs inoculated intramuscularly gave positive results.
Titers of neutralizing antibodies of dogs that received vaccine intramuscularly were higher than those of the animals vaccinated subcutaneously. Antibodies persisted for a longer period in the group vaccinated intramuscularly.

In the second group of 10 dogs, vaccinated intramuscularly, it was possible to find antibodies in 9 out of 10 animals at the 7th day.

SIGNIFICANCE

Under the conditions of this experiment it has been shown that the intramuscular (IM) route is the most suitable for SMB vaccine when used in dogs.

It seems that neutralizing antibodies appear early (7 days) after vaccination by the IM route. This fact is important for control purposes.

PUBLICATIONS


OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1967-1968
TUBERCULIN TESTING OF CATTLE:
A COMPARISON OF OLD TUBERCULIN AND TUBERCULIN PPD
BY CAUDAL FOLD INOCULATION

PROBLEM

The advantages of tuberculin PPD lie in the use of a purified active principle. Once a correlation has been established between protein content and biological potency for a particular product, successive batches can be accurately specified at any desired potency. This is not possible with old tuberculin (OT).

In view of the advantages of PPD, tests have been carried out in a herd of known tuberculous cattle to determine whether it could be used satisfactorily in the caudal fold site for field testing and to compare it with old tuberculin (OT) in both caudal fold and neck sites.

METHOD

The following tuberculins were used in two separate trials:

1. Weybridge mammalian (human) tuberculin PPD - 2.0 mg per ml.

2. USDA old tuberculin (human) synthetic medium, labeled KOT (USDA).

In Trial 1 each of 50 cows was injected intradermally with 0.1 ml of the Weybridge PPD in one caudal fold and 0.1 ml of the KOT (USDA) in the other fold.

In Trial 2 each of 24 cows received 6 intradermal injections (0.1 ml) of the above mentioned tuberculins. Four of these injections - one of the PPD at full strength (1:1) one of the PPD diluted 1 in 10 (1:10) in physiological saline, one of KOT (USDA) at full strength, and one of KOT (USDA) diluted 1 in 10 - were randomly allocated to four sites, two on each side of the neck. The remaining two injections consisted of the PPD at full strength injected into one caudal fold and diluted 1 in 10 injected into the other caudal fold.

All reactions were measured both by increase in skin fold thickness and by diameter of induration.
RESULTS TO DATE

The mean reactions to the two tuberculins, with their standard errors, were calculated. In Trial 1, of the 50 tuberculous animals tested, the PPD gave a larger mean reaction than the OT in the caudal fold site, in terms both of skin thickening and of induration. Since every animal received both tuberculins, the standard errors of the mean differences were low. Although the mean differences were not significant at the 5% level, in the case of skin thickening the difference between the tuberculins approached significance (P<0.1).

Five of these tuberculous cattle (10%) gave negative reactions to both tuberculins in the caudal fold, a positive reaction being regarded as any swelling with an induration measurement of 10 mm or more. An additional 2 animals (4%) failed to react to the PPD only and another 3 (6%) to the OT only.

In Trial 2 the mean values of the tuberculin reactions, with their standard errors, in the neck and caudal fold sites of the 24 animals were calculated. A highly significant correlation between skin thickening and induration was obtained in both neck and caudal fold sites (P<0.001).

In the neck site, the KOT (USDA) demonstrated approximately 60% the potency of the Weybridge PPD, and this difference was almost significant at the 5% level. In the caudal fold site, the PPD was only one tenth as potent as in the neck.

SIGNIFICANCE

The two trials of Weybridge tuberculin PPD and KOT (USDA) in the neck and caudal fold sites of tuberculous cattle gave similar results. Although the difference between the tuberculins was not significant, the results indicated that the PPD was more potent than the OT in both sites.

The results demonstrate that PPD can be used satisfactorily in the caudal fold of cattle for field testing.

The comparison of the activity of the PPD in the two different sites demonstrates clearly that for the intradermal tuberculin test in cattle the caudal fold is much less sensitive than the neck. The effect of using the caudal fold site instead of the neck is the same as reducing the dose of tuberculin to one tenth.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1967
TUBERCULIN TESTING OF CATTLE: A COMPARISON OF DIFFERENT TECHNIQUES USING HUMAN, BOVINE, AND AVIAN PPD TUBERCULINS

PROBLEM

The purpose of the trial is to determine the levels and types of tuberculin sensitivity present in random cattle going for slaughter and to compare the efficiency of single and comparative tuberculin tests using either human or bovine PPD in the neck and caudal fold sites, assessed by increase in skin fold thickness and by diameter of induration.

METHOD

A total of 100 low-grade cattle of mixed breed, age, and sex, which were going for slaughter, were held for tuberculin testing and subsequent post-mortem examination.

Each animal received five intradermal injections (0.1 ml) of the following tuberculins:

1. Weybridge avian tuberculin PPD 0.5 mg per ml.
2. Weybridge mammalian (human) tuberculin PPD 2.0 mg per ml.
3. Weybridge bovine tuberculin PPD 1.0 mg per ml.

The standard comparative test with avian and human tuberculins was carried out on one side of the neck, and the bovine PPD was injected on the opposite side; in addition, each animal was injected with avian PPD in one caudal fold and either human or bovine PPD in the other fold.

Post-mortem examinations were carried out the day after the reading of the tuberculin tests. The animals were classified as either tuberculous or nontuberculous as a result of a search for macroscopic lesions. Samples of lesions were taken for confirmatory diagnosis and typing in the laboratory.

RESULTS TO DATE

Post-mortem examination showed 35 animals to be tuberculous and 65 nontuberculous. Mean tuberculin reactions of these two groups were determined with their standard errors. Paraspecific tuberculin sensitivity,
as indicated by reactivity to avian tuberculin, was present in 18 of the 65 nontuberculous animals. Three of the 65 nontuberculous cattle (4.6%) gave false positive results to the comparative test in the neck using avian and human tuberculins, and one to the test using the avian and bovine tuberculins. Two of these nontuberculous cattle (3.0%) were also positive to either single or comparative tests in the caudal fold. An additional 9 of the 65 nontuberculous animals (13.8%) would have been wrongly classified as positive to a single injection in the neck with human PPD, and 3 of these (4.6%) to a single injection with bovine PPD. Of the 18 nontuberculous cattle that gave positive reactions to the avian tuberculin in the neck site, only 2 were positive to avian tuberculin in the caudal fold, though neither reacted to mammalian PPD in the opposite fold.

