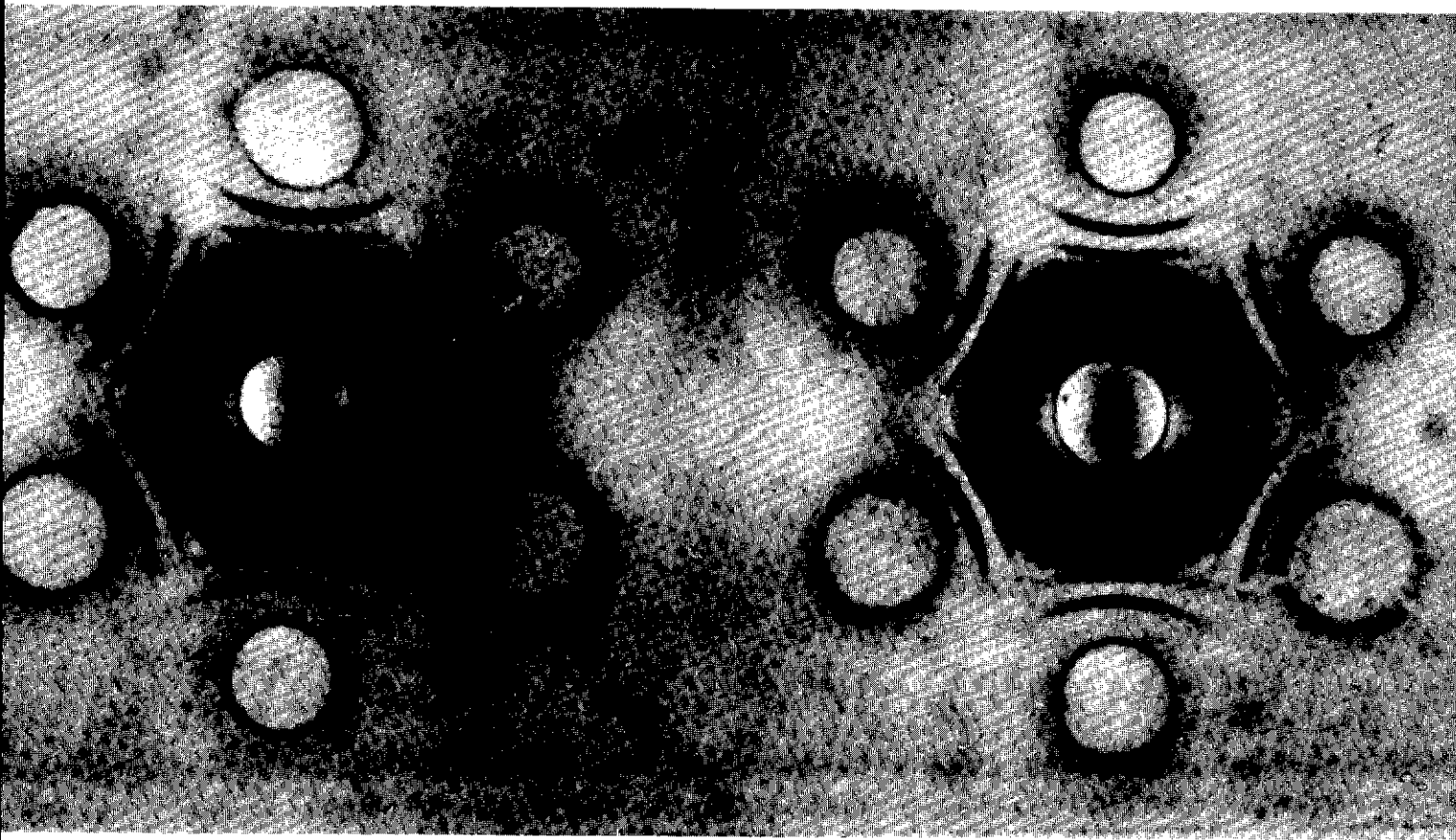


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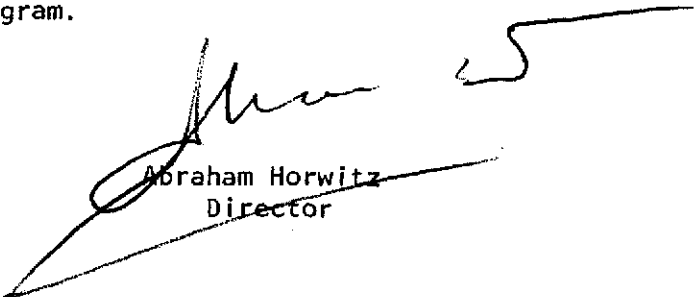
FOREWORD

In 1961 the Pan American Health Organization established a then quite modest program to support research in biomedical areas of significance to Latin America. In the past 13 years the program has appreciably expanded, both in the size of its financial support and in areas of investigative interest. Much of this expansion may be traced to the increasing interest in biomedical research of the governments of Latin America and the Caribbean, which are becoming ever more aware that the future welfare of their peoples rests on scientific and technologic advances. The development of an adequate research infrastructure has therefore become of great importance in the Americas, and the Organization is pleased that it has been able to further that development.

In addition to advising the governments of the Hemisphere on national research efforts and supporting an increasing number of independent investigators and research teams, the Organization also conducts its own research program. It supports a network of research centers concerned with biomedical problems such as the zoonoses, foot-and-mouth disease, perinatology, nutrition, and health planning, operates a rapidly expanding Regional Library of Medicine and the Health Sciences to coordinate and strengthen the provision of bibliographic and audiovisual materials to the Hemisphere's health workers, two centers on the technology of education in health, sponsors scientific meetings and conferences that present invaluable opportunities for exchanges among specialists in specific fields, and undertakes a sizable scientific publishing program.

The Organization believes, as reflected in the following pages, that it has contributed to foster new knowledge in the health sciences; it also believes that much remains to be done. Thus, in the years ahead, it will give particular attention to priority areas, including the administration and delivery of health services as a system, Chagas' disease, mycotic infections, tuberculosis, venereal diseases, heart disease and cancer, environmental problems, and human reproduction and development.

This volume, compiled by the Department of Research Development and Coordination, summarizes the information available on 154 projects which the Organization is now sponsoring and provides a panoramic view of our research program.



Abraham Horwitz
Director

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PROTEIN-CALORIE MALNUTRITION: EFFECTS, MECHANISMS OF ADAPTATION, PATHOPHYSIOLOGY, AND TREATMENT

PROBLEM

Protein and calorie deficiencies are interrelated and highly prevalent in developing countries. There is a definite need to better understand: (a) the effects of various degrees of protein and calorie deficiencies; (b) the interaction between malnutrition, diarrhea, and malabsorption; (c) the interrelationships between protein and calorie utilization, both during depletion and during recovery, including the effects of calorie balance, expenditure, and activity pattern; (d) the interactions between food intake, hormonal regulation, and body composition during recovery from malnutrition; and (e) muscle metabolism during caloric restriction.

In recent years efforts have concentrated on: (a) gastrointestinal physiology in protein-calorie malnutrition (PCM) and during recovery, in the presence or absence of diarrhea; (b) caloric intake, activity patterns, and body composition; (c) hormonal interactions in PCM; and (d) muscle metabolism in calorie deficiency.

METHOD

Longitudinal studies on gastrointestinal alterations were conducted in children admitted to INCAP's Clinical Center with severe PCM, with and without diarrhea. The studies included morphology, microbiology, and physiology of the small intestine at different levels. Intubations of the gastrointestinal tract were performed under basal conditions and after stimulation with a fat meal or intravenous administration of cholecystokynine. Samples of intestinal contents were studied to determine intraluminal events of fat absorption with emphasis on micelle formation, lipolytic activity, and bile composition in conjugated and free bile acids, as well as bacterial flora. Measurements of bile acid pools and turnover rates were also accomplished.

Effects of caloric intake, growth increase, and recovery from PCM were investigated in an animal model and a methodology was set up to study children recovering from PCM. The methodology included various activity regimens, energy balance, and body composition studies. In conjunction with this experimental design, growth hormone and insulin production were measured in response to physiologic as well as to maximal stimuli.

Muscle metabolism in caloric restriction was studied in rats through a hind-limb perfusion technique. Two groups of rats were studied: a control group and a group of adult males fed one-third of the controls' caloric intake. Arterial venous differences in metabolites and muscle composition were measured in both groups, before and after electrical stimulation of the sciatic nerve.

RESULTS TO DATE

Children with protein-calorie malnutrition, with or without diarrhea, had a decreased concentration of conjugated bile acids and a moderate increase in free bile acids, both primarily and secondarily. This was associated with increased anaerobic and facultative flora in the upper gastrointestinal tract and with a decreased capacity to form lipid micelles. This general characteristic was enhanced or made more severe by the presence of diarrhea. As the child recovered, intestinal flora decreased, conjugated bile acid concentration increased, and free bile concentration remained essentially constant. As a result, lipid-micelle formation was also increased. The presence of diarrhea in fully recovered children was accompanied by an increase in upper gastrointestinal flora, a marked increase in free bile acids, and a decrease in conjugated bile acids. Micelle formation from lipid meal was also reduced. The taurocholate pool in malnourished children was markedly reduced but attained normal values when the child recovered. The presence of diarrhea reduced the half-life of taurocholate to one-fifth of the normal value, suggesting a marked fecal loss of bile acids in diarrhea. All the above changes were also present, but to a lesser degree, in healthy children from low socioeconomic settings with poor hygiene. In this population, alterations in the morphology of the intestinal mucosa and in bile acid composition appeared as early as 3 months after birth.

Results of studies in the rat showed a marked decrease in efficiency of calorie and protein utilization and a decreased activity. A method to stimulate adequate activity patterns through games and progressive exercise regimens was developed for children recovering from malnutrition. Growth hormone and insulin radioimmunoassays are now standardized and samples of these substances, taken from children recovering from kwashiorkor under different activity patterns, are being studied.

To conduct more exhaustive studies in this area, we have successfully reproduced kwashiorkor and marasmus-like pictures in the rat.

Studies of muscle metabolism in calorie-deprived rats have shown that these animals break their muscles at a very fast rate when electrically stimulated, liberating a large fraction of myofibrillar nitrogen. At the same time, lactic acid and ammonia production are markedly enhanced. Amino acid analysis of the perfusates is under way.

SIGNIFICANCE

The significance of the research carried out in this project is multiple. Most important is an understanding of the basic mechanisms and effects of protein and calorie deficiency with emphasis on diarrhea and malabsorption. This should result in fundamental knowledge necessary for a rational treatment and the adoption of certain preventive measures. The possible benefit of physical activity in terms of efficiency of food utilization and growth and the reverse, the adverse effects of restricted activity, should be further investigated. The interactions between caloric and protein deprivation are becoming more evident as a consequence of these studies.

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OTHER DATA

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LONGITUDINAL STUDY OF THE EFFECTS OF MALNUTRITION ON PHYSICAL GROWTH AND MENTAL DEVELOPMENT

PROBLEM

Human growth in general is considered as an increase in size and as a change in function. Although hereditary factors determine the characteristics of growth to a large extent, growth is affected in one or more ways by disease and/or nutrition, both environmental factors.

The purposes of this study are to determine: (1) the modifications in physical growth and mental development that occur in preschoolchildren as a result of varying degrees of nutritional deficiencies; (2) at what age adequate nutritional supplements promote favorable changes in physical growth and mental development; and (3) which cultural and socioeconomic factors affect physical growth and mental development.

METHOD

Two groups of preschoolchildren, including newborn infants entering each group during that period, will be compared in physical growth and mental development on a longitudinal basis over 6 years. Both groups were drawn from communities previously matched for the main variables. The experimental group in one community is being given a nutritionally adequate supplement that provides 130 percent of the allowances recommended by INCAP. The control group in the other community is subsisting on its usual (deficient) dietary regimen.

Food supplementation is principally directed toward subjects under 3 years of age, pregnant women, and lactating mothers in the experimental communities.

The subjects are assessed periodically at predetermined age levels, by anthropometric measurements, biochemical determinations, dietary surveys, demographic and sociocultural studies, and neurologic and psychologic examinations.

RESULTS TO DATE

Food supplementation produces a significant increase in birth weight. For every 10,000 calories of food supplement a mother ingests during pregnancy, her child will weigh about 30g more than expected at birth. Moreover, food supplementation during pregnancy is associated with advanced psychomotor development in infancy. Food supplementation of the preschoolchild is related to a significant improvement in physical growth. The relationship between food supplementation and preschool mental development is currently being investigated.

Duration of diarrhea is inversely related to physical growth in infancy and the preschool years. In addition, fetal and maternal morbidity during pregnancy is associated with retarded psychomotor performance.

Socioeconomic status is a powerful predictor of birth weight, physical size in childhood, and psychologic test performance. Nutritional status, as estimated by attained size, is related to psychologic test performance, independently of the socioeconomic status of the child. Preliminary analyses indicate that nutritional and socioeconomic statuses differentially affect various cognitive abilities and both must be considered in assessing mental development in rural Guatemala.

SIGNIFICANCE

The majority of preschoolchildren in developing countries, which encompass over 66 percent of the world population, are now malnourished to varying degrees. Growth and development rates, which are fastest in preschoolchildren, are seriously impaired by the protein and calorie deficiencies as well as the infectious diseases prevalent in such children.

This study is yielding valuable data on the effects of environmental conditions on human growth and development potentials, including the influence of age of onset, duration, and severity of malnutrition.

If it is proved that malnutrition during pregnancy and the first years of life has lasting detrimental effects on human intellectual development, immediate measures to prevent malnutrition during those years should receive highest priority in public health programs.

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85. ----- et al. Testing for mental development. In: Swedish Nutrition Foundation, Uppsala, Almquist and Wiksells, 1974, in press.

OTHER DATA

Grantee: Dr. Robert E. Klein, Division of Human Development,
Institute of Nutrition of Central America and
Panama (INCAP), Guatemala City, Guatemala.

Funded by: National Institute of Child Health and
Human Development.

Timetable: 1965-1977.

THE SOCIAL BACKGROUND OF MALNUTRITION IN URBAN KINGSTON

PROBLEM

The purpose of this study is to gain insight into the social background of childhood malnutrition in urban Kingston. The study aims in particular at discovering if there are frequently recurring social problems common to families of children with protein-calorie malnutrition as compared with families of similar income and environment, whose children are relatively adequately nourished. It is designed to ascertain to what extent these problems can be relieved and perhaps eliminated by nonmedical means.

METHOD

Fifty infants between the ages of 1 month and 3 years and their mothers are being followed from date of discharge from two hospitals in Kingston. Another group of mothers and infants of corresponding age, sociocultural and economic background, but emotionally and functionally healthy are serving as controls; they are from the same areas of town, and often the same yards as the study sample.

Monthly anthropometric examinations of the infants are made. Psychosocial, cultural, and economic data are being collected by an anthropologist. Intensive family case studies will be conducted on six families.

RESULTS TO DATE

None thus far.

SIGNIFICANCE

(1) The project is significant for the Jamaican nutrition program, in respect of diagnosis and program planning;

- (2) For the future of management and prevention of malnutrition, particularly in the poor urban areas of developing countries. It is anticipated that this research will give us a more effective means of helping deprived children who have been in hospital than does the conventional medical outpatient follow-up.

PUBLICATIONS

1. Cook, R. A new proposal for nutrition education: an anthropological perspective, in preparation.

OTHER DATA

Grantee: Ms. Ida Daum, Department of Anthropology,
University of Washington, Seattle, Washington
and Research Fellow, Caribbean Food and Nutrition
Institute (CFNI), Kingston, Jamaica.

Funded by: No support received as of 1 February 1974.
CFNI provides an office base with the usual
services (secretarial, library, assistance
with data analysis).

Timetable: 1973-1975.

EVALUATION OF THE EFFECTS OF NUTRITION EDUCATION ON NUTRITIONAL STATUS

PROBLEM

Nutrition education has always been a part of programs to combat malnutrition. Its specific contribution to the betterment of nutritional status has been difficult to assess, however, because it has usually been integrated with other inputs such as food aid or specially recruited personnel.

Not only is it important to try to define the role of nutrition education per se, but it is equally, if not more important, to make models of nutrition programs that will be easily replicable in other regions where childhood malnutrition occurs.

Consequently, the need exists for devising a program in which the dominant input is nutrition education provided as economically as possible, and having as its main contributors people indigenous to the region.

METHOD

A rural area, the Lambs River district of the parish of Westmoreland, Western Jamaica, was selected as the study area, and a similar area, Darliston, as a control.

Socioeconomic and relevant anthropometric data were collected from both areas during the first 6 months of the project. Such data have established the basic similarities between the two areas.

A staged program of nutrition education will now be developed in the study area, principally involving schools, the health service, agricultural extension services, community development services, voluntary organizations, and volunteer groups. Meanwhile, no similar activities are planned for the control area.

During the project we intend to use child anthropometry, nutrition knowledge and practices, especially in relation to home food production and use, and morbidity trends, as objective parameters of change. In addition, there will of necessity be some aspects that only allow subjective assessment.

RESULTS TO DATE

No results will be available before the final evaluation, which will take place during the last 6 months of the project.

SIGNIFICANCE

Since the goal of nutrition education is to achieve favorable change in the nutrition behavior and practices of people, the involvement and participation of the latter in formal programs are vital to its attainment. Hence, by eschewing the more expensive and sophisticated, but not easily duplicated teaching methods, an inexpensive and workable model of nutrition education will be created. It will be appropriate to the needs of many of the more remote and depressed "pockets" of malnutrition to be found in the Caribbean and elsewhere in the developing world.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Caribbean Food and Nutrition Institute Kingston, Jamaica.
Funded by:	Freedom from Hunger Campaign, United Kingdom.
Timetable:	1971-1974.

FORCES OF INFECTION ACTING ON THE CENTRAL AMERICAN POPULATION

PROBLEM

Infectious diseases interact with malnutrition in a synergic manner. Knowledge of the forces of infection that act on the Central American population is important in health planning, particularly because they are of great significance as to morbidity and mortality and their interaction with malnutrition.

The purpose of the present research is to analyze, on a large scale, a serum bank representing the various ethnic and socioeconomic groups of the six Central American countries for antibodies to several infectious agents that are of known public health significance.

METHOD

A large bank with approximately 17,000 serum specimens was assembled in 1965-1967. The bank is split into two identical collections, one located at the Institute of Nutrition of Central America and Panama (INCAP), and another at the Middle America Research Unit (MARU) in Panama.

Analysis of sera collected in a long-term study conducted in a Guatemalan highland community was started in 1973. The purpose of this analysis is to determine the development patterns of antibodies to specific agent such as Streptococcus, Shigella, rubella virus, herpes simplex virus, cytomegalovirus, Entamoeba histolytica, and Toxoplasma gondii.

Very small amounts of serum are analyzed to determine the prevalence and titer of antibodies to syphilis, Brucella, Salmonella, Shigella, Streptococcus, enteroviruses, adenoviruses, the rubella agent, arboviruses, vesicular stomatitis virus, Toxoplasma gondii, Trypanosoma cruzi, malaria, and Entamoeba histolytica. Microtechniques are used to process the several thousand sera.

RESULTS TO DATE

A profile of antibodies to the various agents investigated has been obtained for the various populations of Central America.

Data have been analyzed by country, age, sex, locality, occupation, and other parameters.

The study of epidemics, and even of their anticipation, has been possible through analyses of the serum bank.