Of the 35 tuberculous animals, 7 (20%) gave false negative reactions to the caudal fold test, 3 with the human PPD and 4 with the bovine. Four of these cattle (11.4%) were also negative to the neck test with bovine PPD, and 2 of these (5.7%) to the test using human PPD.

SIGNIFICANCE

The results demonstrate that in a group of cattle with a high prevalence of tuberculosis infection the comparative tuberculin test carried out in the neck has a much higher efficiency than a single injection of mammalian tuberculin in the caudal fold. Because of the lesser sensitivity of the site, the caudal fold test fails to reveal a high proportion of tuberculous animals of low allergic status. In addition to the lower general level of response, a softer type of reaction is obtained in the caudal fold.

The bovine PPD, at the strength used in this trial, also has a high false negative test error. A better result could be obtained by a more severe interpretation of a comparative neck test using the avian and the bovine PPD. After examination of the data it is recommended that in cattle with a high prevalence of tuberculosis or in known tuberculous herds in the environment of this trial, a bovine reaction 2 mm or more greater than the avian reaction should be considered positive for tuberculosis.

The small number of avian tuberculin reactions obtained in the caudal fold of nontuberculous animals shows that the use of avian PPD at this strength in a comparative caudal fold test is of no value in indicating the presence of paraspecific sensitivity.
In these tests, measurement of induration, which showed less variation than skin thickening, has proven to be a satisfactory method for reading tuberculin reactions in cattle under field conditions. In view of the low general level of reactivity in the trial animals, measurement of skin thickening as well as induration increased the sensitivity and specificity of the test.

The results indicate the value of carrying out trials of similar design on a large scale and in different areas of Latin America. Such investigations would provide information on the extent, levels, and types of tuberculin sensitivity present in the various countries and would demonstrate the efficiency of single and comparative tuberculin tests using either human or bovine PPD in the neck and caudal fold sites.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Zoonoses Center, Ramos Mejía (Buenos Aires), Argentina


Timetable: 1967
THE INTER-AMERICAN INVESTIGATION OF MORTALITY

PROBLEM

The statistics of various countries show 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 made regarding the effects of biological, social, or environmental determinants of disease, these obstacles to interpretation must be removed.

The primary objective of the investigation, therefore, was to provide a comprehensive account of adult mortality in selected populations, which would be as accurate and comparable as possible. Such an account would serve 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.

METHOD

Small teams of physicians and home visitors collected on standard forms all available information relevant to establishing the cause of death of persons between the ages of 15 and 74 over a two-year period in the following cities: Bogotá, Colombia; Bristol, England; Cali, Colombia; Caracas, Venezuela; Guatemala City, Guatemala; La Plata, Argentina; Lima, Peru; Mexico City, Mexico; Ribeirão Preto, Brazil; San Francisco, U.S.A.; Santiago, Chile; and São Paulo, Brazil. About 4,000 deaths were investigated in each city, constituting either all deaths of residents in the age range or a systematically drawn sample thereof. The completed questionnaires were sent for review to the central office in Washington so that the causes of death could be assigned in a uniform manner following established international practice. For deaths from cardiovascular conditions or deaths in which more than one disease seem to be involved, the final assignments of cause were made by two medical referees working independently, and their separate opinions were combined by using weights.
RESULTS TO DATE

Questionnaires corresponding to 43,298 deaths in the 12 cities were used in the analysis. The principal analysis of the results has been completed. The English version of the report on the investigation was released in September 1967 under the title Patterns of Urban Mortality (PAHO Scientific Publication 151). The Spanish edition, Características de la Mortalidad Urbana, is being prepared for release in May 1968.

Some of the results are summarized below.

1. Death rates per 1,000 population between the ages of 15 and 44 show marked differences: the high rate in a given age and sex group in one city is often two to three times as great as that in another city. Between the ages of 45 and 74, however, the disparities are much less. Age-adjusted death rates for the 60-year age span range from 3.2 to 6.3 per 1,000 population for females and from 6.2 to 9.8 per 1,000 population for males.

2. In studying the principal causes, the age-adjusted death rates were grouped according to established procedures. In general, the two leading causes are disease of the heart and malignant neoplasms. For males, diseases of the heart are the leading cause in eight cities, malignant neoplasms in three, and cirrhosis of the liver in one. In contrast, among females, malignant neoplasms hold first place in nine cities and diseases of the heart in three.

3. Mortality from tuberculosis ranges from 3.7 to 67.0 per 100,000 population. It is particularly high in Lima, Santiago, and Cali.

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, Guatemala City, Santiago, and Lima; cancer of the lung (among males) is high in Bristol and La Plata; cancer of the cervix uteri is high in Cali and Lima; cancer of the bladder (among males) is high in La Plata.

5. The death rate from diseases of the heart is three times higher in San Francisco than in Guatemala City among males, but the rate is only 26 per cent higher in San Francisco than in Guatemala City in females.

6. Mortality from bronchitis among males in Bristol is over 22 times the rates in Guatemala City and Lima. By contrast, death rates among females are low.
7. Mortality from cirrhosis of the liver is unusually high in Mexico City, San Francisco, and Santiago among both males and females. In Mexico City and Santiago, the mortality of males from this cause exceeds that of malignant neoplasms of all sites.

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

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

SIGNIFICANCE

Certain populations are distinguished by unusually high death rates from many specific causes, thus indicating 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.

PUBLICATIONS


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: 1962-1967
STUDY ON MULTIPLE CAUSES OF DEATH

PROBLEM

The purpose of this study of the questionnaires completed in connection with the Inter-American Investigation of Mortality for Bristol and San Francisco is twofold. First, the completeness and accuracy of reporting of causes of death on death certificates will be evaluated by comparing such causes with those reported on the clinical records and/or the autopsy reports. Second, the data will be examined for frequency of combinations of causes in relation to age, sex, and residence.

METHOD

Data on the questionnaires from the two cities will be used for recording the multiple causes involved in deaths in which an autopsy was done. One of the medical referees will designate the causes that are to be coded. A program will be developed for tabulations by computer. Advice will be obtained from similar studies being carried out in England.

RESULTS TO DATE

None are reported.

SIGNIFICANCE

Plans are being made for the coding of multiple causes of death in several countries beginning in 1968. Also, in the Inter-American Investigation of Mortality in Childhood the underlying and other associated causes will be analyzed. Thus the data made available from the questionnaires will have application in the near future.

PUBLICATIONS

None thus far.
OTHER DATA

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

Funded by: National Center for Health Statistics, U.S. Public Health Service

Timetable: 1967-1968
INTER-AMERICAN INVESTIGATION OF MORTALITY IN CHILDHOOD

PROBLEM

The over-all aim is to carry out an Inter-American Investigation of Mortality in Childhood in selected communities in order to develop death rates as accurate and comparable as possible, taking into account the nutritional, sociological, and environmental factors responsible for excessive mortality.

One phase of the investigation will involve collecting data on deaths of children under 5 years of age, following standardized definitions and procedures, in areas of the Americas for which present data are incomplete. Such data will be used to compare underlying and contributory causes of death and to study inter-relationships with infectious diseases, nutritional deficiency states, and sociological factors.

Information on approximately 35,000 deaths will be collected over a two-year period in 13 widely separated areas in Latin America on the basis of visits to mothers and certifying physicians, hospital and health center records, and autopsies, in accordance with uniform procedures.

In order to study and compare biologic and social differences between those who die in early childhood and those who live, another phase of the investigation is designed to obtain data on approximately 22,000 living children under 5 years of age through household surveys in which probability sampling techniques have been used.

Finally, it is hoped that the project will stimulate and strengthen medical research on the part of schools of medicine and public health in the area of infant and child health.

METHOD

All deaths of infants and children under 5 years of age, or a sample of the total deaths, are investigated through interviews in the home, at the hospital or clinic, and with the physician to obtain as complete a record as possible of the fatal illness, including results of laboratory and other examinations and autopsy. In order to fully evaluate the factors resulting in the death of these infants and young children, the information collected will include the pregnancy history, the status of the infant at birth, breast feeding, growth and development, and social and environmental conditions.

In order to compare the data on the children who died with similar data for the general population, information will be collected on a sample basis of living children in the study areas.
RESULTS TO DATE

A working group met from 17 to 20 October 1966 to discuss and agree on procedures for the pilot tests to be carried out in 1967. Arrangements were made by the Organization for pilot testing in Brazil (Recife and Ribeirão Prêto), Colombia, Guatemala, and Jamaica. The University of North Carolina School of Public Health also used the questionnaire for pilot tests by interviewers of the National Morbidity Survey.

Suitable principal collaborators were selected from 12 areas in Latin America on the basis of proposals submitted. The proposals were compiled in a single document and presented to AID in July 1967. A small planning meeting was held during the week of 4 December 1967 to review the questionnaire and the results of the pilot tests.

The investigation has been given a high priority by AID, and the document for support of the entire project was signed on 30 January 1968. The Planning Conference of Principal Collaborators was held in Cali, Colombia, from 25 to 29 March 1968 and was followed by a meeting of medical interviewers in Ribeirao Prêto from 3 to 9 April 1968.

Twelve areas in Latin America have been selected for the investigation, and agreements are being signed to provide for the employment and training of staff in June 1968, with the field work to start on 1 July 1968 which will continue for 24 months. In addition to the areas in Latin America, two places in the United States have been proposed for similar studies.

SIGNIFICANCE

1. The present investigation of urban and rural populations living under widely differing conditions would make a unique contribution to the understanding of the underlying and contributing causes of death in infancy and early childhood, including nutritional deficiency diseases. The analysis of mortality on the basis of social and environmental conditions should clarify the factors responsible for excessive mortality and indicate appropriate means of prevention.

2. Malnutrition continues to be a serious problem in many areas of the world, and yet its effect on morbidity and mortality has not been satisfactorily measured. In this project not only will deaths from multiple protein deficiency (kwashiorkor) and other nutritional deficiency states be studied and well documented, but data on weight at birth, length of breast feeding, addition of supplemental foods, and growth and development of children will be obtained and analyzed as well.

3. The introduction of probability sampling for selection of a control group of children will permit an evaluation of the socioeconomic
conditions in these areas and the nutritional state of living children. Also, the continued use of scientific sampling techniques by schools of medicine and public health is an important by-product.

4. Records of previous pregnancies of the mother of the dead child will permit the study of previous pregnancy loss through abortion, fetal deaths, and early childhood mortality.

5. As a result of the study of multiple causes, important contributions can be made to the planning for the 1975 revision of the International Classification of Diseases.

6. Precise information on the magnitude, distribution, and etiology of nutritional deficiency will enable health authorities to plan realistic programs for control.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: U.S. Agency for International Development

Timetable:

Planning conference of principal collaborators 25-29 March 1968
Initiation of 12 field projects 1 June 1968
Collection of data on children who died under 5 years of age and of samples of households and living children under 5 years of age for 24 months 1 July 1968 - 30 June 1970
Completion of field work 1 September 1970
Completion of analysis of data 1 March 1972
ECONOMIC ASPECTS OF WATER SUPPLY AND SEWERAGE SYSTEMS

PROBLEM

One of the biggest problems encountered in health programs in general, and in sanitary engineering in particular, is the quantitative expression of cost-benefit relationships. Account is seldom taken and very little is known regarding the impact of economic activity generated by water supply programs within a country or group of countries. The purpose of this research project is to obtain some specific information on this subject in the case of Colombia. The following elements will be evaluated: (a) the status of industries whose products are used in connection to water supply and sewerage systems; (b) public investments in water supply and sewerage systems; and (c) the social implications, in terms of numbers of people reached, of investments in the water supply and sewerage field.

METHOD

The research has been planned in two stages. First, the existing data will be covered by a review of the technical literature on the subject; compilation of a bibliography from materials existing in libraries, public offices, universities, and so forth, and development of a data collection and tabulation system; actual collection of the data; and organization, tabulation, computation, and analysis of the statistical material collected. The second phase will involve preparation of a full report by the working team; review by the Director of the Economic Development Studies Center (CEDE) and by a consultant; and publication of the final results.

RESULTS TO DATE

A progress report was presented on 28 November 1967. The research is at the data-collection step of the first stage.

SIGNIFICANCE

The results of this research project will provide important data and quantitative parameters on the economic significance of water supply and sewerage programs.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Miguel Urrutia, Economic Development Studies Center, Bogotá, Colombia

Funded by: Pan American Health Organization and the University of the Andes, Bogotá

Timetable: 1967-1968
BASIC FACTORS FOR WATER SUPPLY DESIGN

PROBLEM

Very few countries in Latin America base their design standards and specifications for water supply systems on national experiences and accumulated statistical data. In general, they have tended to rely on the considerations and data offered in "textbooks," usually based on practices followed in the United States. The objective of this research project is to evaluate and revise the basic design standards for water supply and sewerage systems in Chile.