SIGNIFICANCE

This is the first survey attempting to describe the characteristics of a representative sample of the population of Central America by standard and accurate techniques of serologic analyses.

The information obtained will be helpful in unveiling public health problems, in recognizing the relative importance of the various infections, and in directing certain public health measures as vaccination.

PUBLICATIONS

1. Gangarosa, E. J. et al. Epidemic Shiga bacillus dysentery in Central America. II. Epidemiologic studies in 1969. J Infect Dis 122:182-90, 1970.
2. Mata, L. J. et al. Epidemic Shiga bacillus dysentery in Central America. I. Etiologic investigations in Guatemala. J Infect Dis 122:170-80, 1970.
3. ----- et al. Epidemic Shiga dysentery in Central America. Lancet 1:600-01, 1971.
4. ----- and R. Wyatt. The uniqueness of human milk. II. Breast-feeding and host resistance to infection. Am J Clin Nutr 24:976, 1971.
5. ----- et al. Epidemia de disentería Shiga en Centroamérica. I. Investigaciones etiológicas en Guatemala, 1969. Bol Of Sanit Panam 71:93-107, 1971.
6. ----- et al. Avances sobre el conocimiento de la disentería en Guatemala. Rev Latinoam Microbiol, vol. 14, 1972.

OTHER DATA

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

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

Timetable: 1968-1974.

MALNUTRITION, INFECTION, AND HUMAN LEUKOCYTE METABOLISM

PROBLEM

The purpose of the present investigation is to study in children, the impact of protein-calorie malnutrition (PCM), iron deficiency, and infection on the metabolic capacity of fresh blood-derived polymorphonuclear leukocytes (PMN) and on their phagocytic and bactericidal capacity.

METHOD

The above functions are measured by the rate of oxygen uptake in resting and phagocytosing leukocytes, the rate of bacterial engulfment and killing, and by assay of the enzymes' myeloperoxidase system. All of these studies are carried out in PMN leukocytes isolated from blood and purified. The microorganisms used for bactericidal capacity testing are E. coli and staphylococcus.

RESULTS TO DATE

Fifty children have so far been studied. Twelve of them are healthy and well nourished; 3 are well-nourished but infected; 12 have clinically recovered from PCM; 5 have clinically recovered from PCM but are infected; 9 are severely malnourished; and 9 are severely malnourished and infected.

In these children, the resting leukocytes' oxygen uptake was a good index for detecting the presence of infection, and the leukocytes' oxygen uptake during phagocytosis was related to their nutritional state. Leukocytes of children clinically recovered from protein-calorie malnutrition had lower phagocytosis and apparent killing rates. When these parameters are combined, they show that the efficiency of the killing process is reduced in these children.

With respect to the intracellular mechanism responsible for bacterial killing, preliminary evidence has been obtained that points toward a defect in myeloperoxidase.

SIGNIFICANCE

These results permit a better understanding of infection-nutrition relationships and open up the possibilities for systematic studies of the impact that PCM, whether alone or in combination with iron deficiencies or others, may have on the susceptibility to infection of large groups of population at risk, especially in underdeveloped areas of the world.

PUBLICATIONS

1. Pineda, O. Malnutrition, infection and human polymorphonuclear leukocyte metabolism. Fed Proc 31:710, 1972.

OTHER DATA

Grantee:	Dr. Oscar Pineda, Division of Physiological Chemistry, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.
Funded by:	National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.
Timetable:	1973-1975.

ANALYSIS OF DATA FROM AN ECOLOGIC STUDY OF INFECTION, MALNUTRITION, AND GROWTH OF CHILDREN IN A GUATEMALAN INDIAN VILLAGE

PROBLEM

The purpose of this study is to analyze from a long-term investigation of children's infections with different microorganisms, conducted by INCAP's Division of Microbiology from 1963 to 1971 in a semiisolated rural Indian community of the highlands of Guatemala. The 9-year investigation examined (1) the correlation between infection and microbial colonization of the intestinal tract with episodes of illness, and (2) dietary habits of children from the time of birth to the age of 7.

The main objective of the present study is to assess the effect of these variables on the state of nutrition and on the growth and development of the children in the community studied.

METHOD

Data on growth, dietary habits, infections and infectious diseases, as well as on many aspects related to the ecology of malnutrition have been transferred to IBM cards and magnetic tapes in order to be processed.

RESULTS TO DATE

All this information has been analyzed by the Department of Epidemiology of the University of Washington in Seattle, Washington, since August 1973. Results of the analysis will be published in a special monograph.

SIGNIFICANCE

It is expected that the study findings will lead to a better understanding of the etiology of diarrheal disease and to the establishment of adequate control and preventive measures in areas with poor socioeconomic development.

PUBLICATIONS

1. Mata, L. J. et al. Environmental factors and fetal growth in a low socioeconomic highland community. Presented at: 101st Annual Meeting of the American Public Health Association, San Francisco, California, November 7, 1973.
2. ----- et al. Survival and physical growth in infancy and early childhood of Guatemalan Indian infants, by birth weight and gestational age. Presented at: Workshop on: Malnutrition and Infection During Pregnancy as Determinants of Growth and Development of the Child. Institute of Nutrition of Central America and Panama (INCAP), Guatemala, January 9-11, 1974.
3. ----- . Enteric infections of children: factors determining host susceptibility. In: Global Impacts of Applied Microbiology. Proceedings of the IV International Conference, São Paulo, Brazil, July 23-28, 1973.

OTHER DATA

Grantee: Dr. Leonardo J. Mata, Division of Microbiology, Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala.

Funded by: U.S. National Institute of Child Health and Human Development, National Institutes of Health.

Timetable: 1972-1974.

PROTEIN REQUIREMENTS OF CHILDREN OF PRESCHOOL AGE

PROBLEM

Previous studies at the Institute of Nutrition of Central America and Panama (INCAP) have shown that the best protein diet for preschool-children is a mixture of corn and beans, each of these ingredients contributing about 50 percent of the protein (food ratio by weight, 2.6/1). This mixture of cereal and leguminous proteins causes maximum complementation of the deficits in lysine and tryptophan in corn, and methionine in beans. Results in animals have been fully confirmed in children of preschool age under the conditions of the Metabolic Unit. In view of this, two further studies have been carried out:

- 1) To evaluate the nutritional value of a vegetable diet predominantly based on corn and beans, in children living in an environment similar to that of low socioeconomic rural populations of Central America, particularly with poor sanitation and poor clinical control conducive to high morbidity. The diet acceptability and tolerance under more natural conditions is being assessed.

- 2) To determine the effect of changes in diet caloric density, on children's ability to consume enough to satisfy their energy requirements.

METHOD

Part 1. The study included 16 children aged 2-4 years who lived in a high-morbidity institution. It lasted 3 months. The children's diet was planned to provide 1.6 g/kg/day of protein in proportions of 60 parts of corn protein to 40 parts of bean protein. These two foods contributed 95 percent of the N. The remainder came from common vegetables and fruits, and sugar, bringing the caloric intake to 90 cal/kg/day. Menus were consumed ad libitum. To assess the effect of infection on nutrition, morbidity was recorded daily, and the children were divided into three morbidity groups: I, low; II, intermediate; and III, high.

Part 2. Four children aged 1-2 years, completely recovered from protein-calorie malnutrition, received corn-bean protein diets (50:50) as only source of N in quantities adequate to meet nitrogen and amino acid requirements. The amount of fat was varied to provide 3, 12, or 21 percent of their intake. After they had consumed the prescribed

diet, children were given the opportunity to eat additional amounts ad libitum. The total caloric and protein intake were calculated. Weight and height gain and N balance were measured during each 3 week-diet period.

RESULTS TO DATE

Part 1. The 2 to 4 year-old children generally showed a deterioration in their nutritional status. Analysis of results from the different morbidity subgroups I, II, and III, showed an inverse correlation between morbidity and nutritional status: Children with higher morbidity suffered the largest deterioration in their nutritional status. A close relationship was also found between morbidity and anorexia, indicating that repeated infections caused a decrease in food intake. It may be concluded that a reduction in disease rates in low socioeconomic rural groups consuming this type of diets, would produce by itself an improvement in nutritional status.

Part 2. With meals containing 3, 12, or 21 percent fat, the children tolerated and consumed enough corn-bean based diet to meet their energy needs, and their protein and amino acid requirements were amply met. In general, however, the energy intake with the diet of 21 percent fat was higher. N balances showed no clear advantage in the higher fat diet over the lower ones.

SIGNIFICANCE

The first part of the study helped demonstrate that nutritional programs alone may be of little or no value, if the problem of high rate of disease is not attacked successfully and simultaneously. This concept is important in the establishment of priorities of programs at local, regional, or national level. The second part seems to confirm the concept that the combination of cereals and leguminous seeds, if properly combined, can be the basis for breaking the vicious cycle of protein-calorie malnutrition in underdeveloped areas.

PUBLICATIONS

1. Arroyave, G. Amino acid requirements and age. In: Proceedings of the International Symposium on Protein-Calorie Malnutrition. Chiang-Mai, Thailand, January 8-11, 1973, in press.

2. Parrilla, C. M. Valor nutritivo global de dietas cuyas proteínas provienen predominantemente del maíz y del frijol negro en niños de dos a siete años. Interrelación entre morbilidad y nutrición. Trabajo de tesis de maestría en el Curso de Postgrado en Bioquímica y Nutrición Humana. Institute of Nutrition of Central America and Panama, Guatemala City, 1973, Talleres Gráficos del INCAP, 1973, 116 p.

OTHER DATA

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

Funded by: World Health Organization.

Timetable: 1968 - continuing.

IMPROVEMENT OF THE NUTRITIVE QUALITY OF CEREAL-BASED DIETS

PROBLEM

Cereals constitute the principal sources of calories and provide about two-thirds of the dietary proteins of the population of Central America. We recognize that this situation will long continue, particularly in tropical countries, because cereals have a relatively high calorie yield per unit of cultivated area, especially in view of the advances that have recently been made in improving their yield.

The problem with diets of a high cereal content is that their protein concentration is generally low and their protein quality inadequate. This is particularly serious for small children. INCAP has identified in previous studies the limiting amino acids of the proteins in cereals commonly consumed in Central America, and has demonstrated that these deficiencies can be corrected through the addition of either synthetic amino acids or proteins that provide the amino acids in which the cereals are deficient. Our studies now underway are oriented to exploring ways in which these findings can be put into practice within the framework of today's cereal processing and consumption habits in Central America.

METHOD

We have tried to determine the most effective formulas to improve the protein quality of different cereals through the addition of protein concentrates with or without synthetic acids, and we are studying ways to put this type of enrichment into practice either in centralized industrial flour processing or under the conditions of cereal processing and consumption that obtain in rural communities. Infusion techniques have been investigated as a means of enriching cereal grains with amino acids.

RESULTS TO DATE

A corn formula has been developed that contains a protein concentrate of soya plus lysine, minerals, and vitamins. The addition

of this concentrate to corn flours results in improvement not only of protein concentration and quality but also of the general nutritive value of the product. Studies have been under way to determine the feasibility of enriching corn with this formula in the small mills that operate in Guatemala's rural communities and the resulting effects on the nutritional status of the population of the community. Infusion techniques with amino acids have proved very effective in enriching corn and rice. Grains of either cereal have been prepared with high concentrations of the limiting amino acids. Additional studies have shown that cereal-based diets may be improved in quality through the addition of oil. Results have also shown that very small amounts of animal proteins consumed daily are also very effective in increasing the quality of the diets.

SIGNIFICANCE

As previously noted, cereals constitute a very important part of the diet of large sectors of the Central American population. It would be very difficult for economic reasons and even in certain cases because of dietary habits to improve the diet through the addition of other products. But if cereals could be improved without changing their retail price or taste characteristics, a significant nutritional advance would be achieved.

PUBLICATIONS

1. Bressani, R. La calidad proteínica del maíz opaco-2 como ingrediente de dietas rurales de Guatemala. XVII Reunión PCCMCA, Panamá, March, 1971.
2. ----- . El valor nutricional del arroz en comparación con el de otros cereales en la dieta humana de la América Latina. Seminario sobre Políticas Arroceras de la América Latina. CIAT, Cali, Colombia, October, 1971.
3. ----- and L. G. Elías. La calidad proteínica del maíz opaco-2 como ingrediente de dietas rurales de Guatemala. Arch Latinoam Nutr 22:577, 1972.
4. Elías, L. G. Mejoramiento tecnológico de la calidad proteínica del maíz. II Cong Nac Asoc Técnicos de Alimentos de México. March, 1971.

5. ----- and R. Bressani. Improvement of the protein quality of corn-bean diets by the use of fortified corn or opaque-2 corn. III Western Hemisphere Nutrition Congress. Bal Harbour, Florida, August-September, 1971.
6. ----- and ----- . Valor proteínico de los subproductos de la industria del trigo. II. Complementación y suplementación del granillo de trigo con concentrados proteicos. Arch Latinoam Nutr 23:95, 1973.
7. Gómez Brenes, R. Valor proteínico del arroz. XVII Reunión del PCCMCA. Panamá, March, 1971.

OTHER DATA

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

Funded by: International Development Research Center, Ottawa, Canada.

Timetable: 1970-1975.

CORN FORTIFICATION: A FIELD DEMONSTRATION MODEL

PROBLEM

The shortage and poor quality of food consumed in many developing areas will not be rapidly overcome by orthodox methods. Other measures must therefore be investigated, particularly those with a theoretical potential significance. Metabolic ward experiments in animals and humans have shown that the addition of small amounts of the limiting amino acids to foods such as corn results in a remarkable improvement in their quality. Such fortification has not been investigated extensively under field conditions, however.

The purpose of the present research is to conduct a fortified corn field trial in a whole rural community in which a significant amount of information on growth, nutrition, and infection has been collected during the past 8 years. Fortification will make the biologic value of corn comparable to that of meat. It is expected that if this measure is effective, an improvement in fetal growth, infant mortality, and general health will be observed in the community within 2 years.

METHOD

The methods to be followed will be basically those used in the study of intestinal infection and colonization by microorganisms. The supplement consists of soya flour and synthetic lysine added to corn during the milling process. Corn represents 70 percent or more of the protein and calorie intake of villagers. The supplementation will be monitored by frequent chemical analyses of tortillas. Fetal growth will be measured by determining time of gestation and birth weight. Morbidity will be determined by periodic clinical examinations. Postnatal growth will be measured anthropometrically.

RESULTS TO DATE

Preliminary analysis of data collected since 1972 is showing changes in the pattern of postnatal growth in that children are growing better than before corn fortification was started. Infant mortality and mortality of children 1-4 years of age have decreased in families accepting the fortification. There has not been any increment

in the average birth weight at the community level. However, mothers who fortified their corn during pregnancy had fewer babies with birth weight under 2,500g than did mothers who did not accept fortification. Corn fortification has been accepted by 40 percent of the community families. They fortify their corn from 75 to 100 percent of the times they mill it. A more detailed analysis of this program will be made after 2 years of fortification.

SIGNIFICANCE

If corn fortification works at the community level, its introduction in a whole region or even nationally, might dramatically change the nutritional status and growth pattern of the population. The supplement itself does not cost significantly more than corn.

PUBLICATIONS

1. Mata, L. J. et al. A model for maize fortification with soy bean flour, lysine and other nutrients in a low socioeconomic rural community. In: Bressani, R., E. Braham, and M. Béhar (eds.), Proceedings of the Conference on Nutritional Improvement of Maize, Institute of Nutrition of Central America and Panama (INCAP), Guatemala, March 1972.

OTHER DATA

Grantee:	Leonardo J. Mata, Division of Microbiology, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.
Funded by:	U.S. Agency for International Development (USAID).
Timetable:	1971-1976.

CONTROL OF HYPOVITAMINOSIS A (FORTIFICATION OF SUGAR WITH RETINOL PALMITATE)

PROBLEM

The Institute of Nutrition of Central America and Panama (INCAP) has been conducting extensive research into the solution of the widespread vitamin A deficiency problem among the populations of Central America. In 1973 INCAP proposed a method for hypovitaminosis A control which, though still subject to improvements, was considered adequate to be implemented at the national level. It was decided, however, that more research was needed to improve the physical stability of the proposed mixture and to facilitate even further the process of nutrient addition at industrial scale.

METHOD

Research was carried out along the following lines:

- 1) Preparation of a retinol palmitate premix with sugar (1:10) that did not segregate.
- 2) Development of a process of nutrient addition that would ensure adequate, even distribution of the fortifying material in the vehicle (sugar).

RESULTS TO DATE

A very adequate premix has been prepared in cooperation with the research laboratories of Hoffmann-LaRoche, Basle, Switzerland. It contains 50,000 IU/g of vitamin A, is in crystalline form, and does not separate from the sugar. This premix has been tested under a variety of conditions. Industrial-scale pilot trials have been conducted using a newer, simpler process than the one previously described, and which did not entail the addition of equipment or labor in the factories. A colorimetric method for the control of the efficiency of the fortification process and for the regular inspection of the program has also been developed. It requires no laboratory equipment such as a colorimeter, takes approximately 1 minute for simple determination, and costs about two cents. This method is performed with a portable apparatus.

SIGNIFICANCE

A new, simple technique like the one just described, promises to wipe out vitamin A deficiency from all areas of the world suffering from it where conditions permit its application. This approach is badly needed in underdeveloped countries where complex and expensive investments are a block for the implementation of improvement programs.

PUBLICATIONS

1. Arroyave, G. and C. de Funes. Enriquecimiento de azúcar con vitamina A. Método para la determinación cuantitativa de retinol en azúcar blanca de mesa. Arch Latinoam Nutr, in press.
2. ----- et al. Enriquecimiento de azúcar con vitamina A. Método rápido para la fácil inspección del proceso. Arch Latinoam Nutr, in press.
3. ----- . Fortification of sugar with retinol: proposed approach for control of hypovitaminosis A in population groups. In: International Sugar Research Foundation, Proceedings of the Fifth International Sugar Research Symposium, Mexico City, Mexico, September 6, 1972. Bethesda, Maryland, U.S.A., 1972, pp. 22-29.
4. Instituto de Nutrición de Centro América y Panamá. Fortificación de azúcar con vitamina A en Centro América y Panamá. Guatemala, C.A., 1974. INCAP Publication V-36.

OTHER DATA

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

Funded by: Hoffmann-LaRoche and Co., Basle, Switzerland.

Timetable: 1971 - continuing.

DEVELOPMENT OF UNCONVENTIONAL PROTEIN SOURCES FOR HUMAN OR ANIMAL NUTRIMENT

PROBLEM

A marked scarcity of proteins to satisfy the biologic needs of the population still exists in Central America and areas with similar ecologic conditions. Even though in many countries there have been increases in the production of foods of animal origin, particularly beef, the increases have been assigned for economic reasons to exportation and the domestic availability and consumption of such products by large sectors of the population has been really diminishing. Even the availability and consumption of traditional vegetable protein sources such as beans has also been decreasing. Because of this situation and independently of the efforts being made to correct these problems, exploring the possibility of using available but not commonly used protein sources is imperative. INCAP's previous studies along these lines led to the development of vegetable mixtures such as Incaparina. Ways to better use cotton seed are still being studied, while other sources of vegetable protein are being explored at the same time.