METHOD

Investigation is being based on the following sets of factors: per capita water consumption, including averages and variations in the averages; waste water flow, including averages and variations in the averages; waste water quality, BOD, settleable solids, and per capita contribution; and population growth trends.

RESULTS TO DATE

Delays in the purchase of necessary equipment have postponed the start of the project. No progress report has been submitted yet.

SIGNIFICANCE

The research project will help to establish design regulations for water supply and sewerage systems in the light of national conditions and experiences.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Ing. Isaac Faiguenbaum A., Sanitary Engineering Section, University of Chile, Santiago, Chile

Funded by: Pan American Health Organization and the University of Chile

Timetable: April - August 1967
RATE OF FILTRATION IN WATER TREATMENT PLANTS
AND ITS INFLUENCE ON WATER QUALITY

PROBLEM

The demand for water for drinking, municipal, and industrial purposes has tripled between 1940 and 1965. A new water treatment plant similar in design and operation to the old "Las Vizcachas" water treatment plant is being built. The purpose of this research project is to ascertain whether the "Las Vizcachas" plant in Santiago has the capacity to absorb a larger load than it is now handling, so that this additional load could successfully be imposed on the new plant.

METHOD

Hydraulic models will be used in this project. Three small-diameter filters will be designed to test water of the same quality as that actually being tested at the plant. Records will be kept of loss of load, run of the filters, and the quality of the filtered water.

RESULTS TO DATE

No progress report has been issued yet by the executive agency.

SIGNIFICANCE

If the research project shows that a greater load could be applied to "Las Vizcachas" plant, the capacity of both the old and new plant would have been greatly expanded.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Ing. Isaac Faiguenbaum A., Sanitary Engineering Section, University of Chile, Santiago, Chile

Funded by: Pan American Health Organization and the University of Chile

Timetable: March 1967 - September 1967

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DETERMINATION OF LEAD CONTENT IN WATER
THAT HAS BEEN IN CONTACT WITH PLASTIC PIPES

PROBLEM

Between 1962 and 1967 more than 1,000 million dollars has been invested in Latin America in the field of water supply. A considerable portion of these funds has been used for pipelines and water distribution systems in small communities, especially in the rural areas. The pipes used in these small water supply systems are usually 2 inches or less in diameter. Plastic pipes could be used extensively in such systems. The Polyvinyl chloride plastic pipe (PVC) makes use of lead as a stabilizer. In certain amounts, lead is considered toxic when dissolved in water. The purpose of this research project is to find out whether water flowing in these pipes can dissolve lead from the constituent materials and, if so, to establish whether the concentration of dissolved lead goes beyond the permissible limits for drinking water.

METHOD

Samples of PVC pipes from the 5 plants in Lima that manufacture this product were used. Extraction of the water that had been in contact with the plastic pipes was organized into two series. Date of manufacture was taken into account. Tests were planned to carefully determine the absorption of lead by water and to relate this degree of absorption with the lead content in the plastic pipes. PVC pipes actually in service were tested, and the water coming from these pipes was subjected to the same analysis established for the other PVC pipes.

RESULTS TO DATE

The research shows that in PVC pipes with lead used as stabilizer, such as those manufactured in Peru, the concentration of lead in the water that comes in contact and passes through them is very small and does not approach the lower permissible limits for drinking water standards.

The amount of lead that "rubs off" the pipes diminishes with time. It has been experimentally proved in the laboratory and by analysis of samples taken from Latin American water supply systems in service that all the "extractable" lead from the pipes is depleted for potable purposes within a period of 90 days at the most.
It is considered that the use of pipes of this kind in normal service conditions does not offer any risk to the health of the users in the community.

SIGNIFICANCE

The use of PVC pipes is widely open for community water supply purposes. The savings effected with these pipes will make it possible to build a larger number of water supply systems. Public agencies responsible for national water supply programs (especially those responsible for rural water supply programs), credit agencies, industries, and other agencies concerned will benefit from the findings of this research project.

PUBLICATIONS


OTHER DATA

Grantee: Ing. Augusto A. Navarro P., National Engineering University, Lima, Peru

Funded by: Pan American Health Organization and the National Engineering University, Lima, Peru

Timetable: April - August 1967
STUDIES ON THE IMPROVEMENT OF FILTRATION RATES IN WATER TREATMENT PLANTS

PROBLEM

Water treatment plants are designed to cover water demands on the basis of what is called a "design period" - 10, 20, 30, or more years. A very common problem arises when a design period is over and no possibilities for immediate physical expansion of the plant exist, usually because of financial difficulties. To improve the rate of flow through the plant without improving the quality of the water might be a good temporary solution. The present project is intended to study the elasticity of the rapid filters at the "Lo Castillo" water treatment plant in Santiago, Chile, and to investigate their possibilities for increasing filtration rates.

METHOD

A new experimental filter having a surface of 0.806 m² will be built. Different dosages of coagulants will be compared with varying rates of flow through the filters. Correlation will be established between these studies and different sizes of sand and the uniformity coefficient in the sand media used for the filtration bed.

RESULTS TO DATE

It is too early to report any results.

SIGNIFICANCE

If the results are positive, the higher rates of filtration will mean an increase in the capacity of the water treatment plant, and thus an extension of the design period. Such a "cushion" period would help to solve financial problems and permit the addition of new treatment units to the plant.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Prof. Francisco Unda, School of Public Health, University of Chile (in cooperation with the Catholic University of Chile), Santiago, Chile

Funded by: Pan American Health Organization and the University of Chile

Timetable: 1968
PROBLEM

Mixing equipment for flocculation purposes in water treatment is expensive and demands extra time and personnel for its operation and maintenance. The objective of the present research is to study the possibility of accomplishing an efficient flocculation using the kinetic energy of water under proper control.

METHOD

Special prismatic chambers will be used to force water to flow in an helicoidal pattern. Determinations will be made on loss of lead, velocities at different depths, velocity gradients, and parameters related to geometry, turbulence, and stability.

RESULTS TO DATE

The first stage of the project has been completed; one progress report and a final report on the first stage have been submitted. The second stage involves the construction of a settling tank and the operation of the whole model.