METHOD

Seeds from the rubber tree (Hevea brasiliensis), the calabash (Crescentia alata), and the annatto (Bixa orellana) have been studied. The first two seeds contain significant quantities of oil. For that reason the nature and characteristics of their oil and proteins have been studied since only the rational use of both products would economically justify the industrial exploitation of these seeds. The annatto seed is really a byproduct of the extraction of the dye bixina, which is widely used industrially. If the seed as a protein source as well as bixina could both be used, the economic exploitation of the annatto would be stimulated and at the same time another protein source for animal or human nutriment would exist.

RESULTS TO DATE

We have found that rubber seed oil is toxic and for that reason cannot be used for either animal or human nutriment, although it has semisiccative properties that make it of interest to some industries,

e.g., painting. The seed's protein is of medium nutritive value, with a PER of 1.9 compared to casein's 2.7. Calabash seed oil is fit for human consumption and the seed's protein has a moderate nutritive value similar to that of the rubber seed, which could be improved either through combinations with other proteins or with amino acid supplementation. There thus exists a possibility of using this widely available seed in Central America, that will continue to be explored. The annatto seed's protein content is relatively low at about 15 percent. Nevertheless it contains appreciable quantities of carbohydrates and inasmuch as it is a bixina industry byproduct it could suitably be used to feed animals, thus replacing cereals that are now diverted from human needs. We will continue to explore this possibility.

SIGNIFICANCE

These studies are oriented toward a more rational utilization of Central America's natural resources in solving one of the area's most serious nutritional problems, the scarcity of proteins for human as well as animal nutriment. The results of this investigation may be of importance not only in public health and nutrition but also in the Central American countries' economic development, inasmuch as certain protein sources especially soya for animal feed, are now being imported.

PUBLICATIONS

1. Bressani, R. Efecto de la substitución del nitrógeno de la proteína de leche por nitrógeno de urea en terneros no rumiantes. III Reunión Sociedad Latinoamericana de Producción Animal. Bogotá, April, 1971.
2. ----- . Fuentes vegetales ricas en proteína para consumo humano en América Latina. II Cong Nac Asoc Técnicos de Alimentos de México. Tecnología de Alimentos (México) 6:29, 1971.
3. ----- . Application of food science and technology to the exploitation of new protein sources. III West Hem Nutr Cong, Bal Harbour, Florida, August-September, 1971.
4. De la Fuente, G. Composición química y valor proteínico de la torta de palma africana y corozo. II Reunión Centroam Tecnología de Aceites, Grasas y Proteínas. Guatemala, October, 1971.

5. Elías, L. G. Evaluación nutricional del aceite y de la torta de la semilla de hule. II Reunión Centroam Tecnología de Aceites, Grasas y Proteína, Guatemala, October, 1971.
6. Gómez Brenes, R. Evaluación nutricional del aceite y de la torta de la semilla de jícara o morro (Crescentia alata). II Reunión Centroam Tecnología de Aceites, Grasas y Proteína, Guatemala, October, 1971.
7. Jarquín, R. et al. Evaluación del valor nutritivo de sub-productos del camarón en la alimentación de pollos. Turrialba 22:160, 1972.

OTHER DATA

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

Funded by: International Development Research Center, Ottawa, Canada.

Timetable: 1970-1975.

PROCESSING OF BEANS TO PRODUCE AN INSTANT BEAN FLOUR

PROBLEM

The common bean (Phaseolus vulgaris) has been the traditional protein supplement in cereal diets based on corn or rice that are consumed by the majority of the Central American population. Because of changes in agricultural economics, however, this product is becoming ever more expensive and its consumption is therefore declining. Moreover, small rural producers have problems storing this grain. As a consequence, great fluctuations are observed in bean prices, from the relatively low ones of the harvest period to the periods of much scarcity and high prices before the following harvest.

We have considered the possibility of processing beans to obtain a flour that once mixed with water would yield a product similar to the paste that is the most common form in which the populations of Guatemala and other areas consume this grain. This processing would also allow the improvement of the beans' nutritive value through the addition of methionine, the limiting amino acid of beans and a product that already exists in synthetic form at a very low price.

METHOD

INCAP has a wide research program on legume foods, which include the following aspects: variability in chemical composition and nutritive value; effect of storage on cooking quality and nutritional value; factors responsible for the low-protein digestibility in beans; effect of maturation and germination and industrial utilization of beans.

We have investigated what would be the most favorable cooking process as to economy and the effect of processing on the nutritive value of beans as well as ingredients causing adverse physiological effects that they are known to contain. Different drying methods are also being studied to obtain a flour with desirable qualities of taste at a reasonable processing cost.

RESULTS TO DATE

We have determined that a soaking period of 8 hours reduces steam-pressure cooking to 10 minutes, allows better retention of the

beans' nutritive value, and destroys their undesirable substances. With the product thus cooked, flours have been prepared through drying at low temperature. We have studied the taste characteristic of these flours, which appear to be favorable, and their storability in different types of packaging and in different atmospheric conditions. Satisfactory results have been achieved with storage periods no longer than 5 months up to now. We believe this to be insufficient and are investigating ways to overcome the alteration the flours undergo, which is chiefly due to the rancidification of their fats. These studies will be continued. Methods have been developed to measure hardness in beans using a penetrometer. This instrument has been very useful in measuring the effects of storage under different environmental conditions. Biologic studies have shown a protein fraction soluble in water to be important in determining digestibility.

SIGNIFICANCE

We hope through these studies to resolve an economic and industrial problem that is of great significance in animal nutrition in areas such as Central America where beans constitute a very important source of dietary protein in human nutriment.

PUBLICATIONS

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OTHER DATA

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

Funded by: International Development Research Center, Ottawa, Canada.

Timetable: 1970-1975.

UTILIZATION OF COFFEE BYPRODUCTS

PROBLEM

In processing coffee two subproducts are obtained, pulp and skins. Dry pulp has a chemical composition similar to that of cereals, as a result of which the possibility of using it for animal feed has been considered. The skins, on the other hand, are a product with a high cellulose content that could also be used as a bulk material for feeding cellulose-using ruminants.

Previous studies at INCAP and other laboratories have nevertheless shown that coffee pulp contains products that are toxic or adversely affect animals physiologically. The purpose of this investigation is to determine if there are processes easily applied at field level that would allow the elimination or neutralization of coffee pulp's toxic products so that it could be used in feeding animals, and also to determine to what degree coffee skins could be used as ruminant food.

METHOD

Two possible ways to use coffee pulp as animal feed have been considered, the first that of processing it so as to destroy harmful ingredients and the second that of extracting its proteins so they would be nontoxic. The effect of ensilage on the pulp has been studied to test the first possibility, and protein extraction through the use of suitable enzymes and inorganic solvents is being studied to test the second possibility.

RESULTS TO DATE

It has been demonstrated that ruminant performance is inversely related to coffee-pulp level in the diet. Coffee-pulp silage is a convenient way to feed this coffee byproduct to cattle. Ruminants fed diets containing 30 percent fermented pulp (ensilage) grow as well as those fed diets of 30 percent dehydrated pulp. Animals fed 30 percent ensilaged pulp also have higher fatty acid blood levels than do controls or those fed 30 percent dehydrated pulp. This effect is similar to that observed with insulin, epinephrine, or caffeine.

Coffee-pulp feeding reduces nitrogen balance, protein digestibility, and increases urine volume.

The use of enzymes and inorganic solvents has allowed the extraction of about 70 percent of protein from coffee pulp. Chemical analysis of the protein indicates that in essential amino acids it is comparable to protein of good biologic value, which means that the process would be of great use if a practical extraction method is achieved. Swine have been fed diets of up to 24 percent of coffee pulp. Growth, feed conversion efficiency, and nitrogen balance decreases as the amount of coffee pulp in the diet increases. The factors responsible for these effects are not known, but there is some evidence that they might be caused by the caffeine, phenolic compounds, and the lack of nutrient availability in the pulp. It has been demonstrated that coffee bean skins can form up to 30 percent of the bulk material in the diet of ruminants. Pelletization of the feed improves the utilization of the skins still more. Contacts have already been established with the animal feed industry so that it may consider applying this finding.

SIGNIFICANCE

If methods can be established for the suitable use of coffee subproducts in animal feed, great economic advantages would accrue for coffee-producing countries that have enormous quantities of the subproducts and, at the same time, such use would spur the development of the livestock industry and thus results in more efficient and cheaper production of food from that source.

PUBLICATIONS

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2. ----- . Utilización de pulpa de café. Valor nutritivo en monogástricos. III Reunion Asoc Latinoam Producción Animal. Bogotá, April, 1971.
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OTHER DATA

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

Funded by: International Development Research Center. Ottawa, Canada.

Timetable: 1973-1975.

HEMATOLOGIC INVESTIGATIONS IN THE CENTRAL AMERICAN POPULATION

PROBLEM

Epidemiologic studies and therapeutic trials in population groups in Central America have indicated that iron deficiency is the most important cause of nutritional anemia. Folate deficiency, evident by biochemical analysis, apparently has no direct influence on hemoglobin levels, although it may limit the response to iron administration. In continuation of previously reported studies, we have concentrated on finding an adequate form of iron supplementation to the population. Various forms of iron have been tested, both at the Clinical Center and at the population level, with emphasis on an iron salt that would behave more like the hem iron than like the inorganic (cereal-vegetable) iron.

METHOD

At the Clinical Center patterns of iron absorption have been studied after the administration of ferrous sulfate, ferric orthophosphate, sodium iron pyrophosphate, hemoglobin, and sodium iron EDTA administered alone and in combination with corn and beans or with purified corn starch. At the population level, a coastal group of anemic individuals were given therapeutic doses of iron to bring to normal their iron nutrition. The population was then divided into two similar groups: one receiving sugar fortified with ferric orthophosphate and the other receiving unfortified sugar. This test lasted 9 months.

RESULTS TO DATE

Ferric orthophosphate and sodium iron pyrophosphate are essentially nonabsorbable. The pattern of absorption of ferrous sulfate was different from that of sodium iron EDTA which resembled more the pattern of the hem iron. Administration of ferrous sulfate mixed with corn and beans changed the iron absorption and also apparently decreased it, when compared to the effect of ferrous sulfate mixed with purified corn starch. In contrast, the iron from hem and from sodium iron EDTA was better absorbed with corn and beans or with corn starch; there appeared to be no difference between the effects of these two nutrients. We are now testing sodium iron EDTA

and ferrous sulfate at a population level. The previous population level study on iron supplementation with sugar fortified with ferric orthophosphate has corroborated the Clinical Center's findings that the iron in ferric orthophosphate is essentially nonabsorbable.

SIGNIFICANCE

Iron fortification and supplementation has not been accomplished in populations who consume primarily cereals and legumes. The discovery of a form of iron that would be absorbed similarly to the iron in hem would greatly facilitate the implementation of iron-supplementation programs.

PUBLICATIONS

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2. Cook, J. D. et al. Nutritional deficiency and anemia in Latin America. A collaborative study. Blood 38:591-603, 1971.
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8. ----- and E. Cifuentes. Capacidad física en trabajadores agrícolas de la costa de Guatemala antes y después de terapia con hierro. Presented at the III Reunión de la Sociedad Latinoamericana de Nutrición y Seminario sobre Ambiente Biológico y Nutrición. Resúmenes de trabajos. Institute of Nutrition of Central America and Panama (INCAP), Guatemala 11-14 September 1972, p. 63.
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11. ----- and E. Cifuentes. Physical fitness in anemic Guatemalan agricultural laborers before and after iron therapy. Submitted for publication.

OTHER DATA

Grantee: Dr. Fernando E. Viteri, Biomedical Division, Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala.

Funded by: World Health Organization and other sources.

Timetable: 1968-1975.

INTERACTION BETWEEN NUTRITION AND PRODUCTIVITY OF AGRICULTURAL LABORERS. EFFECT OF CALORIE AND IRON SUPPLEMENTATION

PROBLEM

Agricultural laborers' physical working capacity is limited by the availability of calories for activity derived from their total caloric intake, minus basal energy expenditure. We have previously shown that an increase in daily calorie intake from 2,700 to 3,500/cal produces a substantial increase in daily energy output. We have also shown that even mild degrees of iron deficiency anemia decreases the capacity of agricultural laborers to perform near maximal exercise.

We propose to test the hypothesis that calorie and iron supplementation in the diet of agricultural laborers will result in increased working capacity and actual productivity. Furthermore, we propose that better earnings and more energy off-the-job will result in an improvement in the nutrition and the general quality of life of the family of such laborers.

METHOD

We are studying two communities in the lowland areas of Guatemala through anthropometric and hematologic measurements, physiologic performance in standardized tests, energy expenditure in actual field situations, and socioeconomic, morbidity, and dietary surveys of the family. Work output, income, and expenditures are constantly evaluated throughout one agricultural cycle. Both communities will receive vitamin supplementation but in one community, workers will have in their diet supplement 800 extra calories per day. The measurements outlined before will be continued for at least one full agricultural cycle.

RESULTS TO DATE

All basal data were collected during 1973 and supplementation will start in the middle of 1974. Findings will be published at a later date.

SIGNIFICANCE

This research is fundamental in guiding the distribution of scarce resources in nutrition programs. Improving the work output of laborers and the quality of life of their families through supplementation of the main provider is a new and, most probably, very effective nutrition policy.

PUBLICATIONS

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10. ----- and B. Torún. Anemia and physical work capacity. In: Anaemia and Hypoxia, vol. 3, No. 3, Clinics in Haematology. In press.

OTHER DATA

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

Funded by: Bank of Guatemala, National Science Foundation, INCAP, and private donations.

Timetable: 1973-1975.

ASSOCIATION BETWEEN NUTRITION AND FERTILITY VARIABLES

PROBLEM

In most developing countries where poor nutrition exists, rapid population growth is also seen. The two problems are multifactorial, interactive, and affect similar groups. Much attention has been focused on the effects of nutrition on the likelihood of conception, cause of pregnancy, and outcome of pregnancy as to infant morbidity, mortality, and child growth and development. Yet little has been done to show the converse relationship--the association between fertility and nutritional status. This study will help show the effects of fertility on family health as manifested by nutritional status.

METHOD

Information derived from the extensive and comprehensive nutrition surveys carried out by the Institute of Nutrition of Central America and Panama (INCAP) during 1965-1967 will be used to:

1. Determine what are the correlations, if any, between fertility and nutrition variables.
2. Select indices for the measurement and assessment of these relationships.
3. Suggest the basis for the development of large-scale experimental intervention programs, considering both fertility and nutrition variables.

RESULTS TO DATE

In accordance with the terms of the agreement signed by the collaborating groups, INCAP has made available to Columbia University the basic information required for the analyses. These data have been completely edited and compiled into a data system for one country, El Salvador. Analyses of this information have been completed and a detailed report is currently in preparation. Briefly, the results are as follows:

1. Age-specific parity is a correlate of preschoolchild survival.
2. An important correlate of the nutritional status of surviving children is the mother's previous parity history.
3. Within family groups, there is a correlation between child mortality patterns and the nutritional status of surviving children.
4. For the Salvadoran mothers studied, if the first pregnancy occurred before 18 years of age, the youngest child at the time of the nutrition survey was likely to be in poor nutritional status.

In a second stage, this information was used to examine the channels of action and relative weights of maternal variables using probabilistic analysis.

SIGNIFICANCE

The implications of these findings for intervention programs are of particular importance. To say that women's age-specific parity throughout child-bearing years is correlated with the health and survival of their children is to emphasize the importance of short interval between births. High age-specific parity contrasts with merely high parity, regardless of age, primarily because mothers have had more births within a given time span. Our study gives a lesser, though significant degree of importance to the age of the mother at first pregnancy.

High mortality among young children means shorter lactation span, earlier first postpartum ovulation and menstruation, and shorter inter-birth interval. Therefore, child loss does lead to a replacement effect, whether for social, psychologic, or physiologic reasons or combinations thereof. To say then, that a mother's malnutrition adversely affects her children's health and survival, points to the same shortening of inter-birth interval.

The need for a dual approach to achieve the common objectives of improved nutrition and modified fertility emerges clearly when these findings and trends are simulated in different hypothetical communities.

PUBLICATIONS

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2. Wishik, S. M. and M. A. Guzmán. Association between nutrition and fertility variables. PAHO/INCAP Document, in preparation.

OTHER DATA

Grantee: Dr. Samuel M. Wishik, Division for Program Development and Evaluation, International Institute for the Study of Human Reproduction, Columbia University, New York, New York, and Dr. Miguel A. Guzmán, Division of Statistics, Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala.

Funded by: Pan American Health Organization and Agency for International Development.

Timetable: 1972-1974.

STANDARDIZING METHODOLOGY AND COMPUTER ANALYSIS FOR ASSESSING NUTRITIONAL STATUS

PROBLEM

Nutritional deficiency diseases are still considered a major public health problem. They are responsible for the high infant-mortality and the high morbidity in preschool children. Knowledge of the nature, magnitude, cause, and potential remedial and preventive measures is still fragmentary.

The purpose of this study is to define and provide a standardized system for conducting nutrition surveys; for recording and analyzing the data in a uniform manner; providing a mechanism for a continuing data bank and retrieval system; reducing and eliminating duplication of computer survey data formats, codes, edits, and programs for analysis. To identify and provide a mechanism to correlate nutritional health data with other sociologic, economic, environmental, and disease factors indicative of high-risk groups.

METHOD

Consultation was provided in designing nutrition surveys for Argentina, Chile, Peru, Northeastern Brazil, and São Paulo, Brazil.

Consultation and training of local personnel were provided for sample definition and selection, data-recording forms, guidelines for interpretation of the data, and formats for coding, verification, tabulation, and analysis of the data.

RESULTS TO DATE

A manual, General Guidelines for Establishment of a Data System for the Assessment of Nutrition and Health Status was published by PAHO and circulated in 1973. Field studies are being conducted in Northeastern Brazil by the Institute of Nutrition of Pernambuco and by the Escola Paulista de Medicina's Department of Preventive Medicine in São Paulo.

Likewise, the survey samples have been identified and personnel standardized and trained to conduct nutrition surveys in the Northern Region, Salta, and Northeastern Region, Corrientes, Argentina, and national surveys of Chile and Peru. These will be conducted in 1974-1975.

SIGNIFICANCE

The project will assist in developing a data retrieval system for surveillance and evaluation of nutritional status throughout the Region, for use within countries, and for comparison of problems and their solutions in the various countries. It will provide a continuous updated source of information on nutrition and health status.

PUBLICATIONS

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OTHER DATA

Grantee: Dr. A. E. Schaefer, Nutrition Consultant to the Pan American Health Organization, Nutrition Program, Health Services Department, Pan American Health Organization, Washington, D.C.

Funded by: Pan American Health Organization and Ford Foundation.

Timetable: 1971-1975.

SYSTEMS ANALYSIS IN NUTRITION AND HEALTH PLANNING

PROBLEM

A principal responsibility of INCAP is to assist the governments of Central America and Panama in planning intervention programs that will meet specific nutrition and health goals. At present, these planning efforts are severely limited by the lack of a suitable methodology that starts with a systematic framework and extends to the semiquantitative evaluation of various potential programs. The main purpose of this project is to develop a simplified method that government-planning groups could conveniently apply to the evaluation of various potential programs for the achievement of specific nutrition and health objectives, while satisfying certain constraints (e.g., budget, personnel, facilities).

METHOD

The general approach is to utilize the methods of systems analysis (including operations research, decision analysis, econometrics, and mathematical optimization techniques), when appropriate. The emphasis is on evolving an analysis that is sufficiently simple to be accepted by planning groups.