SIGNIFICANCE

Hydraulic flocculators of simple construction and operation with efficiencies comparable to those of regular mechanized equipment could be used in small towns. This would avoid the necessity of borrowing currency in order to import the mixing equipment.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Ing. Pedro Martinez Pereda, Center of Sanitary Engineering, University of Mexico, Mexico, D. F. Mexico

Funded by: Pan American Health Organization and the Faculty of Engineering, University of Mexico

Timetable: September 1966-1967
RESEARCH ON STABILIZATION PONDS IN ARGENTINA

PROBLEM

Stabilization ponds seem to offer an adequate solution for the treatment of sewage coming from middle-sized and small communities. It is a relatively low-cost process, it is efficient, and it is simple to operate and maintain. It can be used successfully to treat waste from slaughterhouses, which is a problem of great importance in Argentina.

Nevertheless, the effects of particular geographical conditions—seasonal variations in temperature, hours of sunshine, altitude, and so forth—on the efficiency of the process need to be investigated.

METHOD

Two stabilization ponds will be built and put into operation. The efficiency of the lagoons will be studied, with focus on the reduction of the oxygen biochemical demand. Flow rate control will be established, and samples will be taken for laboratory analysis. Rainfall, temperature, evaporation, sunlight hours, etc., will be recorded.

RESULTS TO DATE

No significant results can be offered yet. The building of the lagoons, originally scheduled for 1967, was delayed for administrative reasons, and the data collected so far are not sufficient to yield any interpretations.

SIGNIFICANCE

The research will provide information on the efficiency of natural treatment of sewage through the stabilization pond process in the area of the Province of Buenos Aires.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Ing. Carlos Carrique, School of Sanitary Engineering, University of Buenos Aires, Buenos Aires, Argentina

Funded by: Pan American Health Organization and the University of Buenos Aires

Timetable: April - December 1967
RESEARCH ON STABILIZATION PONDS IN MEXICO

PROBLEM

Even though the design of stabilization ponds to treat raw sewage has evolved remarkably in the last ten years, this process is still used very little, if at all, in Latin America. Mexico is in an almost ideal situation with respect to the basic design factors: it has the proper temperatures, luminosity is high, and land may be purchased at reasonable cost. The purpose of this research project is to try to establish optimum criteria for the design of stabilization ponds in Mexico.

METHOD

Studies will be made on hydraulic loads, biological loads, geometry, recirculation, efficiency, costs, etc. A first step will be the compilation and study of bibliography. Next, the groundwork for the project will be prepared, and two lagoons will be constructed on the campus of the University of Mexico. Controlled analysis will be carried out in a number of items: dissolved oxygen, biochemical oxygen demand, algae growth, etc. In the second stage of research an effort will be made to discover whether the data can be adjusted to a particular mathematical law. When this possibility has been tested, the findings from the two lagoons on the University campus will be correlated with the results obtained from the actual operation of a number of existing lagoons.

RESULTS TO DATE

The first stage has been completed, and according to the progress reports some excellent preliminary results have been obtained on loads, BOD efficiency, and flora growth. The second stage is now in full operation.

SIGNIFICANCE

It is believed that this research will bring to light a number of basic issues on stabilization ponds. New knowledge and better understanding of the process are looked for, and, most important, extensive use of stabilization ponds in Mexico is expected as a consequence of the project.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Ing. Francisco Montejano U., Faculty of Engineering, University of Mexico, Mexico, D. F., Mexico

Funded by: Pan American Health Organization and the Faculty of Engineering, University of Mexico

Timetable: 1966-1967
STUDIES ON REFUSE DISPOSAL

PROBLEM

The adequate disposal of refuse and garbage is a major problem in every city. In spite of this fact, however, economic data on the subject are practically nonexistent in Latin America. In addition, there are few, if any, parameters for measuring the sanitary significance of the several procedures used. The purpose of the present research is to investigate the costs and sanitary aspects of the various garbage disposal methods employed in certain cities of Brazil.

METHOD

A survey is being conducted in selected cities to obtain and classify information concerning the respective garbage disposal methods used in each case. The first circuit consists of São Paulo, Campinas, Piracicaba, São Carlos, Araraquara, Baurú, São Paulo; the second circuit, São Paulo, Curitiba, Londrina, Presidente Prudente, Araçatuba, Lins, São Paulo; the third circuit, São Paulo, Rio de Janeiro, Miterói, Juiz de Fora, São Paulo; the fourth circuit, São Paulo, Belo Horizonte, Brasília, Salvador, Recife, Fortaleza, and São Paulo.

RESULTS TO DATE

The research was begun in February 1968. No progress report has been issued yet.

SIGNIFICANCE

The research will provide valuable information on the sanitary and economic aspects of various garbage disposal methods used in a number of Brazilian cities.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Dr. Walter Engracia de Oliveira, Department of Environmental Sanitation, University of São Paulo, São Paulo, Brazil

Funded by: Pan American Health Organization and the University of São Paulo

Timetable: 1967-1968
RESEARCH ON DOMICILIARY GARBAGE INCINERATORS

PROBLEM

A study being conducted by the Environmental Engineering Research Center of the School of Sanitary Engineering is attempting to determine the amount of settleable solids in the atmosphere in the city of Buenos Aires. The average of 96 analytical tests shows that 150 kgs/ha/month of settleable solids fall on the city. The main source of this fallout, at least in the residential districts, has been traced to domiciliary garbage incinerators.

The purpose of the present research project is two fold: (a) to ascertain by statistical methods such basic data as per capita volume of garbage disposal, volume per weight unit, noncombustible components by weight, weight of ashes per kilogram of garbage, etc., and (b) to determine the variation of settleable particles in the combustion gases.

METHOD

Samples are being collected and analyzed according to three types of sources:

(a) Rented houses
(b) Owner-occupied houses
(c) Offices

Daily determinations are being made of garbage weight and volume, noncombustible component by weight, weight of ashes, settleable particles in combustion gases and incinerator temperatures.

A system of collecting, weighing, measuring, sampling, and analyzing garbage from each of the source groups is being established.

RESULTS TO DATE

The first progress report is being prepared.
SIGNIFICANCE

The research will establish the degree of importance of domiciliary garbage incinerators in the fallout of settleable solids in certain sections of Buenos Aires and will help to improve design factors.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Ing. Juan C. Garaventa, School of Sanitary Engineering, University of Buenos Aires, Buenos Aires, Argentina

Funded by: Pan American Health Organization and the University of Buenos Aires

Timetable: April - December 1967
RESEARCH ON THE DESIGN AND ECONOMIC AND FINANCIAL ASPECTS OF A COMPOSTING PLANT

PROBLEM

Industrialization is one of the processes for the proper disposal of garbage. It has a number of advocates, but at the same time some authorities are skeptical about its efficiency and practicability. Composting is one of the methods used in processing the garbage for industrial purposes. Some of the municipalities around Santiago are contemplating the installation of garbage digestion plants based on foreign patents. The aim of the present project is to study the feasibility of building a composting plant using national equipment, with no patents involved, in order to effect as substantial an economy as possible.