RESULTS TO DATE

An essential element of the model is an approximate description of malnutrition and mortality in terms of those variables that may be affected by intervention programs. We have initially concentrated on using the results of two INCAP longitudinal studies to develop preliminary models relating the incidence of malnutrition to birth weight and age. The two studies are for rural Guatemalan communities with considerable variability in ethnic, socioeconomic, and ecologic conditions.

The resulting models may serve as baselines to estimate the consequences of potential programs aimed at different target groups, such as pregnant and/or lactating women, and children whose weights at birth or thereafter fall below prescribed levels. We have prepared several illustrations of the possible benefits of using models of this nature in nutrition and health planning.

SIGNIFICANCE

In the initial phase of this project, we have: (1) described the role of systems analysis in a methodology for nutrition and health planning; (2) proposed a method for designing the models of malnutrition needed in the analyses; (3) designed models based on two INCAP longitudinal studies; (4) illustrated, in a very preliminary matter, how these models may be used to evaluate the relative benefits of potential intervention programs.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. R. E. Stickney, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA, and Dr. I. D. Beghin, Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala.

Funded by: The U.S. National Science Foundation, and the W. K. Kellogg Foundation.

Timetable: 1973-1976.

EPIDEMIOLOGIC AND METABOLIC STUDIES OF CHOLELITHIASIS IN CHILE

PROBLEM

Cholesterol gallstones are unusually frequent in the Chilean population, and the complications of cholelithiasis constitute a major cause of morbidity and mortality in this country.

The purposes of this investigation are (1) to study the incidence and natural history of the disease, and the dietary habits of patients with cholelithiasis, and (2) to investigate in humans and experimental animals some of the metabolic disturbances that could play a role in cholesterol gallstone formation.

METHOD

1) Epidemiologic studies are being performed in a sample of urban population. Subjects are submitted to clinical examination, cholecystography, and study of their dietary habits.

2) Daily excretion of biliary lipids and bile acid pool size will be measured in selected patients. A plan to obtain these parameters from young women with gallstones and from old women with normal gallbladders has been designed.

3) The biliary excretion of cholesterol and the dynamics of the enterohepatic circulation of bile salts are being studied in rats under different experimental conditions.

RESULTS TO DATE

The project was approved in November 1973. Epidemiologic studies and investigations in experimental animals are in process. A plan for the metabolic studies in patients has been prepared.

SIGNIFICANCE

Cholesterol gallstones constitute a serious health problem in Chile. A better understanding of the epidemiology of cholelithiasis and of the metabolic disturbances that explain the precipitation of biliary cholesterol, is clearly needed. The search and application of rational methods for the medical dissolution of gallstones and for the prevention of the disease must be founded on this basic information.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Vicente Valdivieso, Unidad de Gastroenterología, Escuela de Medicina, Universidad Católica de Chile, Santiago, Chile.

Funded by: Pan American Health Organization.

Timetable: 1974.

CORRELATIVE MORPHOLOGIC AND BIOCHEMICAL STUDIES ON EXPERIMENTAL EPILEPSY

PROBLEM

The aim of the present project is to systematically study the adjacent and contralateral motor cerebral cortex of cats implanted unilaterally with alumina cream to produce focal motor seizures at different time intervals by using light and electron microscopy and biochemical techniques. Since accumulating data in the literature have indicated that biochemical reactions involved in γ -aminobutyric acid (GABA) synthesis at nerve endings are one of the mechanisms controlling excitability in the central nervous system, and since GABA has been postulated to be an inhibitory transmitter at central synapses, the methods in the present project will be particularly oriented to study the role of inhibitory mechanisms in the regulation of cerebral cortex excitability in relation to convulsions, by using various biochemical techniques.

METHOD

Adult cats (3 kg) will receive intracerebral injections of alumina cream (0.02-0.04 ml), stereotaxically oriented according to a previously established model (Velasco et al. Epilepsia [Amsterdam] 14:3-14, 1973).

The various morphologic and biochemical studies will be carried out in representative areas of the perilesional and contralateral motor cortex at the three stages of the experimental model (latency, convulsions, and remission) using the electroencephalographic recordings as a particular reference.

All experiments will also be performed in control animals not receiving injections and in animals receiving silicon injections, according to previous work (Velasco et al. Epilepsia [Amsterdam] 14:15-27, 1973). The project will be divided into four stages: Stage I. Systematic light microscopic study for neuronal population in the various cortical layers, in formalin-fixed material by perfusion. Stage II. Correlative ultrastructural and biochemical study in glutaraldehyde-fixed material by immersion. Representative fragments will be employed to investigate glutamic decarboxylase, acetylcholinesterase, choline-acetyl transferase, and lactic and succinic dehydrogenase activities, total proteins,

and DNA. Stage III. Serial laminar study of the motor cortex for electron microscopy and biochemical determinations. Stage IV. Investigation of the blood-brain-barrier phenomenon in this model using some enzymatic tracers.

RESULTS TO DATE

None thus far.

SIGNIFICANCE

Although precise epidemiologic studies on epilepsy are difficult to carry out, various works indicate an overall incidence of 0.5 percent in the general population involving all age groups. Almost 75 percent of epileptic patients have the so-called idiopathic type of the disease. By knowing more about the mechanisms involved in the production of convulsions in some experimental conditions using laboratory animals, we will have more data which, put together, will permit us to have a better understanding of the disease. The present project is relevant as it is expected to evaluate the role of the inhibitory system of the cerebral cortex in the regulation of brain excitability. It is also important because if findings are conclusive in regard to convulsion-producing mechanisms, a great field of research on the various substances related to some neurotransmitters in the control of different types of idiopathic and acquired epilepsy would be open.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Alfredo Feria-Velasco, Section of Neurobiology and Neuropathology, Scientific Research Department, Instituto Mexicano del Seguro Social, México, Mexico.

Funded by: Pan American Health Organization.

Timetable: 1974-1976.

DENTAL CARIES IN COLOMBIAN COMMUNITIES

PROBLEM

The prevalence of dental caries is universal and has reached such proportions that in some areas of Latin America up to 99 percent of schoolchildren suffer from this disease. In recent years convincing proof has been adduced to show that caries is an infectious disease and that certain strains of streptococcus have a causative role. In this context the Department of Dental Science of the Royal College of Surgeons of England has conducted investigations into the components of the oral flora associated with active caries and with the absence of caries. This work has been carried out on nonhuman primates in which caries, indistinguishable from that seen in human children, has been produced by infecting the animals with cariogenic streptococci and maintaining them on a high sugar diet.

In Colombia a semiisolated community has been identified by scientists from the University of Antioquia in which the degree of caries resistance is totally at variance with what would be expected by those who regard sugar consumption as the primary cause of caries. In this community sugar (in the form of panela) is a staple part of the diet. The hypothesis to be tested is that the resistance to caries in this community is attributable to the absence of an infective agent capable of fermenting sugar to form acid which initiates tooth decay; or, alternatively, that this relative immunity may result from the presence of a factor which neutralizes the caries-promoting potential of streptococci.

METHOD

Two similar communities in Colombia will be studied. In Heliconia there is a low caries prevalence and in Don Matias, which is otherwise comparable, the caries prevalence is high. Two hundred children in each community were examined dentally and divided into 7 to 10- and 10 to 14-year-old age groups. Samples of dental plaque were obtained from each child and subjected to bacteriologic, chemical, and electron-histochemical analysis. Follow-up samples have also been taken for comparison with initial findings. Analyses of water from the low caries community are being undertaken.

RESULTS TO DATE

In the community with low caries, dental plaque is present in relatively small amounts and, in some instances, it is difficult to gather sufficient quantity for analysis. By contrast, plaque from the community with high caries is abundant and more cohesive; it is also more difficult to suspend in bacteriologic media. The capacity of plaque to form acid was measured and the pH value reached after 5 minutes' exposure to a sucrose solution was lower in samples from the community exhibiting higher prevalence of caries. Early studies of Gram-stained smears of plaque from the lower caries town showed the presence of ameba-like bodies, but this finding has not been repeated. An unusual difference has been observed in the physical nature of polysaccharide formed by a standard strain of Streptococcus mutans when grown on sucrose-containing media made up with water from Heliconia, Don Matias, and London. When the culture medium is made up with Heliconia water the degree of adherence of the polysaccharide appears to be substantially less than is the case when the media are prepared with water from the other two sources. This suggests that elements are present in Heliconia water that influence the formation of polysaccharide from sucrose by Streptococcus mutans. Analyses of water are as yet incomplete. Thus far it has been ascertained that Heliconia water is unusually hard and possesses an unusually high buffering capacity. It does not seem likely that these features alone could be responsible for the phenomenon of increased resistance to dental caries. More information is required and is in the process of being sought.

SIGNIFICANCE

The existence of a community enjoying relative freedom from dental caries is unremarkable, even though the prevalence of this disease in most urban populations has approached saturation. What is extremely unusual in the situation under investigation, is that the degree of freedom from caries occurs in a population whose diet is rich in readily fermentable carbohydrate. Whatever factor is operating to reduce the caries incidence in Heliconia is able to do so despite a dietary regimen that would normally be classified as highly cariogenic.

PUBLICATIONS

None thus far.

OTHER DATE

Grantee: Dr. Bertram Cohen, Royal College of Surgeons of England, London, England, and Dr. William H. Bowen, National Caries Program, National Institute of Dental Research, Bethesda, Maryland.

Funded by: International Sugar Research Foundation, Bethesda, Maryland, U.S.A. (in part).

Timetable: 1972-1974.

MECHANISM OF THE DIABETOGENIC ACTION OF PITUITARY GROWTH HORMONE

PROBLEM

The diabetogenic action of pituitary growth hormone (GH) has been the subject of an extensive research work. This action has implications for the peripheral insulin resistance, diabetic angio-pathy, and renal damage. The GH's mechanism of action in these different pathologic conditions has not, however, been elucidated. Our research in this field led us to the isolation of GH-dependent insulin antagonist, localized in some α_2 -glycoproteins from human fasting normal plasma called α_2 -inhibitor. Under the present project, we propose to purify the α_2 -inhibitor and to study its diabetogenic activity in relation to GH increases. The specific aim is to produce transient diabetes mellitus by administration of pure α_2 -inhibitor. The word transient is used because diabetes would be the consequence of diminished peripheral consumption and not of the lesion of the beta cells of Langerhans' islands.

METHOD

a) Purification. All the classic procedures to isolate glycoproteins have been used. In every instance the α_2 -inhibitor has been demonstrated by the hemidiaphragm bioassay (diminution of labeled glucose uptake). However, plasma-protein fractionation by trichloroacetic acid, 0.15 M at 4°C, added through dialysis, gave the highest inhibitory specific activity in the supernatant. We propose to pass this supernatant, which contains five α_2 -glycoproteins components, through chromatographic CM-cellulose column.

b) Functional characterization. Local and systemic stress in the rat in which hypersecretion of GH will be induced will be used; local, by subcutaneous turpentine abscess and systemic, by administration of cold plus restraint in normal and partially pan-creatctomized rats (P-rat), nondiabetic but with insulin deficiency under emergency stress conditions. The P-rat will be a more sensitive biologic model for the diabetogenic potentiality of α_2 -inhibitor.

RESULTS TO DATE

The purification of α_2 -inhibitor was postponed to a later date.

Results on functional characterization will be published in Horm Metab Res.^{3/} Administration of rat-GH or systemic stress (endogenous GH secretion), caused the appearance of the α_2 -inhibitor (normally absent in the rat). A time-stress-response curve showed a peak appearance 3 hours post-stress, with disappearance at 8 hours. These results suggested an insulin hypersecretion capable of neutralizing the α_2 -inhibitor and its potential diabetogenic power. Reduction of the endocrine pancreas put into evidence a transient diabetic poststress response (4-6 hours of glycosuria). Local stress with turpentine or cotton inflammation did not induce the α_2 -inhibitor in intact-normal rat, but 3 hours after inflammation it was near $P < 0.5$. In systemic stress produced by restraint plus cold (-17°C), cold could be omitted. This opened the study of the role of immobilization or confinement as a stressor agent.

Antistress experiments with drugs that could block the brain adrenergic or 5-hydroxytryptaminergic system have been started. In some instances, the poststress diabetic response has been prevented with chlorpromazine and oxyperline.

SIGNIFICANCE

1. Conceptual incorporation of growth hormone as a neuro-endocrine stress component with diabetogenic activity through the endogenous production of an insulin-antagonist (α_2 -inhibitor).
2. New explanation for the appearance of diabetic response when prediabetic patients have inflammatory processes.
3. Better understanding of the unbalance of diabetic patients submitted to surgical stress.
4. New hypothesis for the peripheral insulin resistance observed in burned or traumatized patients.
5. Opening of a preventive antistress drug treatment to inhibit the neuroendocrine response at the hypothalamic level, blocking the GH discharge and the hyperproduction of α_2 -inhibitor.

PUBLICATIONS

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OTHER DATA

Grantee: Dr. Luis Vargas, Department of Cell Biology,
Institute of Biological Sciences, Catholic
University of Chile, Santiago, Chile.

Funded by: Pan American Health Organization.

Timetable: 1973-1974.

ENDEMIC GOITER AND CRETINISM IN BOLIVIA

PROBLEM

Endemic goiter is a major public health problem in Bolivia. The entire country is iodine-deficient and the goiter incidence has been estimated at 40 to 50 percent among the general population. In many isolated communities virtually everyone has a goiter. There is considerable variation in the incidence of goiter and cretinism among different ethnic groups.

This investigation has the following objectives: (1) to ascertain the true incidence of goiter in various parts of Bolivia; (2) to elucidate the causes of endemic goiter and cretinism; (3) to clarify the role of genetic and ethnic factors.

METHOD

Surveys have been conducted among 10,420 adults and children in the Department of Chuquisaca. The incidence of goiter and cretinism, and urinary iodine levels are being determined. We are carrying out special studies of thyroid function, including iodine uptake and clearance, perchlorate discharge, plasma inorganic iodine, and absolute iodine uptake.

RESULTS TO DATE

Radioiodine thyroid uptake increases with the severity of the goiter and is also higher in nodular than in diffused goiters.

The same kind of relation was found for PBI 131.

Thyroid clearance for radioiodine increases with the severity of the goiter and is a little higher for men than for women.

SIGNIFICANCE

This study will give detailed information on the prevalence and severity of goiter and cretinism in a large area of Bolivia. Factors that may influence the development of goiter and cretinism will be assessed.

PUBLICATIONS

1. Pardo, A. et al. Estudio tiroideo con radioiodo en zona de bocio endémico (Sucre). I Cong Nat Med Int, April 1973.
2. ----- et al. Clearance tiroideo para iodo en zona de bocio endémico (Sucre). I Cong Nat Med Int, April 1973.

OTHER DATA

Grantee: Dr. Antonio R. Pardo Subieta, University of San Francisco Xavier and Santa Bárbara University Hospital, Sucre, Bolivia.

Funded by: Universidad de San Francisco Xavier.

Timetable: 1972 - continuing.

ENDEMIC GOITER AND THE PATHOPHYSIOLOGY OF THYROID DISEASE IN CHILE

PROBLEM

We have shown that iodine-deficient subjects have a diminished T₄ and an increased T₃ secretion of their thyroid glands. KI administration normalizes the thyroid secretion of both hormones.

We are also studying: (1) The factors that influence the development of goiter as a consequence of the iodine deficiency; (2) the hypothalamic-pituitary unit role in the iodine-deficient goiters' genesis; and (3) the liver's role in the thyroid hormone metabolism in dietary-iodine deficiency.

METHOD

The studies involve: (a) evaluation of the thyroid clearance changes in relation to iodide plasma concentrations and (b) induction in normal subjects of acute iodine deficiency with manitol and furosemide to determine thyroid clearance changes and correlate them with the pituitary response (TSH response to TRH administration) and with sex.

1. Chronic iodine-deficient subjects with and without goiter. The TSH basal concentration and the TSH response to TRH administration are measured and the procedure repeated after KI administration.

2. Normal subjects. The thyroxine disappearance rate is assessed in relation to the different plasma concentrations obtained by administering different amounts of thyroxine.

3. Iodine deficient subject. The relationship between the thyroxine plasma concentration and its fecal excretion is studied.

4. Iodine deficient rats. The hepatic and fecal clearance of thyroxine is determined to define the relationship between plasma concentration and fecal excretion.

RESULTS TO DATE

(A) Relationship between thyroid clearance, plasma iodide concentration (PII) and goiter: there is a negative linear correlation between log iodide thyroid clearance and log plasma iodide concentration, which is independent of the goiter type ($r:-0.67$, $p=0.001$).

The iodide clearance is proportional to the size of the goiter ($r: +0.56$, $p=0.001$). The absolute iodine uptake is significantly higher in nodular goiters than in diffuse ones (2.30 ± 0.28 (SE) versus 1.63 ± 0.20 , $p=0.05$).

The increased absolute iodine uptake of nodular goiters is caused by the greater iodine thyroid clearance. Data indicate that nodular goiters secrete a nonhormonal kind of iodine, the hormonal iodine secretion being the same in both kinds of goiters. The presence of a goiter could be caused by an intrinsic thyroid capacity to respond to iodine deficiency and to the hormonal synthesis efficiency, increasing the iodine thyroid clearance, per gram of gland.

(B) We have not seen changes either in the thyroxine fecal clearance with acute loads of thyroxine or in other excretion routes.

(C) In chronically iodine-deficient rats the thyroxine concentration and consumption diminished to half its control value (from 1.45 to 0.76 $\mu\text{g/day} \times 200\text{g bodyweight}$).

In the same rats the fecal excretion diminished 4 times (from 0.83 $\mu\text{g/day} \times 200\text{g bodyweight}$ to 0.19 $\mu\text{g/day} \times 200\text{g bodyweight}$).

These facts suggest that thyroxine fecal clearance diminishes when its plasma concentration also does.

SIGNIFICANCE

We are also studying various adaptation mechanisms to iodine deficiency, other than those already known, i.e. the increase of triiodothyronine which we previously described. In this category we are particularly interested in studies of thyroxine enterohepatic circulation.

We think that this research project will contribute to the knowledge of why some people living in dietary iodine-deficient conditions do not develop goiter and why women do develop it with much higher frequency than men. From this kind of information we believe we can increase our knowledge about endemic goiter genesis.

PUBLICATIONS

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OTHER DATA

Grantee: Dr. Carlos Stevenson, Facultad de Medicina Oriente, Universidad de Chile, Hospital Salvador, Santiago, Chile.

Funded by: Partially supported by the Chilean National Council for Scientific and Technological Research.

Timetable: 1974-1976.

PATHOPHYSIOLOGY OF ENDEMIC GOITER AND CRETINISM IN ECUADOR AND THEIR PREVENTION WITH IODINATED OIL

PROBLEM

Well documented studies on endemic cretinism have demonstrated that this disorder is found in geographic association with endemic goiter. Traditionally, "endemic cretinism" refers to patients with obvious mental retardation and defects in hearing, speech, and walking. Besides, as has been demonstrated in the Ecuadorean Andes, the incidence of endemic cretinism is more related to the socioeconomic situation of a community than to the magnitude of iodine deficiency. Questions remain to be answered as to: (1) the extent of intellectual deficiency in areas where endemic cretinism is highly prevalent; (2) whether iodine deficiency alone, which may lead to cretinism, can also cause less obvious intellectual defects; (3) the time factors of the effects of iodine deficiency on intellectual development.