METHOD

The over-all investigation contemplates four phases: (a) a study of the effects of humidity content and number of turnovers; (b) analysis of the final product and testing of its "acceptability" by prospective clients; (c) design of a plant based on the collected and analyzed data; and (d) a study on how the construction of the plant should be financed. The present project will consider only the first two phases.

The Municipality of Los Condes in Santiago will provide the garbage needed (10 to 12 cubic meters twice a week for a period of 6 months) and the personnel to handle the selection, conditioning, turnover, and storing. A special lot will also be provided by the municipality.

Samples from the piles will be taken for chemical and physical analysis. Turnovers will be applied systematically and humidity will be changed artificially to observe the results. Stabilization times will be recorded and milling procedures will be used. Tests on the acceptability of the product will be run on the basis of cost and probable market price.

RESULTS TO DATE

It is too early to report any results.
SIGNIFICANCE

If the research establishes the feasibility of composting, a plant will be designed and the Municipality of Santiago will finance its construction.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Prof. Francisco Unda, School of Public Health, University of Chile, Santiago, Chile

Funded by: Pan American Health Organization and the University of Chile

Timetable: April - November 1968
REGIONAL LIBRARY OF MEDICINE

PROBLEM

A survey of library resources in South America conducted by the Pan American Health Organization in 1965 revealed serious deficiencies in the supply of information to the biomedical community and an urgent desire on the part of most of the institutions concerned to resolve the problem.

METHOD

In 1967, as an initial step toward meeting this need, PAHO established a Regional Library of Medicine in São Paulo, Brazil, to provide library support on a regional basis for biomedical education, research, and practice using modern communication techniques, and to train biomedical librarians at advanced levels.

RESULTS TO DATE

A library building provided by the Escola Paulista de Medicina has been adapted for use by the project, and equipment is being procured as necessary.

The systematic acquisition and maintenance of bibliographic materials, serial publications, monographs, and documents is under way and will continue.

The staff is being organized so as to make the best use of special techniques for international library service. Its role in the improvement of local services is also being developed.

A Scientific Advisory Committee on the Library has been appointed, and its first meeting is scheduled for August 1968.

SIGNIFICANCE

By providing specialists in the health sciences with access to a comprehensive collection of scientific and professional literature, the Regional Library will contribute to the acceleration and improvement of biomedical education, research, and practice in Latin America.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Health Organization; Project Coordinator - Dr. M. Martins da Silva, Washington, D.C.

Funded by: The Government of Brazil, the U.S. National Library of Medicine, the Commonwealth Fund, and the Pan American Health Organization

Timetable: 1967 - continuing
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, it was considered desirable to establish a Reference Laboratory and Training Center in Santiago, Chile. The Center's purpose is to provide facilities for standardizing laboratory procedures and iodine determinations and to train personnel in these techniques.

METHOD

Duplicate samples of biological fluids (blood and urine), food, water, soil, and other materials from collaborating laboratories are checked at the Center for iodine content. Standardized test samples are 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 are being trained at the Center in the laboratory techniques to be used in the study.

RESULTS TO DATE

Two biochemists and three laboratory technicians were trained at the Center during 1967.

The laboratory has undertaken standardization procedures in collaboration with the Boston Medical Laboratory to assure adequacy of its techniques and validity of results.

A total of 2,138 iodine content determinations were carried out in biological samples from local and international collaborators.

Efforts have been made to recruit additional international trainees for 1968.
SIGNIFICANCE

The Reference Center facilitates current research on endemic goiter being conducted under PAHO sponsorship by the 12 collaborating laboratories. The Center also provides 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 the biological fluids of the people receiving the prophylaxis.

PUBLICATIONS

None thus far.

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

PROBLEM

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

METHOD

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; the results are then forwarded to the respective investigator. In this way, he is able to maintain a check on his own methodology and results. Standardized test samples are 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 laboratory technician from collaborating laboratories receive orientation and training in the study design and the laboratory methodology, respectively, by means of short training visits to the Reference Laboratory.

RESULTS TO DATE

A total of six collaborating investigators have received training in hematological methods for the Inter-American Study of Nutritional Anemias. A total of 1,782 serum folate, 1,429 B₁₂, and 200 serum iron determinations have been carried out for collaborating laboratories. Standardization procedures have been undertaken with other laboratories.
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 diseases.

PUBLICATIONS


OTHER DATA

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

Dr. Miguel Layrisse, Venezuelan Institute of Scientific Research, Caracas, Venezuela

Funded by: Williams-Waterman Fund

Timetable: 1965 - continuing
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 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.

METHOD

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 for 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). An annual basic course on the theory and laboratory techniques of immunology has been inaugurated. WHO and PAHO are facilitating visits by foreign immunologists to participate in the course and in the research.

RESULTS TO DATE

The main objective of the Center is to provide postgraduate training in basic immunology for candidates from Latin American countries and to develop immunological research projects directly or indirectly related to public health problems in these countries. Two training courses have been organized: the first lasted a full year from March 1966 to March 1967; the second course began immediately afterwards in April 1967 and lasted for eight months. The 1968 course started in May.
The first two courses were attended exclusively by Brazilian students, but the current course has a student from Peru - the first step in extending the benefit of the Center's activities to other countries in the area.

Visiting lecturers and examiners from the United States and Europe have been invited to participate in the Center's activities. They have assisted in the teaching program and have provided guidance for the research projects undertaken by the students. Studies on autoantibodies discovered in *Pemphigus foliaceus* and on the effect of immunosuppressive drugs in the treatment of this disease have been initiated. In addition, research on immunochemical characterization of snake venom enzymes, blister fluid in Brazilian *Pemphigus foliaceus*, enzymes in cercarial extracts of *Schistosoma mansoni*, and guinea pig immunoglobulins has been undertaken. Studies of the mechanisms of the Arthus reaction and of the immunoglobulins responsible for anaphylaxis in the guinea pig have been begun, as well as characterization of the immunoglobulins in guinea pig serum responsible for cytotoxic activity in nephrotoxic serum nephritis.

**SIGNIFICANCE**

Experienced scientists agree that applied research will flourish only if it is constantly nourished by ideas, techniques, and findings derived from basic research. It is therefore shortsighted, in any region of the world, not to devote a reasonable share of the resources available to the support of basic research, particularly in the field of immunology, where there is often only a short step between basic findings and their application to 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 that call for pressing action in the developing countries: the development of immunodiagnostic 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.
PUBLICATIONS


OTHER DATA

Grantee: Dr. Otto Bier, Escola Paulista de Medicina, São Paulo, Brazil

Funded by: Pan American Health Organization/World Health Organization

Timetable: 1966 - continuing
RESEARCH TRAINING PROGRAM IN VIROLOGY, ORNITHOLOGY, ECOLOGY, AND TROPICAL MEDICINE

PROBLEM

There is a widely acknowledged shortage of trained investigators in many fields of medical research, especially investigators competent in planning and carrying out programs that include both field and laboratory research. Hence, the purpose of this training program is to develop research investigators in virology who are at home not only at the laboratory bench but also in the field. It is hoped that the predoctoral and postdoctoral trainees, in addition to receiving their primary training in virology, will also develop a keen appreciation of entomology, ornithology, mammalogy, and other areas of zoology.

METHOD

Extensive training in virology is given at the Department of Microbiology, Cornell University Medical College; the curricula for predoctoral and postdoctoral trainees is rounded out with courses in other departments at Cornell and, in some cases, courses offered at other graduate institutions in the New York area. Field training has been given through an eight-week graduate field research-training course in virology, entomology, vertebrate zoology, and ecology held at sites on the tropical Atlantic lowlands of Mexico, and through graduate student participation in field research programs in Mexico, Guatemala, Belize, and Honduras. The students at Cornell may choose to orient their thesis research around this field work or they may follow the materials collected during field periods by participating in weekly seminars at which current research progress is reported.

RESULTS TO DATE

During the period from mid-1966 through 1967 one trainee from Jamaica received his Ph.D. degree. A postdoctoral student from Mexico concluded her two-year training period in 1967 after having completed several major studies, including a comparison of the virulence of over 30 strains of Venezuelan virus from Mexico, Panama, Colombia, Trinidad, and Florida; a comparative study of over 20 strains of group C virus isolated in Mexico; a study of the relationship of a strain of Patois virus from Mexico to the prototype Patois virus from Panama and to other antigenically related viruses; and a study of a nonarthropod cycle for Venezuelan virus in cotton rats. She is now actively continuing studies on arboviruses at the
National Institute of Virology in Mexico City. Another predoctoral fellow spent a summer training period in Guatemala and Honduras and is now actively involved in research on the effects of mosquito passage on virus virulence, especially in Venezuelan encephalitis. Two new predoctoral trainees initiated their studies at Cornell University Medical College in September 1967.

SIGNIFICANCE

This research training program is adding to the corps of 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, Belize, and Honduras), the eventual long-term productivity of the trainees, once they become independent investigators and educators, will provide the really significant rewards of the program.

PUBLICATIONS


OTHER DATA

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

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

Timetable: 1962-1967
INTERNATIONAL CENTER FOR IDENTIFICATION OF SNAILS
FOR THE STUDY OF SCHISTOSOMIASIS

PROBLEM

It is planned to establish a center for identification of the snail hosts of schistosomiasis and to encourage research on snail identification.

METHOD

A grant, received annually, is used to defray laboratory operation costs and to cover the expenses involved in collecting data on distribution and identification of the species.

RESULTS TO DATE

In 1967 four snail samples from other countries besides Brazil were identified. Snails of several species were supplied to three foreign laboratories.

Three PAHO fellows were trained in the laboratory.

As a result of a collecting trip to the Caribbean area, 42 species of snails were collected and identified. Numerous studies in the laboratory clarified the status of various species in the collection.

Studies on the susceptibility of strains of Biomphalaria were conducted using two strains of S. mansoni.

SIGNIFICANCE

Identification of the snails is a highly specialized and difficult procedure. Maintenance of a special laboratory for this purpose not only secures high quality results but also saves a great deal of time, since local workers are relieved of the need to perform this demanding task.

PUBLICATIONS

None thus far.
OTHER DATA

Grantee: Dr. W. Lobato Paraense, National Institute of Rural Endemic Diseases, Belo Horizonte, Brazil

Funded by: Pan American Health Organization

Timetable: 1967 - continuing
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 1 January 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 (NCHMHII) 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.

METHOD

Data are obtained from the following sources: 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 Center's scientific reference analyst 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 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) have been prepared. In addition, lists have been compiled 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 made up.

A preliminary survey of mental health legislation in Latin America has been made on the basis of information available in the Washington, D.C., libraries. The data have been tabulated to show areas of law affecting mentally disabled persons in each country.

A provisional directory of psychiatrists in all Latin American countries has been completed.

A survey of existing programs for education in psychiatry and related sciences has also been completed.

SIGNIFICANCE

Information that heretofore could not be obtained in compiled form will be made available to psychiatrists and other mental health workers. Communication and exchange of information among persons in this field will be facilitated; as a result, there will be less duplication of effort and improved coordination of mental health activities.

PUBLICATIONS

The Directory of Psychiatrists in Latin America is being made ready for publication.
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: 1963-1967
PROBLEM

The purpose of the grant is 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.

METHOD

For the 18-month period from 1 July 1964 to 31 December 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.

In July and August 1964, the Organization’s staff made initial exploratory visits to Santiago, Chile; São Paulo and Ribeirão Prêto, Brazil; and Caracas, Venezuela to investigate the status of 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, held on 7 January 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, 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 on 18 and 19 March 1965. Interest was expressed in a training program in medical demography at the University of Chile School of Public Health and at the University of São Paulo School of Hygiene.

RESULTS TO DATE

During the two-week period from 2 to 13 August 1965, a senior research demographer from Princeton University’s Office of Population Research
serving as consultant to PAHO, met with staff from the School of Public Health of the University of Chile Faculty of Medicine to investigate the possibilities of a training program. The document prepared as the basis for the program, Health and Population Dynamics, was discussed on 12 and 13 August, 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 30 November 1965, and an announcement of the program was issued in December 1965.

The Consultant spent the two weeks from 8 to 20 November 1965 at the University of Sao Paulo's School of Hygiene and Public Health to assist in the planning of 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 (10 to 13 August), 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 at such a center had been discussed with several potential members of the staff - a pediatrician, two sociologists, and an economist, among others. A document was prepared presenting the needs, the multidisciplinary nature of the problem, present trends and new dimensions, goals and plans, the phasing of developments, organization and staff, and research and teaching programs to be conducted at the Population Center.