This part of the general program aimed at evaluating the role of iodine in intellectual development in endemic goiter areas and specifically the role of iodine in endemic mental retardation, including obvious and lesser degrees of mental retardation.

METHOD

The study was conducted in Tocachi and La Esperanza, two isolated, entirely comparable neighboring rural Andean communities. Severe chronic iodine deficiency, protein-caloric malnutrition, and high incidence and prevalence of goiter and cretinism, are the most important characteristics shared by these Spanish-speaking villages.

1. Two groups of patients were studied. One of them, the "defective group", comprised 77 persons ranging from 9 to 60 years of age. Almost everyone in this group had at least one of the following abnormalities in walking, speaking, hearing, and mental capacity, or a combination of them. The other group, the "normal group", included 50 adults chosen at random and by purpose: local leaders, farmers, etc. In these two groups, intelligence was scored by using adaptations of the Gesell, Leiter, and Binet-Simon tests.

2. In 1966, iodized oil was injected to every person in Tocachi. In 1968, all women in the reproductive years were reinoculated, as were all children born after 1966. La Esperanza remained as the control village.

The children born in Tocachi, during the 7-year study period, were divided in two groups: TG1, comprising children in whom iodine deficiency correction, directly by intramuscular administration, occurred from the 4th to the 7th month of fetal life or during nursing; and TG2, comprising children in whom correction occurred before conception or during nursing, as a result of intramuscular administration of iodized oil to the mother. The children born in La Esperanza during the same period were matched for chronologic age and divided into EG1 and EG2 groups. All children were tested with an adaptation of the Stanford-Binet intelligence Scale. These adaptations included picture recognition and vocabulary which have local characteristics. A total of 216 children were tested: 103 (60 males and 43 females) in Tocachi, the treated village, and 113 (57 males and 56 females) in the control village of La Esperanza.

RESULTS TO DATE

1. According to the Stanford-Binet classification, the "defective group" scored in the idiocy, imbecility, mental weakness, and borderline defective levels. The "normal group" scored in the mental weakness, borderline defective, low average, normal, and high average levels. Thus overlapping was found between these two groups, when they were compared for mental age and IQ's. These results also indicated that in the studied communities a significant percentage of the population considered normal belonged in fact to the mentally weak and borderline defective group.

2. The mean IQ in the TG1 group was 71, as compared to 69 in the EG1 group. In the TG2 group, the mean IQ was 83, as opposed to 72 in the EG2 group. From a statistical analysis, the difference between the IQ's of the TG2 and EG2 was highly significant ($p < 0.002$). The IQ difference between TG1 and EG1 lacks significance. Since children whose IQ scores are below 70 are considered to be mentally retarded, there was in the Tocachi and La Esperanza groups a significant prevalence of mentally defective children. In TG2, however, only 9.5 percent of the children scored in the mentally defective range. Furthermore, there was no case in that group of a child scoring below 50, the range that corresponds to idiocy-imbecility.

The physical development of the children from Tocachi and La Esperanza was retarded in relation to that of well-nourished children from developed countries. This was illustrated by their height, weight, and annual increment of height curves, obtained from the longitudinal study conducted on them from birth.

SIGNIFICANCE

In the endemic goiter area studied the extent of intellectual deficiency went from the most severe cases (idiocy-imbecility, obvious mental deficiency, and IQ below 50) to normality. If, for purpose of definition and tradition, we consider endemic cretins those subjects whose mental capacity corresponds to idiocy-imbecility, we may state that the correction of chronic iodine deficiency from conception prevents endemic cretinism. On the other hand, correction of iodine deficiency after the third month of intrauterine life appears to have no effect on the future intellectual abilities of the newborn. Consequently, we may speculate that the future intellectual capacity of a child is related to the maternal thyroid function during early embryogenesis, or perhaps, as an hypothesis to the extrathyroid action of iodine on nervous system development during early embryogenesis.

Since endemic cretinism has not been reported in places where, despite severe chronic iodine deficiency the diet is adequate, the results obtained in this study indicate that iodine deficiency is a necessary condition for the appearance of endemic cretinism in a community. Whether or not iodine deficiency is a sufficient condition could be answered only in the future.

Since in the Tocachi Group 2, a significant number of children presented less obvious mental retardation, it seems apparent that there are other concomitant factors playing a role on the intellectual development of the studied children. Less obvious intellectual deficits in endemic goiter areas should be referred to other causes, i.e. protein-caloric malnutrition.

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OTHER DATA

Grantee: Dr. Rodrigo Fierro-Benítez, National Polytechnic School, Department of Radioisotopes, Quito, Ecuador.

Funded by: Pan American Health Organization.

Timetable: 1969-1974.

MANGANESE POISONING: A METABOLIC DISORDER

PROBLEM

Parallel studies of manganese poisoning and Parkinson's disease have shown clinical and metabolic similarities and dissimilarities. We have proved that treatment with levodopa, the precursor of the neurotransmitter dopamine, was successful in both diseases. We expect that the information generated will provide insight into the nature of extrapyramidal disorders induced by manganese. Therefore, in the last 2 years our efforts have been concentrated in the following areas:

- 1) Analysis of the influence of protein intake on the off-on phenomenon observed in parkinsonian and manganic patients.
- 2) Correlation of electroencephalographic patterns during sleep in parkinsonian and manganic patients. Correlation of these results with growth hormone output during sleep.
- 3) Long-term evaluation of levodopa treatment in patients with manganese poisoning.
- 4) Effects of manganese administration to pregnant rats on the concentration of norepinephrine and dopamine in the brains of newborns and their mothers.

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 were sent from Chile to the Brookhaven National Laboratories in the United States.

Levodopa was administered orally to manganic patients for periods up to 4 years. Healthy miners have received levodopa for periods of 30 days. Several biochemical maneuvers have yielded insight as to the mode of action of levodopa; dopa decarboxylation inhibitors, nicotinamide, and fusaric acid have been tested. Furthermore in-vivo decarboxylation rates of amino acids have been measured, and finally evaluation of blood brain barrier to manganese in relation to the susceptibility to manganese poisoning is subject to experimental work.

RESULTS TO DATE

1) Protein intake and intermittent refractoriness to levodopa. A metabolic difference between parkinsonian and manganic patients has emerged. High-protein intake enhances the intermittent refractoriness to levodopa (off-on phenomenon) of unstable Parkinson's disease patients but not of patients with manganese poisoning. At the Brookhaven National Laboratories, the morning motor function scores of eight patients with Parkinson's disease were impaired from 26 ± 2 to 40 ± 3 (normalcy: 0; maximal impairment: 100); $p < 0.001$. On a high-protein diet (2 g/kg), these same patients' morning motor function scores on the same amount of levodopa, were impaired from 26 ± 3 to 54 ± 3 , $p < 0.001$. A low-protein diet of 10 g of protein per day, however, achieved a stability of motor function; morning scores were 25 ± 3 and scores at 3 pm were 24 ± 4 , $p < 0.9$. Protein-intake effects were blocked when a peripheral decarboxylase inhibitor, MK-486, was used. In five manganic patients, similar dietary maneuvers failed to produce morning or afternoon changes in the response to levodopa.

Furthermore, measurements of total body decarboxylation of levodopa ^{14}C , showed no significant differences between parkinsonian and manganic patients, with and without the peripheral decarboxylase inhibitor, MK-486.

2) Electroencephalographic changes in manganese poisoning. Continuous electroencephalographic and myographic records of seven normal controls and five manganic patients were gathered during the hours of sleep for two consecutive nights. Manganic patients were studied while off levodopa at basal clinical level, and while on levodopa at optimal control. Significant changes in the distribution of REM sleep (rapid eye movements period) were observed. About 13.9 percent ± 1.7 of the controls' sleep represented REM periods, as compared to only 5.5 percent ± 0.6 , $p < 0.001$ for manganic patients not taking levodopa. On optimal doses of levodopa, the same manganic patients increased their

REM periods to $8.9 \text{ percent} \pm 0.7$; $p < 0.001$, vs patients without treatment, and $p < 0.02$ vs controls. The growth hormone concentrations of these patients were $4.95 \pm 1.4 \text{ ng/ml}$ while off levodopa and $1.64 \pm .5$ while on levodopa, $p < 0.05$.

3) Chronic levodopa treatment of manganic patients. Treatment schedules of 5-6 years were completed. Nine patients, seven of them predominantly hypokinetic and two dystonics, received optimal amounts of levodopa in alternation with a placebo, and six patients were kept off levodopa for extended periods. Their basal levels were evaluated; no progression has been observed. During these years, no episodes of involuntary movements or mental changes have been evident.

4) Catecholamine concentrations in the brain and manganese intake in pregnant rats and newborns. Pregnant rats were placed on a Hurley (exclusively manganese-deficient) diet, to which different concentrations of manganese during the entire period of pregnancy were added. Following delivery, a significant diminution of dopamine was observed in the brains of mothers kept on a manganese-deficient diet, $528 \pm 69 \text{ ng/brain}$ vs $848 \pm 87 \text{ ng/brain}$ in rats with a normal Mn intake, $p < 0.05$; Noradrenaline concentrations showed a similar decrease of $198 \pm 31 \text{ ng/brain}$ in mothers on a manganese-deficient diet as opposed to $448 \pm 42 \text{ ng/brain}$ in rats on a control diet, $p < 0.001$. Offsprings of rats on a manganese-deficient diet had brain dopamine concentrations of 38.9 ± 2 ; as opposed to 51 ± 6 for offsprings of rats with a normal manganese intake. Norepinephrine concentrations were also diminished in manganese-deficient animals.

SIGNIFICANCE

Dissimilarities between parkinsonism and manganese poisoning lie in the absence of levodopa-induced dyskinesia and mental aberrations in manganic patients. Furthermore, high-protein intake does not impair levodopa effects in manganic patients, as it does markedly in parkinsonian patients. New similarities between both extrapyramidal disorders consist of a marked reduction of REM sleep. Experimental information shows the significant influence of manganese intake on brain catecholamine concentrations, both in pregnant rats and in their offsprings.

In addition, if manganese can drastically influence brain concentrations of the neurotransmitters dopamine and noradrenaline in newborns, it follows that this population could be potentially susceptible to environmental changes of manganese. Increases of environmental manganese can be envisioned if manganese is to replace lead in gasoline. Therefore, the problem of susceptibility to manganese poisoning should be a matter of further study.

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OTHER DATA

Grantee: Dr. Humberto Torloni (Project Coordinator), Department of Research Development and Coordination, Pan American Health Organization, Washington, D.C.; Drs. George Cotzias and Ismael Mena (Principal Investigators), Catholic University, Santiago, Chile; University of California, Los Angeles Harbor General Hospital. Brookhaven National Laboratories, New York.

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

Timetable: 1963-1973.

SUSCEPTIBILITY TO MANGANESE POISONING

PROBLEM

Toxicity of environmental lead from gasoline precludes its further use as an antiknock agent. Manganese is being considered as an alternative, but toxicity of environmental manganese has not been fully assessed. Manganese is a potent neurotoxic metal, which produces psychosis and symptoms similar to those of parkinsonism. At present, its toxicity is confined to industrial exposure. In a study of manganese miners and foundry workers, a marked susceptibility factor was detected. We propose, therefore, to investigate the effects of manganese as an atmospheric pollutant and a new industrial hazard.

METHOD

We have linked manganese poisoning susceptibility to intestinal absorption of manganese and intend to:

- 1) Study intestinal manganese absorption in newborns and infants.
- 2) Measure ^{55}Mn concentrations in the brains of fetuses, newborns, and infants and correlate these values with norepinephrine and dopamine concentrations.
- 3) Study the fate of inhaled manganese and manganese oxide and levodopa decarboxylation in healthy manganese miners.
- 4) Study the effects of manganese and manganese oxide intakes on norepinephrine and dopamine concentrations in the brain of rats and their survival to cold.

The evaluation of intestinal absorption of natural ^{55}Mn in newborns and infants will be performed as a balance study of nonradioactive manganese, measuring the daily intake of manganese in milk and its excretion in urine and feces. Manganese will be measured at the University of California at Los Angeles by neutron activation analysis.

RESULTS TO DATE

None thus far.

SIGNIFICANCE

Efforts to define population risk caused by the possible substitution of lead in gasoline by Mn are acutely necessary. The threat of a potential nonexistent danger could slow down a good industrial solution to a major energy problem. On the other hand, the present study may draw attention to the risk to the young population of Mn poisoning. Findings may prove to be highly effective in securing protection for this sector of our population.

The consequences of an increased Mn body burden in healthy populations will be explored both in adults and animals. Observations on susceptibility would be further strengthened if we also demonstrate increased intestinal absorption of Mn in children. The correlations with catecholamine metabolism appear justified in view of the known connections between manganese and the neurotransmitters. Extrapolation of observations on the effects of an expanded Mn pool in normal population to potential, new industrial risk appears justified.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Dr. Ismael Mena, University of California at Los Angeles, Harbor General Hospital Campus, and Catholic University, Santiago, Chile, and Dr. Humberto Torloni (Project Coordinator), Department of Research Development and Coordination, Pan American Health Organization, Washington, D.C.
Funded by:	Research project submitted to the National Institutes of Health, U.S. Public Health Service.
Timetable:	1974-1977.

MULTIDISCIPLINARY STUDIES ON PRIMITIVE POPULATIONS IN LATIN AMERICA

PROBLEM

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

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

Over the past 10 years we have published some 50 papers reporting on various aspects of this program. The findings fall in two categories. On the one hand, we have worked extensively on the genetic structure of unacculturated Indian populations, developing therefrom a number of arguments concerning the constraints under which man has been operating during some three million years of human evolution. On the other hand, we have pursued a number of aspects of Indian health, with particular reference to how the biologic pressures bearing on Indian populations differ from those bearing on civilized populations. Of particular interest at the moment is the matter of mineral balance. An entirely unexpected finding is evidence that mutation rates among Indian populations may be higher than anticipated. A vigorous effort is in progress to confirm this finding.

SIGNIFICANCE

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

It seems almost certain that during the next several decades, modern man will attempt to extend his activities into many parts of the tropics still relatively untouched. If he does this in such a way as to leave the tropics relatively untouched--and this seems necessary if the tropics are to survive as an exploitable ecosystem--then he will inevitably be subject to many of the same biomedical pressures which we are studying in the Indian. Thus a knowledge of these pressures could be of value to modern man in the tropics.

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Grantee: Dr. James V. Neel, Department of Human Genetics,
The University of Michigan Medical School, Ann
Arbor, Michigan.

Funded by: U.S. Atomic Energy Commission and the National
Science Foundation.

Timetable: 1973-1975.

FETAL MATURATION, CONDITION, AND GROWTH, IN RELATION TO AMNIOTIC FLUID PARAMETERS

PROBLEM

The specific aims of this project are to develop, apply, and evaluate methods for the diagnosis of fetal maturation and growth, by using amniotic fluid parameters.

Starting with the hypothesis that maternal or fetal pathologic conditions or both may alter the values of the parameters commonly used in fetal maturation diagnosis, the following objectives are postulated:

- 1) To establish the evolution of different parameters of amniotic fluid, in relation to amenorrhea in pregnancies with and without abnormalities;
- 2) To establish the possible differences in both groups, for the main parameters;
- 3) To combine mathematically the parameters, to obtain more precision with the least error; and
- 4) To establish correlations between amniotic fluid parameters and fetal weight and height, as well as the newborn's Apgar score at the fifth minute of life.

METHOD

Samples of amniotic fluid were obtained by transabdominal puncture made during the third trimester of pregnancy, in women with known gestational ages.

The parameters studied in the samples are percentage of orange-stained cells, bilirubin-like pigments, creatinine, total proteins, glucose, pH, total volume, and electrolyte concentrations, (sodium, potassium, calcium, phosphorus, and chloride).

The following method was used in the analysis of the data:
(a) tendency of values, from one puncture to another, in patients with more than one amniocentesis; (b) correlation between each parameter and amenorrhea, for the different groups with normal pregnancies, diabetes, other pathologic conditions and all the patients; (c) correlation of

each parameter (in each group) with weight, height, and Apgar score; the patients who had less than one week elapsing between puncture and delivery were the only ones considered; and (d) analysis of parameters, with multiple regression techniques, which consider the whole information obtained from amniotic fluid for each patient in relation to amenorrhea.

RESULTS TO DATE

For the same amenorrhea, differences in the values of different parameters were found fundamentally by comparing the group without pathologic symptoms with the diabetic group. Remarkable differences in bilirubin-pigment concentration, creatinine content, total protein, pH, and glucose levels, indicate that fetal kidney function in diabetic gravidas, matures earlier than in healthy pregnant women.

Through the study of multiple regression, an equation is reached for each group, that estimates amenorrhea with the least number of parameters (orange-stained cells and bilirubin pigments) and with a calculated error.

Precision reached in the estimation of amenorrhea through amniotic fluid studies, is similar--in regard to its error--to that obtained by direct clinical examination of the newborn.

SIGNIFICANCE

The lack of accurate diagnostic methods to determine the age of the fetus, when the date of the last menstrual period is not known, or is not reliable, is a common cause of errors. Unknown or unreliable amenorrhea is found in a large percentage of the hospital pregnant patients (from 10 to 30 percent). This situation, added to the lack of accurate diagnostic methods, have led to the adoption of interventionist policies, which frequently are the cause of iatrogenic prematurity, with consequent high-risk mortality and morbidity.

A practical, safe, and inexpensive technique, for estimating gestational age, could be a helpful advantage in the management of high-risk patients.

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Grantee: Dr. Rubén Belitzky, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Pan American Health Organization.

Timetable: 1972-1973.

SODIUM PARA-AMINOHIPPURATE IN AMNIOTIC FLUID. A NEW TEST FOR THE EVALUATION OF FETAL CONDITION DURING PREGNANCY

PROBLEM

Previous reports have demonstrated that sodium para-amino hippurate (PAH) appears rapidly in amniotic fluid when this substance is administered to the mother either orally or intravenously. In addition, its concentration increases continuously for 2 hours and reaches values several times higher than those found in maternal plasma.

Experiments performed in pregnant ewes have demonstrated the importance of fetal urine in determining the increments in amniotic fluid concentration of substances eliminated by the kidney.

Taking into account the above mentioned results, it seems that some aspects of fetal kidney functions during pregnancy may be studied using PAH, since this substance is almost exclusively eliminated by this organ. Also, deterioration of this function could be investigated if standards for normality are established in advance.

METHOD

a) Lowest increment expected for normality

Seven healthy pregnant women were studied between 1 and 6 days before delivery. Their gestational ages ranged from 35 to 42 weeks.

Newborns were vigorous at birth and their neurologic examinations at 48-72 hours of life were also normal.

Each mother received a single intravenous injection of PAH (8 mg/kg) followed by an infusion of the same substance at a rate of 25 mg/min during 30 minutes.

In this group nine samples of amniotic fluid were obtained from each subject. The first, before PAH administration, and the others every 30 minutes after the beginning of the infusion, for 4 hours.

The average hourly increment was calculated using the linear regression coefficient.

b) PAH test during pregnancy

Forty-five pregnant women with live fetuses were studied. Of these, 27 were diabetic, 3 were Rh(D) sensitized, 4 had a prolonged pregnancy, 1 had a severe preeclampsia, 1 had a septicemia, 1 was chronically hypertensive, and the remaining 8 were clinically normal.

Gestational ages in this group ranged from 34 to 44 weeks.

Each mother received PAH in the manner described above.