SIGNIFICANCE

The Pan American Health Organization is now actively assisting in the development of research training programs in health and population dynamics at the University of Chile School of Public Health and at the University of Sao Paulo School of Hygiene and Public Health.

In Chile, the first-four month course on health and population dynamics was given from August to November 1966, and the second from August to December 1967. The third course is scheduled for August to December 1968. Beginning in 1968, the course previously given for specialization in biostatistics was rearranged and divided into one principal course and three others 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.

In Sao Paulo, the first course in population dynamics at the School of Hygiene was given from October to December 1967 and the second will be from October to December 1968. A one-year program for population specialists can be initiated in 1969.
Funds from the U.S. Agency for International Development have been received for the two-year period 1 July 1966 to 1 July 1968 and have been used, in supplement to what has been made available by the two schools of public health, for the training of faculty, fellowships, simultaneous interpretation, and research equipment.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Pan American Health Organization
Funded by: U.S. Agency for International Development
Timetable: 1964-1968
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 relationship between health and population dynamics; to disseminate knowledge on the process of population dynamics and its implications 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 intersectoral planning.

METHOD

A four-month course in health and population dynamics, primarily for medical school faculty, was given from 1 August to 30 November 1966. This was followed by a second course from 21 August to 15 December 1967. The curriculum covered, among other topics, the design and conduct of research and methods of analyzing health and demographic data. The teaching staff included visiting professors from Harvard, Columbia, North Carolina, Princeton, and São Paulo Universities. Guidelines for the teaching of demographic statistics in medical schools were developed during the courses. A third course is scheduled for 19 August to 20 December 1968.

The 16-month program in biostatistics starting in March 1968 includes courses on demography, among other subjects. Thus there will be a wider offering of material for students in the health and population dynamics course.

RESULTS TO DATE

The 1966 and 1967 courses were attended by a total of 40 persons from 12 countries (Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Peru, and Venezuela).
SIGNIFICANCE

This program is preparing Latin American medical school faculty for teaching and research in the areas in which health and population dynamics are related.

PUBLICATIONS


OTHER DATA

Grantee: Dr. Guillermo Adriasola, School of Public Health, University of Chile, Santiago, Chile

Funded by: Pan American Health Organization/World Health Organization, and the United Nations Development Program

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. The rate of population increase involves highly complex variables - mortality, as affected by the availability of scientific and technological developments, by economic productivity, and by education; fertility, as affected by educational, social, and economic levels of development; and reproduction.

It is reasonable to assume, therefore, that development planning will view economic, social, and human advances as an interrelated process in which population trends deserve the most intensive and scientific consideration.

The proposed center for studies in health and population dynamics at the University of São Paulo will provide for research, teaching, and consultation in these fields, with courses in the medical, health, social, economic, and mathematical sciences. Responsibilities for planning and organization within the university are being shared initially by the health and social sciences schools.

METHOD

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 required courses for all students and diversified and specialized courses for those with different backgrounds and interests. The graduates of the 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 students who satisfactorily meet the educational requirements for admission and who successfully complete the one- and two-year courses.

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, are cooperating on this program. The Department of Sociology consists 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.

Persons designated to serve on the staff will receive training during the first 18 months both at the Department of Applied Statistics in São Paulo and in the United States to prepare them for their specific functions at 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

The first course was given from 23 October to 20 December 1967. Forty-five students from eight countries were enrolled. Harvard and Columbia Universities each sent a visiting professor from their Schools of Public Health, and several other members of the faculty had received graduate training in the United States.

The University of São Paulo has approved the New Center of Population Dynamics, under the Faculty of Hygiene and attached to the Department of Applied Statistics. A sociologist has been appointed director for the first two years. The Center has five faculty members, all trained or being trained. Besides, it expects to have five research assistants and to have additional financial support for several research projects from a Special Fund for Research established by the State Government of São Paulo. Five research projects are being planned or are already under way. The New Population Center in São Paulo is becoming a recognized center for research and training in demography.

SIGNIFICANCE

This research training program prepares faculty for teaching and research in medical and other professional schools in Brazil and in other Latin American countries. As a result, courses on health and population dynamics will be conducted in other medical centers, and research will be encouraged.
PUBLICATIONS

None thus far.

OTHER DATA

Grantee: The Faculty of Hygiene and Public Health, University of São Paulo, São Paulo, Brazil

Funded by: Pan American Health Organization/World Health Organization

Timetable: 1966-1970 (with probable extension)
PROBLEM

Proper attention has not been given in Latin America to the field of reproductive biology, with the result that this complex area of science has lagged behind. The need for well-trained academic personnel in both basic and applied research is constantly increasing.

The fact that population growth, which is directly related to this area of study, exceeds the capacity to increase the food supply is a serious hindrance to the improvement of socioeconomic conditions in Latin America.

It is the intent of this project to contribute to the betterment of these conditions by improving the scientific manpower resources in the area of human and animal reproduction.

METHOD

Ten centers, from three different Latin American countries, have pooled their teaching and research facilities to develop a training program aimed at providing, through the scientific approach, indispensable general knowledge in the field of reproductive biology and population dynamics. After a general training period, each of the fellows in the program engages in a 17-month original research project, working with an experienced mentor on a tutorial basis.

The following institutions are participating:

Argentina: Laboratory of Neurobiology, Institute of Biology and Experimental Medicine; Laboratory of Endocrine Research, University del Salvador; and the Departments of Histology and Obstetrics of the University of Buenos Aires.

Chile: School of Public Health, University Maternity Hospital, Institute of Physiology, and the Barros Lacto Maternity Hospital, all of the University of Chile; and the Centro Latinoamericano de Demografia.

Uruguay: Service of Obstetric Physiology, University of Uruguay.
RESULTS TO DATE

The first course, which was held in 1967, had 56 participants from 12 Latin American countries - Argentina, Bolivia, Brazil, Chile, Colombia, Dominican Republic, El Salvador, Mexico, Nicaragua, Peru, Uruguay and Venezuela. After the first seven months of general background study, eight research projects were undertaken at the various participating institutions.

For the second course, 49 applications have already been received. These have come from Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Honduras, Mexico and Peru.

SIGNIFICANCE

In addition to fulfilling the aims of the project, it is hoped that by bringing different groups together to study this subject the training program will set an example of integration among the Latin American countries and will contribute to the development of collaborative research projects among the participants.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Jorge M. Rosner, Executive Director, Latin American Training Course on the Biology of Reproduction, Buenos Aires, Argentina

Funded by: The Ford Foundation, Pan American Health Organization, and the Population Council

Timetable: 1967-1970