Only three samples of amniotic fluid were obtained from each patient; one before PAH administration and the others 60 and 120 minutes after the beginning of the infusion. The difference between the concentrations of these last two samples was considered as the hourly increment for each subject.

In all cases the test was performed between 1 and 6 days before delivery.

The increment that occurred in each subject was compared with the lowest increment expected for normality, and the test classified as: NORMAL (over 3.60 $\mu\text{g/ml/hour}$), LIMIT (between 3.60 and 3.40 $\mu\text{g/ml/hour}$), and LOW (below 3.40 $\mu\text{g/ml/hour}$).

The newborn's condition was evaluated using the Apgar Score at the 1st, 5th, and 10th minute of life, and the neurologic examination performed between 48 to 72 hours after birth.

Comparisons were made between groups with NORMAL and LOW tests during pregnancy.

RESULTS TO DATE

a) Lowest increment expected for normality

In all patients, the concentration of PAH in amniotic fluid continued to increase after maternal infusion was discontinued. The highest value varied among cases, from 8 to 27 $\mu\text{g/ml}$. The time elapsed to reach the highest concentration varied from 90 to 210 minutes.

As an average, the increment of PAH concentration in amniotic fluid was a linear function of time between 30 and 150 minutes after the beginning of the infusion to the mother ($r = 0.99$, $p < 0.01$). Thus the hourly increment ($b = 5.83 \mu\text{g/ml/hour}$) and its fiducial limits

(95 percent confidence interval) for one given study were calculated (lower limit 3.50 $\mu\text{g/ml/hour}$).

The lower limit of the 95 percent confidence interval was considered the lowest increment expected for normality.

b) PAH test during pregnancy

From the 45 tests performed between 1 and 6 days before delivery, 18 were classified as NORMAL, 25 as LOW, and 2 as LIMIT.

1) Perinatal deaths: In the group with NORMAL test only one death was recorded. It occurred in utero, at 36 weeks of pregnancy, because of intrauterine pneumonia. The PAH test was performed 48 hours before fetal death. The remaining newborns were alive on the 7th day after birth.

In the group with LOW tests, one stillbirth was also observed. Of the 24 live newborns, 8 died within the first 48 hours of life. Six died because of hyaline membrane disease, one had hydrops fetalis, and the other had severe congenital malformations.

Analysis of the hourly increment of PAH only in this last group revealed that the newborn infants who died had statistically significant lower values than did those who survived ($p < 0.01$).

In this group there were no differences in gestational age between fetuses and newborns who died and those who survived ($p < 0.05$).

2) Apgar scores: Excluding neonates who died, in the LOW test group of 16 newborns, 11 were depressed at the first minute, 6 continued depressed at the 5th minute, and 3 were still in this condition at the 10th minute of life.

Of the 17 newborns in the NORMAL group, only 4 were depressed at the first minute and 1 continued in this condition at the 5th and 10th minutes.

Differences between NORMAL and LOW groups were highly significant at these three periods of life ($p < 0.001$).

3) Neurologic examination of the newborn: Of the 16 living newborns with LOW tests, 13 had abnormal neurologic examinations between 48 and 72 hours of life, whereas only 1 newborn of the 17 with NORMAL tests, was found to have neurologic abnormalities in this period.

The association between LOW tests and abnormal neurologic examination of the newborn was highly significant ($p < 0.001$).

Neurologic abnormalities were characterized by a depression or hyperexcitability syndrome, either alone or combined.

4) Gestational age and birth weight: Gestational age at the moment of PAH test was significantly higher in the NORMAL group (276 days \pm 4 S.E.) than in the LOW group (258 days \pm 3 S.E.) ($p < 0.05$).

This parameter was not significantly correlated with the hourly increment of PAH in amniotic fluid in both groups, NORMAL ($r = -0.05$) or LOW ($r = 0.16$), within the range of gestational ages studied.

Differences in birth weights between NORMAL test group (2.978g \pm 0.144 S.E.) and LOW test group (3.00 \pm 0.154 S.E.) were statistically nonsignificant ($p < 0.05$). This parameter was not correlated with the hourly increment of PAH concentration in amniotic fluid both in the NORMAL ($r = -0.21$) or in the LOW group ($r = -0.06$).

SIGNIFICANCE

From our results it is possible to postulate that when PAH is administered intravenously to the mother, increments in the concentration of this substance in amniotic fluid depend on: uterine blood flow, PAH placental transfer, fetal kidney blood flow, urinary volume, and frequency of fetal micturition. If this were so, a LOW test would reveal a disturbance in one or more of the above mentioned factors which in time may be cause or effect of fetal distress. This hypothesis seems to be supported by our results.

Independently of the physiopathologic mechanisms involved in the test, the results themselves give relevance to its applicability in obstetric practice, specifically as a procedure for diagnosis of fetal condition in high-risk pregnancies.

Furthermore, it is important to stress the simplicity of the test which could be carried out almost in every hospital all over the world.

PUBLICATIONS

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Grantee: Dr. Omar Althabe, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Pan American Health Organization.

Timetable: 1970-1974.

FETAL GROWTH AND CORD IMMUNOGLOBULINS IN CENTRAL AMERICAN POPULATIONS

PROBLEM

As many as 40 percent of village children in some areas of Guatemala experience fetal growth retardation and half of the newborns show elevated IgM, a sign of fetal antigenic stimulation. These facts have dramatic implications for survival and postnatal growth. In general, however, there is very little information about the frequency of fetal growth retardation in the various countries of Central America.

The purpose of this investigation is to stimulate workers in the various Central American countries to collect data on birth weight and cord immunoglobulins in rural areas where most of the population lives.

METHOD

Cord blood samples from all newborns examined were analyzed and all births in each community studied recorded; birth weight records of growth and survival are kept by periodic visits.

Children born in the Roosevelt Hospital of Guatemala have also been included in the study since the middle of 1973.

RESULTS TO DATE

Forty percent of the babies examined showed evidence of having suffered intrauterine infection. At the Roosevelt Hospital alone the percentage was around 32. Intrauterine infections by selected agents were as follows:

In the two villages studied, Toxoplasma gondii, herpes viruses, and cytomegaloviruses were responsible for about 10 percent of the intrauterine infections. At the Roosevelt Hospital, Treponema pallidum was responsible for 8 percent of the babies with elevated IgM. Herpes viruses, cytomegaloviruses, and Toxoplasma gondii, were identified with 1.5 percent incidence for each agent.

SIGNIFICANCE

If results obtained in Guatemala are confirmed in other Central American countries, more emphasis should be given to prenatal care, since studies in Guatemala have shown that infant mortality is heavily influenced by fetal growth retardation.

PUBLICATIONS

1. Urrutia, J. J. and L. J. Mata. The influence of infections and infectious disease during pregnancy on infant birth weight. Presented at: Workshop on Latent Effects of Malnutrition and Infection During Pregnancy as Determinants of Growth and Development of the Child. Institute of Nutrition of Central America and Panama (INCAP), Guatemala, January 9-11, 1974.

OTHER DATA

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

Funded by: World Health Organization (1971-1972).

Timetable: 1971 - continuing.

STANDARDS FOR NEONATAL CARE: EARLY OR LATE CLAMPING OF UMBILICAL CORD AT BIRTH

PROBLEM

The immediate clamping of the umbilical cord at birth, or its postponement until a few minutes later, are policies indiscriminately applied in many centers.

At present, no agreement has been reached as to which is the best procedure for the neonate.

A special study was conducted to learn about the immediate and late effects of cord clamping performed at birth, and some minutes after birth.

METHOD

A sample of healthy full-term gravidas was selected. From this sample, only patients with spontaneous onset and evolution of labor were considered. Their newborns had to be vigorous at birth (Apgar score > 6), with appropriate weight for their gestational age. No drugs were administered during labor.

In one group of 23 patients, clamping of the umbilical cord was performed immediately at birth; in another group of 19 patients clamping was done 5 minutes after birth, maintaining the newborn below the level of the mother during this time.

The selection of the procedure to be followed for each case, was made at random. The following parameters were measured in every neonate:

- 1) Hematocrit: from blood samples obtained at 15, 30, 60, and 90 minutes after birth, as well as at 3, 30, and 60 days of life.
- 2) Acid-base balance and pCO_2 of the same samples during the first 90 minutes of life.
- 3) Bilirubin concentration at 72 hours of life.
- 4) Fetal heart rate (FHR) during the last hour before birth.

5) Neonatal heart rate (NHR), continuously recorded and measured during the first 30 minutes of life.

RESULTS TO DATE

The following preliminary results have been obtained to date:

1) A comparison of the two groups showed no differences in birth weight, amenorrhea, Apgar scores, etc.

2) Neonates with late clamping of the umbilical cord, had higher hematocrit values than did those with early clamping. The differences between means were significant at every time considered, until 60 days of life.

3) During the first 30 minutes of life, the early clamping group's NHR was higher than that of the late clamping group.

4) No remarkable differences were found in the acid-base balance, pO_2 , bilirubin concentration, and weight increment of both groups.

These results show that there is an important passage of blood from the placenta to the fetus--through the umbilical cord--in the first 5 minutes after birth.

SIGNIFICANCE

The extra blood supply for the fetus, obtained by late clamping of the umbilical cord, represents an increment in the iron reserve of the neonate, which is reflected by a high hematocrit value, until the second month of life (last follow-up observation).

No side effects were observed when late clamping of umbilical cord was performed.

From these considerations, we recommend late clamping of the umbilical cord for full-term healthy newborns.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Antonio José Duarte Jacomo, Latin American
American Center of Perinatology and Human
Development, Montevideo, Uruguay.

Funded by: Ford Foundation.

Timetable: 1973-1974.

EXPERIMENTAL ACUTE FETAL ASPHYXIA

PROBLEM

Cord compression and occlusion during labor is a recognized cause of acute fetal distress. Looping of the umbilical cord around the fetal neck or the fetal body facilitates this compression, particularly when fetal membranes are ruptured.

A little stretching of the cord and an infrequent cord compression or occlusion of short duration do not cause fetal distress. Even in the absence of fetal metabolic alterations, however, transient falls may be seen in fetal heart rate records.

On the other hand, transient falls in fetal heart rate, similar to those caused by cord compression, may correspond to true fetal distress.

Identification of the conditions in which cord compression may produce or not fetal distress, and its pathogenic mechanism, is to date an unsolved problem that cannot be directly studied in humans.

In approximately 3 of every 10 pregnant women in labor and lying in the supine position, the uterus compresses the maternal aorta, and thus diminishes fetomaternal exchanges. This phenomenon, called the Poseiro effect, is a possible cause of fetal distress.

To investigate the mechanisms of umbilical vessel occlusion and maternal aortic compression and their immediate effects on the fetus, special experiments were devised using animal preparations.

METHOD

Full-term pregnant sheep and goats were selected because they usually have a single fetus, similar in size and volemia to the newborn infant.

Through microcesarean section, a pneumatic wristlet was placed around the umbilical cord, which permitted the occlusion of umbilical vessels for different lengths of time.

Before, during, and after each occlusion, fetal heart rate (FHR) and fetal arterial pressure were recorded and serial arterial blood samples were obtained. Similar data were recorded from the mother.

Short, manual, complete aortic occlusions of different durations were performed, using similar animal preparations.

The same experimental model is now being used to establish the real role of vagal influence. Some occlusions were made after pharmacologic pneumogastric blockade and surgical section.

RESULTS TO DATE

The following preliminary conclusions have been reached so far:

A. Umbilical vessel occlusion

1) Occlusion of the umbilical vessels produced transient falls in FHR, similar to those recorded in cord loop cases when amniotomy was performed.

2) When the duration of umbilical vessel occlusion was shorter than 30 seconds, FHR desaccelerations were recorded, but there was no fetal hypoxemia or acidemia.

3) Occlusions longer than 30 seconds always produced fetal hypoxemia and acidemia, in direct relation with the length of the occlusion.

4) The first sign of umbilical vessel occlusion was a transient fall in FHR record and a significant increase in fetal arterial pressure. Values of pO_2 diminished rapidly, 15-30 seconds after the occlusion had started; the lowest pO_2 values coincided with the lowest value in FHR falls. The pCO_2 values increased more slowly, at a less marked slope than that of pO_2 changes. Fetal arterial pH diminished later (acidemia) and base excess later still, towards the end of the occlusion.

5) When occlusion was interrupted, FHR regained very rapidly its previous values, following a pattern, particular to each animal. Fetal blood pressure fell immediately and later went back to previous levels. Previous levels of pO_2 and pCO_2 were reached 30 to 60 seconds after the end of the occlusion; pH and base excess took several minutes to go back to preocclusion levels.

6) If occlusion was repeated, recovery was incomplete and fetal acidosis occurred.

B. Maternal aortic compression

1) Complete occlusion of the maternal aorta produced transient falls in the FHR base line (Dips), and alterations in fetal homeostasis.

2) In the 15-30 seconds following the beginning of the aortic occlusion, transient falls in FHR and increases in fetal blood pressure were recorded; blood samples showed a marked decrease in pO_2 and an increase in pCO_2 values.

3) The lowest pO_2 values were found after aortic compression ended; pH and base excess changes occurred only towards the end of the occlusion.

4) The lag time between the beginning of aortic occlusion and acute fetal asphyxia was 15-30 seconds more than in cord compression. In addition, preocclusion levels, after the interruption of stimuli, were regained more slowly than in cord compression.

SIGNIFICANCE

Knowledge of the fetal changes occurring when umbilical cord or maternal aorta or both are compressed, may be useful to recognize early symptoms of fetal distress and to prevent its development.

PUBLICATIONS

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OTHER DATA

Grantee: Dr. Hugo Sabatino, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Ford Foundation.

Timetable: 1972-1974.

URINARY ESTRIOL AS A MEASURE OF PERINATAL RISK

PROBLEM

A simple method to diagnose fetal condition, especially in high-risk pregnancies, is needed. The main objective of this work is to study the value of estriol determinations in maternal urine to measure fetal jeopardy.

The fetus plays an important role in the biosynthesis of estriol, a fact which has led investigators to believe that urinary levels of estriol might be indicators of the fetal condition.

At present determinations of urinary estriol levels are widely used. There still exists, however, doubts as to the advantage of this procedure to diagnose fetal jeopardy.

METHOD

Fifty-two pregnant women were studied. They were divided into 3 groups of: (1) 24 healthy women who were examined more than 10 times during pregnancy and who gave birth to normal babies; (2) 19 diabetic gravidas; and (3) gravidas with other complications of pregnancy.

Twenty-four hour urine specimens were periodically collected from the 25th week of amenorrhea. Diuresis was controlled by measurements of urine creatinine levels.

Estriol was measured by Oakey et al's colorimetric method. Other assays were also performed; test of fetal tolerance to uterine contractions, para aminohippurate test, and human chorionic somatomammotropin (HCS) concentration in maternal blood.

The obstetricians and pediatricians taking care of the mothers and newborns, did not know the results of any of the measurements performed.

RESULTS TO DATE

Standard limits for the healthy group were fixed. A patient was considered as having low-estriol excretion when she had more than 20 percent urinary estriol values below the 10 percentile.

The following conclusions may be drawn from this study:

- 1) In normal pregnancies urinary estriol values increased with amenorrhea. This increase followed a parabolic slope. Values in the healthy group were similar to those reported in the literature.
- 2) In diabetic and other pathologic pregnancies, no association was found between estriol values and perinatal mortality.
- 3) Estriol values showed a great variability. This fact may partially explain the poor association between estriol values and fetal condition.
- 4) A significant correlation was found between estriol values and newborn weight. This finding did not hold true for diabetic pregnancies.
- 5) None of the measurements studied significantly correlated with perinatal mortality in the group with diabetes.

The determination of urinary estriol values by a colorimetric method was not found to be a reliable technique for the evaluation of fetal condition. It should always be made in combination with other measurements. In addition, it should not be used as a screening method to identify fetal jeopardy in obstetric patients.

SIGNIFICANCE

As has been mentioned, this technique is widely used in the whole world. Some obstetricians overstress its importance, however, and adopt specific procedures for the management of pregnancies solely on the basis of urinary estriol values.

The present study has shown that such a decision may be very dangerous.

PUBLICATIONS

1. Belizán, J. M. El estriol urinario como indicador de riesgo perinatal. Doctoral thesis in Biology of Reproduction presented to the El Salvador University, Buenos Aires, Argentina. Recognized by the Ministry of Health, 1974.

OTHER DATA

Grantee: Dr. José Miguel Belizán, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Ford Foundation and Pan American Health Organization.

Timetable: November 1971 - April 1973.
July 1973 - March 1974.

ESTIMATION OF GESTATIONAL AGE BY CLINICAL EXAMINATION OF THE NEWBORN INFANT

PROBLEM

The purpose of this study is to develop a quantitative method for the determination of gestational age, using somatic and neurologic parameters of clinical examinations of the newborns.

The method must be painless and easily performed so as to be routinely employed.

METHOD

A group of newborns with the following characteristics were examined, using 21 parameters of the Dubowitz method: the babies were vigorous at birth (Apgar score > 6) and they were borne of healthy mothers who knew accurately the date of their last menstrual period. All examinations were performed between 12 to 48 hours after birth when there was no depression of the newborn (condition III of the Prechtl classification).

A group of 115 newborns of mothers who had had from 205 to 296 days of amenorrhea at birth was studied. Neonatal weights oscillated between 790 g and 4,500 g.

Data from somatic and neurologic examinations were processed by multiregressional analysis. Parameters not significantly related to amenorrhea were eliminated.

Finally only six parameters, four somatic and two neurologic, were selected. The corresponding regression coefficients were used to establish a quantitative score for each parameter.

All data were processed by computer machine (Digital model PDP12).

RESULTS TO DATE

Days of amenorrhea (gestational age) were calculated in function of: skin texture, breast size, ear form, plantar creases, scarf sign, and head lag.

The precision of this method was similar to that of Dubowitz with 21 parameters (SE 8, 4 days).

An optional method using only somatic parameters was developed to permit gestational age determinations when neurologic alterations were present.

The accuracy of the method was tested by different members of the staff in another group of 230 newborns; estimations of amenorrhea were made with similar precision to that of the first group.

SIGNIFICANCE

Knowledge of the newborn's gestational age is extremely useful for the prevention, diagnosis, and treatment of neonatal conditions.

Neonatal mortality and morbidity are directly related to gestational age. The newborn's degree of maturity, per se, determines the type and intensity of care he is to receive. Gestational age is unknown in about 10 to 30 percent of deliveries performed in most centers.

A practical, simple, and inexpensive method to determine gestational age, would be widely used in medical practice.

PUBLICATIONS

1. Capurro, H. Estimation of the gestational age by the clinical exam of the newborn. Doctoral thesis presented to the School of Medicine, Universidad de la República, Montevideo, Uruguay. Printed and distributed by CLAP, 1973.
2. ----- and G. Lieutier. Estimation of the gestational age by the clinical exam of the newborn. Proceedings of the XIV Argentinian Congress of Obstetrics and Gynecology. October 1973, in press.

OTHER DATA

Grantee: Dr. Haroldo Capurro, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Pan American Health Organization.

Timetable: 1972-1975.

HUMAN CHORIONIC SOMATOMAMMOTROPIN (HCS) STUDIES IN NORMAL AND PATHOLOGIC PREGNANCY, LABOR, AND EARLY PUERPERIUM

PROBLEM

The purposes of this study are:

- 1) To determine HCS levels in maternal serum and amniotic fluid, for the diagnosis of fetal health and maturity and of the functional state of the placenta.
- 2) To compare and complement the data obtained with other determinations made in this Center to evaluate:
 - a) fetal maturity
 - b) health of the feto-placental unit
 - c) fetal tolerance to uterine contractions
 - d) newborn maturity and health
 - e) functional state of the placenta

METHOD

Normal and high-risk pregnant women (those with diabetes, toxemia, arterial hypertension, heart diseases, or Rh-immunization, were selected.

The determination of HCS was performed by inhibition of the immunohemagglutination technique, as described by Gusdon (Obstet Gynecol 33:397, 1969). Whenever possible, blood samples were taken (every 3 to 4 weeks) during gestation. Amniotic fluid samples were obtained from patients submitted to amniocentesis for evaluation of fetal maturity or to other diagnostic methods used in the care of high-risk patients.

The following data were also obtained and correlated with HCS levels: (a) fetal tolerance to induced uterine contractions (Pose test); (b) Apgar score of the newborn at the 1st and 5th minutes of life; (c) weight of the newborn at birth according to its gestational age; (d) length and weight/length index of newborn; and (e) weight of placenta

and histologic study of the trophoblast, both by common and contrasted phase microscopy.

RESULTS TO DATE

So far, 199 samples obtained from 56 healthy pregnant women, 100 samples from 25 diabetic patients, and 86 from 20 patients with various pathologic conditions have been studied.

a) In healthy pregnant women, HCS serum levels were similar to those reported in the literature, both in their absolute values and in their evolution during gestation. There was an important disparity among the values found, which at the end of pregnancy were between 6 and 15 $\mu\text{g/ml}$ of serum. No variations were found in HCS levels during labor and delivery.

A correlation was shown between HCS levels and the length of the newborn and placental weight.

b) Diabetic patients: The HCS serum level increased during pregnancy in a similar way to that of normal patients. No correlation was found between the degree of intensity of the diabetes (according to P. White's classification) and HCS levels.

c) Other pathologic conditions: Alterations were found in the absolute serum values of HCS as well as in their evolution only in those patients with small-for-date fetuses. This and other conditions are now being investigated.

SIGNIFICANCE

Perinatal mortality in Latin America and other countries are extremely high.

Many apparently normal pregnancies may result in different types of complications with serious consequences for the fetus or infant. The availability of a rapid, precisely quantified and reliable biochemical screening method is therefore of paramount significance.

Evaluation of HCS serum levels, alone or combined with the determination of other variables commonly used to evaluate fetal health and maturity, and the functional state of the placenta, may be of diagnostic or of prognostic value or both.

PUBLICATION

1. Abusleme, C. et al. Niveles sanguíneos de somatomatotrofina coriónica humana (HCS) en el embarazo normal y patológico. V Reunión de la Asociación Latinoamericana de Investigaciones en Reproducción Humana (ALIRH), Buenos Aires, Argentina, 1972, p. 157.

OTHER DATA

Grantee: Dr. Carlos Abusleme, Laboratory of Protein Hormones, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Ford Foundation.

Timetable: 1971-1973.

HUMAN CHORIONIC SOMATOMAMMOTROPIN.
CHEMICAL STRUCTURE AND BIOLOGICAL ACTIONS

PROBLEM

A. Biochemical Studies

The objectives of this part of the project are:

1) To develop a method for the isolation of human chorionic somatomammotropin (HCS) from human placentas, in conditions such that the native configuration of the protein molecule remains unaltered.

2) To study the chemical, physicochemical, and biologic properties of the native hormone and some appropriate derivatives, using the unaltered preparation of HCS obtained by our mild method. Results will be correlated with those reported earlier by other authors and ourselves for the pituitary growth hormone (HGH) and ovine luteotropic hormone (LTH) in order to assess similarities or differences between them which could be related to their common lactogenic activity.

3) To possibly identify a smaller part of the complex hormone molecule with a biologic activity.

B. Biologic Studies

The purposes of these studies are:

1) To find out what is the action of HCS, alone or combined with human chorionic gonadotropin (HCG) on the alterations observed in the immunologic system at term pregnancy.

2) To determine if this action is related to the prolonged skin allograft survival in pregnant women.

METHOD

A. Biochemical Studies

Isolation of HCS was achieved through: (1) homogenization of washed placentas in pH 4.5 buffer; (2) extraction of the residue with pH 8.6 buffer; (3) precipitation at pH 4.5; (4) dialysation and

lyophilization of supernatant; (5) fractionation of soluble material at pH 8.6 by exclusion chromatography on Sephadex G-100; (6) fractionation by ion exchange chromatography on DEAE-cellulose of the HCS fraction (detected by immunodiffusion techniques); and (7) final purification by preparative electrophoresis on Pevikon.

The HCS preparation thus obtained was submitted to biologic, chemical, and physicochemical characterization, and its lactogenic activity measured by the local crop-sac assay in the pigeon.

B. Biologic Studies

To study the action of HCS and HCG on the immunologic system, the following experiments were performed:

1) Immature adult rats, whole and castrated, received injections of the hormones during 21 days (gestation time for the rat). The doses of these hormones were calculated to reproduce the levels found during the third trimester of pregnancy in humans. On the 22nd day all the animals were bled to death. A blood sample of each animal was obtained and the leukocytic count and formula were studied. After autopsy to discard any infection, the thymus, spleen, and bilateral cervical lymph nodes were excised, weighed, and fixed in 10 percent formalin.

2) Female adult Wistar rats were inoculated daily with saline, HCS, HCG, or a mixture of both hormones, and submitted to skin allograft. Twenty four hours after rejection of the skin allograft, the animals were sacrificed and the thymus and spleen were weighed both wet and dry.

RESULTS TO DATE

A. Biochemical Studies

The HCS preparation obtained (yield = 5 μ g/100 g of placental tissue) was demonstrated to be:

1) Homogeneous, as shown by gel filtration chromatography, disc electrophoresis, thin-layer gel filtration, electrophoresing, double immunodiffusion, and analytical ultracentrifugation.

2) Sensible to high ionic strength solutions. The treatment of the hormone with half-saturated ammonium sulfate produces important alterations of the native molecule and its lactogenic activity is badly diminished.

3) Monomeric, as shown by exclusion chromatography.

B. Biologic Studies

1) There was no statistically significant change in the immature rats' thymus weight. There was, however, a weight increase in the thymus of adult rats, whole and castrated, inoculated with HCS.

2) HCS produced a remarkable increase in leukocytic blood values because of lymphocytosis and possible monocytosis. The administration of the hormone has no effect on the leukocytic count and formula of whole animals. In the case of castrated rats, the hormone produced a depression in the leukocytic values on the 12th day of treatment. This effect, which is significant in all the components of the formula, disappeared at the end of the experiment (day 21).

SIGNIFICANCE

1) To study the chemical, physical, and biological properties of HCS, it is of utmost importance that the native molecule should not be damaged during the isolation and purification procedure.

The method reported here fulfills this requirement, minimizing alterations and aggregations of the protein hormone.

2) The detailed knowledge of the structure of the HCS molecule, and the relationship of its structural characteristics with its biological properties are the basis for the understanding of the molecular mechanism of hormone action. The preparation of derivatives with specific biologic activities or known structural modifications, permits the study of target organ interactions, with pharmacologic or therapeutical significance.

3) The identification of a fragment of the molecule with some activity will greatly diminish the work involved in the synthesis of biologically active compounds, and will enable the study of the effects caused by different activities.

PUBLICATIONS

1. Brovetto-Cruz, J. et al. Relación entre la actividad lactogénica y la composición química de la somatomotrofina coriónica humana. V Reunión de la Asociación Latinoamericana de Investigaciones en Reproducción Humana, No. 102, November 1972, p. 156.

2. ----- et al. Human chorionic somatomammotropin. Studies on the chemical structure and biological activity of the native and modified hormone. In: Int Workshop Horm Prot, Hormone Research Laboratory. University of California Press, 1973, in press.
3. Fraga, A. et al. Acción de la somatomamotrofina coriónica humana (HCS) sobre el sistema timo-linfático. V Reunión de la Asociación Latinoamericana de Investigaciones en Reproducción Humana, No. 104, November 1972, p. 158.

OTHER DATA

Grantee: Dr. Jorge Brovetto-Cruz, Laboratory of Protein Hormones, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: The Population Council and the Pan American Health Organization.

Timetable: 1972-1974.

CONTRACTILITY OF THE NONPREGNANT HUMAN UTERUS IN FOUR PHASES OF THE MENSTRUAL CYCLE. EFFECT OF ADRENOCEPTOR-STIMULATING DRUGS

PROBLEM

Up to the present, little agreement has been reached on the effect of adrenoceptor-stimulating drugs on the contractility of the nonpregnant human uterus recorded in situ.

The purposes of this project were:

- 1) To make a comparative study between three classic methods employed for recording intrauterine pressure.
- 2) To make a quantitative study of the effects of some compounds known to be potent stimulators of the adrenoceptors, and not yet explored in the nonpregnant human uterus in situ.

METHODS

1) Methods of recording. In all studies, intrauterine pressure and heart rate were continuously recorded by accurate and objective methods.

For recording intrauterine pressure we used simultaneously an open-tip catheter; an open-tip catheter with a small terminal sponge; and a catheter closed with a small terminal balloon.

The heart rate was recorded with a cardiometer triggered by the R-wave of the ECG.

2) Methods of administration of the drugs. Each drug was given intravenously for periods of 20 minutes, at three different doses, to the same woman during 4 consecutive weeks of the same menstrual cycle. Values of peak pressure, intensity, tonus, and frequency of the contractions during infusion were compared with those of spontaneous activity.

For each drug, the effect of each dose on the four variables measured was processed by analysis of variance, two-way layout, and two criteria of classification: dose and week of menstrual cycle.

The adrenoceptor-stimulating drugs studied were:

- a. Th-1165-a (Birotec, Pertussin); a stimulant of beta-adrenoceptors. The doses employed were 1, 2, and 4 $\mu\text{g}/\text{min}$.
- b. Orciprenaline (Alupent), stimulant of beta adrenoceptors, although it also has a mild stimulating effect on alpha-adrenoceptors. The doses employed were 5, 10, and 20 $\mu\text{g}/\text{min}$.
- c. Epinephrine (Adrenaline). It stimulates both alpha and beta adrenoceptors. The doses employed were 2, 5, 5, and 10 $\mu\text{g}/\text{min}$.
- d. Ethyl-adrianol (Effortil), stimulant of both adrenoceptors. It was given at doses of 200, 400, and 800 $\mu\text{g}/\text{min}$.

In addition, the sublingual route with two tablets of 5mg of Birotec was tested in four different women at four different weeks of the menstrual cycle.

RESULTS TO DATE

1) Methods of recording.

The most reliable results were obtained with the sponge-tip catheter. The open-tip catheter was very frequently occluded. The closed catheter did not record the real intensity and tonus.

2) Stimulating drugs of alpha and beta adrenoceptors

Birotec given intravenously produced an immediate and very marked uterine motility. This effect was produced at all 4 weeks of the menstrual cycle and at all doses employed. Sublingual administration also caused a long period of uterine inhibition. Cardiovascular side effects were minimal and well tolerated.

Orciprenaline reduced uterine motility at all four weeks of the cycle. The most marked effect was found on the 4th week and at doses of 20 $\mu\text{g}/\text{min}$. Also, it caused a marked increase in heart rate and hypotension.

Epinephrine had no significant effect on uterine motility. It produced tachycardia and hypotension.

Ethyl-adrianol caused a marked increase in uterine motility especially in the intensity and peak pressure of uterine contractions during the first week of the cycle. It caused a mild increase in heart rate, no changes in diastolic, and a mild rise in systolic arterial pressure.

SIGNIFICANCE

The clear inhibitory effect of Birotec on uterine motility and its minimal cardiovascular side-effects, strongly recommend its use in women to block the undesirable side-effects of the intrauterine device (hypercontractility, pain, hemorrhages, expulsion). Besides, it could also be used in the treatment of essential dysmenorrhea. The long duration of uterine inhibition when Birotec is administered by the sublingual route is another point in favor of this recommendation.

The stimulating effect of Ethyl-adrianol on the motility of the nonpregnant human uterus, explained by stimulation of uterine alpha-adrenoceptors, is exactly the opposite to that obtained during pregnancy. This "reversal" in the effect of a drug on the same target organ has been described by us for the first time.

PUBLICATIONS

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2. ----- et al. Effects of adrenoceptor-stimulating drugs on the myometrium and cardiovascular system in non-pregnant women. In: Coutinho, E. and A. Hollander, (eds.) Proceedings of the XIII International Symposium on Basic Biology. "Physiological and Genetical Aspects of Reproductions," Salvador, Bahía, Brazil, 2-7 December, 1973, in press.
3. ----- . Effects of adrenaline and Th-1165-a upon uterine contractility of the human non-gravid uterus during the menstrual cycle. In: Proceedings of the XIV Argentinian Congress of Obstetrics and Gynecology, 21-26 October, 1973, Buenos Aires, Argentina, in press.

OTHER DATA

Grantee: Dr. J. Rodrigo Cifuentes B., Latin American
Center of Perinatology and Human Development,
Montevideo, Uruguay.

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

Timetable: 1972-1974.

DEVELOPMENT OF A NEW METHOD FOR THE ISOLATION OF OXYTOCIN FROM HUMAN BLOOD

PROBLEM

Plasma oxytocin levels along different stages of the reproductive cycle were studied in the Laboratory of Neurohypophyseal Hormones. Some years ago, we described our measurements of oxytocin in labor and lactation. These hormone measurements were made by a combination of physicochemical extraction and bioassay. Other authors have found different results using radioimmunoassay. These discrepancies could be explained by the use of extraction and dosage methods based on different principles. This point could be stressed if the hormone's biologic and immunologic active sites were not located in the same place in the molecule.

We intend to measure the hormone by several techniques but mainly through development of a new method combining immunoadsorption with bioassay. We will thus ensure that the measured substance has the same biologic and immunologic properties as oxytocin.

Some observations, which led us to suspect that the amount of oxytocin adsorbed on siliconized glassware (commonly used in laboratories) could be considerable, have also been systematically studied.

METHOD

The projected method that combines immunoadsorption and bioassay was studied as follows:

a) The oxytocin immunoadsorbent was prepared by polymerizing the gamma-globulin fraction of an oxytocin antiserum by glutaraldehyde. Adsorption of the peptide and its subsequent desorption from the immunoadsorbent was studied.

b) To measure oxytocin eluted from the immunoadsorbent by bioassay, it was necessary to change the hormone from the elution buffer to Tyrode's solution. This was accomplished by ultrafiltration through AMICON UM-05 membrane.

c) The bioassay was performed with the isolated strip of lactating mouse mammary gland, which we previously described.

In addition to this technique, radioimmunoassay and Chard et al.'s extraction method using Fuller's earth were employed. In the radioimmunoassay, the main difficulty was to obtain good antisera. Initially, oxytocin coupled with ovalbumin by glutaraldehyde was used as an immunogen. Recently, some rabbits were immunized with oxytocin coupled to bovine gamma globulin by ethylcarbodiimide.

Finally, adsorption studies of oxytocin and ^{125}I -oxytocin were carried out on glass- and plasticware under various conditions, such as treatment with silicone, sulfochromic mixture, and NaOH. Adsorption isotherms at 4°C were achieved.

RESULTS TO DATE

In immunoadsorption experiments with our best antiserum, low overall yields (both adsorption and desorption of oxytocin) were obtained: 40 percent \pm 2.5. The use of purified antibodies will probably lead to attainment of a higher efficiency.

The change of oxytocin from the elution buffer to Tyrode's solution by ultrafiltration through AMICON UM-05 membrane showed variable results. This was due to the fact that all these membranes do not give the same yields. The latest recoveries obtained with previously controlled membranes were 60.2 percent \pm 9.1. It was concluded that the whole extraction method should be improved, mainly raising the recovery of the immunoadsorption step.

The first attempts to obtain good antisera failed. Rabbits were immunized with oxytocin coupled to ovalbumin. More recently other groups of rabbits were injected with oxytocin coupled to bovine gamma globulin.

One of 12 rabbits produced a good antiserum, with high affinity and specificity. This antiserum enabled us to perform a radioimmunoassay that can detect low concentrations of oxytocin (10 $\mu\text{U}/\text{ml}$ of plasma).

For unspecific adsorption of oxytocin (or ^{125}I -oxytocin) on different surfaces, our investigations showed that the minimum adsorption of the peptide is obtained using unsiliconized glassware previously treated with sulfochromic mixture or 10 percent NaOH. It is inconvenient to work with plasticware or siliconized glassware because of their high adsorption. By means of adsorption isotherms, it was also observed, that adsorption increases with the concentration of the oxytocin solution. From these isotherms, the loss of hormone due to adsorption on the walls of storage vessels may be calculated. These studies are still in progress.

SIGNIFICANCE

The importance of obtaining a reliable method to measure small amounts of oxytocin in plasma should be stressed. Various techniques, though improved, do not seem to be absolutely specific. For instance, radioimmunoassay could measure residues of the oxytocin molecule after its degradation by plasma oxytocinase. A reliable and sensitive method should throw more light on the controversial significance of oxytocin in human reproduction. We intend to continue work on this subject.

From adsorption results it appears that the surface nature of labware should be carefully considered when working with oxytocin, to avoid loss by adsorption. Eventually these findings could be extended to other hormones.

PUBLICATIONS

1. Diano, W. et al. Adsorption of ^{125}I -oxytocin on pyrex glass and siliconized glass and polypropylene, in preparation.
2. Garófalo, E. et al. Obtención y purificación de la Ocítocina ^{125}I . Presented at the V Meeting of ALIRH. Buenos Aires, Argentina, 1972.
3. Roca, R. et al. Improvement in the bioassay of oxytocin by the mouse mammary gland "in vitro". Proc Soc Exp Biol Med 139: 1010, 1972.
4. ----- et al. Desarrollo de un método de aislamiento y valoración de ocítocina en plasma humano. Presented at the V Meeting of ALIRH. Buenos Aires, Argentina, 1972.

OTHER DATA

Grantee: Dr. Juan A. Coch, Laboratory of Neurohypophyseal Hormones, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Population Council and Pan American Health Organization.

Timetable: 1971-1974.

EFFECTS OF RUPTURE OF MEMBRANES DURING LABOR ON FETAL HEART RATE

PROBLEM

It is known that the production of type I dips by uterine contractions is significantly greater after than before amniotomy. Type I dips are attributed to the uneven compression of the fetal head or to the transient occlusion of umbilical vessels during uterine contractions.

The purpose of this paper is to establish the pattern of fetal heart rate (FHR) throughout labor when the membranes remain intact until the second stage, and to compare the incidence of type I dips during advanced labor (cervical dilatation greater than 5 cm) between women with intact membranes and others with ruptured membranes.

METHOD

The study is made in 37 pregnant women without known complications and in whom labor starts spontaneously at term and progresses without need of oxytocic, analgesic, or other drugs. In 17 of these labors, the membranes are ruptured artificially when cervical dilatation reaches 5 cm (early amniotomy). In the other 20 labors membranes remain intact at least until full cervical dilatation is obtained. The decision to perform early amniotomy is made at random. In all 37 labors intra-uterine pressure and FHR are graphically recorded. When membranes are intact, FHR is recorded by means of ultrasound. There are no significant differences between both groups of labors in the duration of labor and in birth weight, cephalic perimeter, and Apgar score of the neonates.

The incidence of type I dips in a given group of labors is expressed by the percentage of uterine contractions which produced type I dips. For this calculation all the contractions recorded in the several labors of the group are pooled together. The significance of the difference in the incidence of type I dips between two groups is determined by a nonparametric statistical test.

RESULTS TO DATE

The incidence of type I dips is low or nil when the membranes remain intact until a few minutes before delivery. If the rupture occurs late, when the fetal head is engaged, a high incidence of type I dips is recorded after the rupture. After early amniotomy, made before head engagement, the incidence of type I dips is low; as the head engages, the incidence increases.

The influence of the following three factors on the incidence of type I dips is analyzed quantitatively: (1) status of the membranes (intact or ruptured); (2) station of the fetal head (before or after engagement); and (3) loop of cord around the fetal neck (present or absent).

During the first stage of labor the incidence of type I dips is significantly lower in the group of labors with intact membranes (3 percent) than in that with early amniotomy (21 percent). This result agrees with the hypothesis that the bag of waters protects the fetal head from the uneven compression received during uterine contraction. A similar result is found when the incidence of type I dips is studied in the period of labors before engagement of the head and also in the period after engagement. In both conditions the incidence of type I dips is significantly lower in labors with intact membranes than in those with early amniotomy. This finding suggests that the protective effect of the bag of waters is present both before and after head engagement. In the group of labors with ruptured membranes, the incidence is significantly higher after the head is engaged than before engagement. This indicates that in absence of the bag of waters the engagement greatly facilitates the production of type I dips. A similar comparison made in the group of labors with intact membranes shows no significant difference before and after engagement of the head, confirming the protection provided by the unruptured membranes even when the head is engaged.

SIGNIFICANCE

Evidence obtained from clinical observations indicates that type I dips may be associated with the uneven compression and deformation of the fetal head, which may cause EEG alterations, cerebral birth trauma, and neurologic sequelae. Experimentation in animals shows that compression of the fetal head reduces cerebral blood flow and oxygen availability to the brain, altering the EEG and increasing vagal tone with the consequent rapid fall in FHR.

All these facts make it advisable to reevaluate the common obstetrical practice of rupturing membranes artificially early in the first stage of labor, as a routine procedure in the management of labor.

PUBLICATIONS

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2. ----- et al. Fetal monitoring in labor. In: Wallace, H. M., E. M. Gold, and E. F. Lis (eds.) Maternal and Child Health Practices. Problems, Resources and Methods of Delivery. Chapter XVI, pp. 332-94, 1973. Charles C. Thomas, Springfield, Illinois.
3. ----- . Effects of Rupture of Membrane on Fetal Heart Rate Pattern. Int J Gynaec Obstet 10:169-72, 1972.
4. Schwarcz, R. et al. Fetal heart rate patterns in labors with intact and with ruptured membranes. J Perinat Med 1:153-65, 1973.

OTHER DATA

Grantee: Dr. Roberto Caldeyro-Barcia and Dr. Ricardo Schwarcz, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Pan American Health Organization and Ford Foundation.

Timetable: 1972-1973.

LATIN AMERICAN COLLABORATIVE STUDY ABOUT THE EFFECTS OF MEMBRANE RUPTURE ON LABOR PROGRESS AND ON CEPHALIC MOLDING OF THE NEONATE

PROBLEM

The objectives of this study are:

- 1) To learn about the influence of ovular membranes and other fetomaternal factors on:
 - a) duration of the different stages of labor.
 - b) evolution and type of termination of labors.
 - c) the condition of the newborn and the molding of its head.
- 2) To establish criteria of normality for the variables included in this study.
- 3) To stress the importance of critical judgement of obstetricians and neonatologists about the possible harmful effect of some medical conducts or actions routinely taken in the management of labor.
- 4) To promote the participation in this project of a great number of maternal and child health centers in Latin American countries, with the aim of achieving a wide experience, discussion, and unified criteria regarding the management of normal labor.

METHOD

1) Clinical material

Study patients had to fulfill the following requirements:

- a) They suffered no known disease and had a normal pregnancy.
- b) They were nulli- and multiparas without cephalopelvic disproportion and with a normal pelvic.
- c) They had between 266 and 294 days of amenorrhea.
- d) They carried live fetuses in cephalic presentation.

- e) They started labor spontaneously (without any medication).
- f) They had intact membranes at the start of the study.
- g) They had a cervical dilatation greater than 3 cm and less than 5 cm at the time they entered the study.

2) Design

In 50 percent of the labors, early amniotomy was performed when cervical dilatation was between 3 cm and 5 cm.

In the other half of the cases, membranes were kept intact until the beginning of the late second stage, and whenever possible until birth.

Each procedure was performed alternatively (for example, one patient underwent early membrane rupture, the next, a late rupture, and so on). Choice of the first procedure in each center was made in an aleatory way. Under no circumstances was the decision maintained if it was contraindicated for a given patient.

Following membrane rupture, patients were kept in a horizontal position but were allowed to change their decubitus as often as they wished.

The patients were not given any drug until the onset of labor. Those who had precise medical indications to take a given drug, were also recorded and the data obtained interpreted in a given section of this study.

3) Control of labor and the newborn

The variables selected to assess the progress of labor and the condition of the newborn were recorded in a coded file history. This coded file was compiled according to an instruction manual covering all the variables.

4) Analysis

The coherence of the information received from the collaborating centers was checked and data processed by electronic computation and statistically treated.

RESULTS TO DATE

Nineteen health centers of nine Latin American countries have joined the research program. So far these 19 centers have provided 1,251 clinical protocols. We are presently processing 1,134 of them, the results of which will be published in our Third Progress Report.

Results of the 478 protocols presented in our Second Progress Report (June 1973), revealed that:

1) Early rupture of membranes caused a neonatal cephalic molding significantly greater than that observed in newborns of mothers with intact membranes. The signs of cephalic molding studied were disalignment between cranial bones and presence of caput succedaneum.

2) There was a great disparity in labor duration. However, when it was measured starting at a given cervical dilatation (5 cm), the mean duration was significantly less in the group of labors with early rupture than in the other.

3) Based on the 1,134 protocols of labors recorded by the collaborating groups, we will be able to know with greatest precision the probabilities that the newborn infant of a mother with early rupture of the membranes has, of presenting cephalic molding. We may also be able to estimate, for example, which labors with early rupture produce greater cephalic molding.

The forthcoming results will probably be useful to establish recommendations that may be adopted as rules in the care of labor.

SIGNIFICANCE

This study will reveal the real effect of early rupture of membranes, or of nonrupture, on the progress of labor and on cephalic molding of the neonate.

Though this fact is still being discussed, there already exists some evidence that intrapartum amniotomy may be harmful to the newborn.

The information we collected has never before been obtained, since most obstetricians routinely perform amniotomy before cervical dilatation reaches 6 cm, to shorten the duration of labor.

In addition, this investigation has provided other important information as follows:

1) Because of its "perinatal" objective, it has involved the teamwork of obstetricians and neonatologists.

2) It has required intrapartum and neonatal control, which is feasible and efficient.

3) It has taught the obstetrician to observe the physiologic phenomena of labor and has prevented unnecessary minor interventions (use of analgesics or oxytocic drugs).

4) It has tended to improve intrapartum and neonatal care.

5) It has permitted the collection of data on the physiology of labor in several Latin American countries with different socioeconomic, ethnic, and cultural conditions.

Finally, this research will lead to the description of normal characteristics of the most important parameters in the physiology of labor and some parameters of the neonate and show the index of perinatal mortality in a low-risk population that has been especially controlled.

PUBLICATIONS

1. Schwarcz, R. L. and S. Konichezky. Latin American collaborative study in the influence of membranes upon labor and the newborn. First Progress Report presented to the Pan American Health Organization and to the Collaborating groups. Latin American Center of Perinatology and Human Development, Montevideo, January 1973.
2. ----- . Latin American collaborative study in the influence of membranes upon labor and the newborn. Second Progress Report presented to the Pan American Health Organization and the Collaborating groups. Latin American Center of Perinatology and Human Development, Montevideo, June 1973.
3. ----- et al. Conservative management of labor. Proc VII World Congr Obstet Gynec, Moscow, August 1973. Excerpta Medica, in press.

OTHER DATA

Grantee: Dr. Ricardo L. Schwarcz, Latin American Center
of Perinatology and Human Development, Montevideo,
Uruguay.

Funded by: Pan American Health Organization.

Timetable: 1972-1975.

PATTERNS OF FETAL AND NEONATAL HEART RATES, ACID-BASE BALANCE, AND BLOOD GASES

PROBLEM

Little is known on the modifications in heart rate patterns and the changes in the blood components which occur during the fetoneonatal period as part of the internal milieu adaptations.

The aim of this study is to establish the normal patterns of these changes and to interpret the possible mechanisms involved.

METHOD

Twenty-six healthy gravidas with full-term pregnancy, spontaneous onset and progress of labor, and spontaneous delivery were studied. They received no drugs during labor, and gave birth to vigorous newborns without fetal distress who had their umbilical cords clamped immediately at birth.

Fetal heart rate (FHR) was recorded by ultrasounds or through a cephalic electrode; neonatal heart rate (NHR) was recorded immediately after birth by a tachometer triggered by the ECG signal (one cephalic electrode and one inserted in the umbilical cord).

Acid-base balance and blood gases were measured in blood of umbilical artery, and from arterialized capillaries in the newborn's heel, at 15, 30, 60, and 90 minutes after birth.

RESULTS TO DATE

1) Fetoneonatal heart rate pattern

Baseline of FHR during the last 90 minutes before birth was very regular; there were no differences among means when values at 60, 30, and 10 minutes before birth were compared.

Heart rate values increased by 32 beats per minute after birth; NHR had significantly higher values 10 minutes after delivery than did FHR 30 minutes before birth. After this immediate increase, NHR showed

a clear tendency to stabilize at values similar to those observed during fetal life. There were no differences between heart rate values 60 minutes before and after birth.

2) Patterns of acid-base balance and blood gases

- a) Blood pH: pH values varied from 7.26 in the umbilical artery at birth, to 7.38 in blood heel samples 90 minutes after birth.

There was a persistent increase in the pH values from birth until 30 and 60 minutes of life.

- b) Base deficit: from delivery until 30 minutes of life, there was a transient increase in base deficit. During the following hour of observation, base deficit values decreased to levels similar to those of umbilical artery at birth.
- c) pO_2 : significantly higher values were observed at birth ($\bar{x}=22.9$ mm Hg) than those at 15 minutes of life ($\bar{x}=53.0$ mm Hg). pO_2 values did not change during the following hour of observation.
- d) pCO_2 : significantly lower values were observed at birth ($\bar{x}=50.0$ mm Hg) than 30 minutes after delivery (heel samples, $\bar{x}=35.7$ mm Hg).

SIGNIFICANCE

Knowledge of the normal patterns of cardiovascular and internal milieu adaptations, from fetal life to neonatal period, has a practical importance in the diagnosis and control of adaptative disorders.

PUBLICATIONS

1. Bejar, R. et al. Acid-base balance, pO_2 , and pCO_2 in fetuses and newborns vigorous and depressed at birth. Proceedings of the XIV Argentinian Congress of Obstetrics and Gynecology, October 1973, Buenos Aires, in press.

2. Bustos, R. et al. Patterns of fetal and neonatal heart rates in vigorous newborns and with mild depression. Proceedings of the XIV Argentinian Congress of Obstetrics and Gynecology, October 1973, Buenos Aires. In press.

OTHER DATA

Grantee: Dr. Raul Bustos, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: Pan American Health Organization.

Timetable: 1972-1973.

EFFECTS OF ATROPINE ON THE HEART RATE OF THE HUMAN FETUS THROUGHOUT PREGNANCY

PROBLEM

The purpose of this work is to elucidate the role played by the vagal innervation of the fetal heart in the control of the heart rate throughout pregnancy.

METHOD

Fetal Heart Rate (FHR) was graphically recorded and measured by a beat-to-beat cardi tachometer in 74 human nonatropinized fetuses between the 14th and the 40th week of gestation.

The cardi tachometer, triggered by an ultrasound emit-receive system, was applied to the abdominal wall of the mother and directed to the fetal heart. This system was activated by each heart beat by means of the Doppler effect.

Thirty-six atropine tests were performed in 14 pregnant women at different gestational ages ranging from the 14th to the 40th week of pregnancy. In five patients, the atropine test was repeated 5 or 6 times during the same pregnancy at 1 or 2 weeks' intervals. All patients were delivered at term in our service, and all fetuses were in good condition at birth. The follow-up of the infants showed no abnormalities in their development.

A dose of 2 mg of atropine sulfate dissolved in 10 ml saline was slowly injected i.v. to the mother in a 2- to 4-min period.

RESULTS TO DATE

In the 74 nonatropinized fetuses, FHR diminished as gestational age increased. According to the regression coefficient there was a fall of 0.8 beats/min for every week of increment in gestational age. The regression coefficient was significantly different from zero ($p < 0.001$). The correlation coefficient between FHR and gestational age ($r = -0.7$) was statistically significant ($p < 0.001$).

In 33 of the 36 fetuses receiving atropine, the medication produced a significant rise in FHR.

The increment in FHR was measured as the difference between the mean values of FHR before and after the injection of the drug.

Postatropine increment of FHR augments with gestational age. The regression coefficient ($b=0.7$) indicates that the effect of atropine increases approximately 0.7 beats/min every week. This regression coefficient is significantly different from zero ($p<0.001$). According to the regression equation [$y=(-4.9) + 0.7 (x)$], the amplitude of the postatropine rise in FHR is 5 beats/min at 15 weeks' and 19 beats/min at 35 weeks' gestation.

The correlation coefficient between the effect of atropine and the gestational age is $r=0.74$; this correlation is significant ($p<0.001$). The determination coefficient ($r^2=0.55$) indicates that 55 percent of the changes in the postatropine increment in FHR are due to variations in gestational age.

SIGNIFICANCE

For each gestational age, the difference in FHR between the non-medicated and the atropinized fetuses is attributed to the presence of a cardiomodulator vagal tone. Assuming that atropine blocks the vagal effect on FHR, the amplitude of the postatropine rise in FHR is taken as a measure of the preexisting vagal tone. According to the results reported in this work, in the human fetus the cardiomodulator vagal tone, although small, is already detectable at 15 weeks of pregnancy and increases significantly as gestation advances. At term of pregnancy and in resting normal conditions, the vagal tone is constantly reducing the heart rate of the human fetus by 20 beats per minute.

PUBLICATIONS

1. Schifferli, P. Y. and R. Caldeyro-Barcia. Effects of atropine and beta-adrenergic drugs on the heart rate of the human fetus. In Boreus, L. (ed.) Fetal Pharmacology. Raven Press, New York, 1973, pp. 259-79.

OTHER DATA

Grantee: Dr. Pierre Ives Schifferli, Latin American
Center for Perinatology and Human Development
(CLAP), Hospital de Clinicas, Montevideo,
Uruguay.

Funded by: University of Lausanne and Pan American
Health Organization.

Timetable: 1972 - continuing.

ACROSOMAL TRYPSIN IN RABBITS

PROBLEM

Our initial purpose was to search for the factors involved in the activation of rabbit acrosomal trypsin occurring in the female genital tract. The initial results about the levels of trypsin activity in ejaculated spermatozoa, in discordance with those existing in the literature, forced us to reinvestigate the enzyme activity in epididymal, ejaculated, and uterine spermatozoa.

METHOD

We measured trypsin activity by the Schwert and Takenake method (hydrolysis of BAEE in buffer Tris-HCl 50 mM, pH8). We established the difference between total trypsin and active trypsin of the extracts by means of the property shown by trypsin of different sources: the complex trypsin-inhibitor is dissociated at acid pH, and newly formed with decrease in trypsin activity when it is returned to alkaline pH.

RESULTS TO DATE

The ratio total trypsin: active trypsin is similar for acrosomal extracts of epididymal and ejaculated spermatozoa, but the active trypsin rises after ejaculated spermatozoa stay in the uterus for 13 hours.

SIGNIFICANCE

This research is a contribution to the knowledge of the components in spermatozoa capacitation.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Manuel García Patrone, Latin American
Center of Perinatology and Human Development,
Montevideo, Uruguay.

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

Timetable: 1972-1974.

EPIDIDYMAL MOTILITY

PROBLEM

Little is known about the spontaneous contractility of the epididymis, its regulation, and about the possible action of drugs upon epididymal motility. To date, no quantitative study has been made in this field.

The purposes of this project were to: (1) develop an original method for recording epididymal motility in mice, (2) identify normal patterns, and (3) quantify the effects of different drugs on epididymal motility.

METHOD

Epididymal contractility of the mouse was recorded, using a transducer for isometric tensions. Effects of different drugs (oxytocin, vasopressin, epinephrine, orciprenaline, acetylcholine, atropine, or propranolol) were tested by studies of epididymal cauda motility in vitro.

Similar experiments were performed in cephalic epididymis of the mouse. Another study was made on the mouse epididymis in situ, by using a transducer for hydrodynamic pressures.

RESULTS TO DATE

The following conclusions have been reached so far:

- a) Cauda epididymis in vitro has a marked contractility.
- b) According to its pharmacologic response, cauda epididymis has only alpha adrenoceptors.
- c) Cephalic epididymis in vitro has practically no contractility, only small peristaltic-like oscillations.
- d) Cephalic epididymis does not respond to alpha or beta adrenoceptor-stimulating drugs.

e) Cauda epididymis in situ has spontaneous contractility, similar to that recorded in vitro. Each contraction recorded in situ is followed by a base-line increase. This phenomena is probably responsible for spermatic wave progression.

SIGNIFICANCE

Previous experiments have indicated that epididymal motility is the main factor in spermatozoid progression.

A reliable method to measure this activity and to test the effects of drugs on epididymal motility is a clear advantage. This study also shows the possibility of future therapeutic applications of these drugs.

PUBLICATIONS

1. Hib, J. et al. Neuro-hormonal control of epididymal contractions. In: Coutinho, E. and A. Hollander, (eds.), Proceedings of the XIII International Symposium on Basic Biology, Physiological and Genetical Aspects of Reproduction, in press, 1973.
2. -----. Epididymal contractility. Doctoral thesis presented to the School of Medicine, Universidad del Salvador, Buenos Aires, Argentina. Printed and distributed by CLAP, 1974.
3. -----. The contractility of the cauda epididymis of the mouse and the effects of oxytocin. J Reprod Fertil, in press, 1974.
4. -----. Effects of oxytocin and vasopressin on spontaneous contractility of the mouse cauda epididymis "in vitro". Biol Reprod, in press, 1974.
5. -----. Effects of autonomic drugs and blocking agents on the spontaneous contractility of the mouse cauda epididymis "in vitro". Submitted for publication to J Reprod Fertil.

6. -----. Effects of histamine, promethazine, serotonin and LSD on the spontaneous contractility of the mouse cauda epididymis studied "in vitro". Submitted for publication to J Pharmacol Exp Ther.

OTHER DATA

Grantee: Dr. José Hib, Latin American Center of Perinatology and Human Development, Montevideo, Uruguay.

Funded by: World Health Organization.

Timetable: 1972-1973.

POLIOMYELITIS AND DENGUE NEUTRALIZATION ANTIBODIES
IN SERA FROM A SURVEY OF BRIDGETOWN, BARBADOS

PROBLEM

a) Poliomyelitis. Preliminary tests on sera from a "well-immunized" population sample in Barbados, West Indies, indicated low or poor antibody levels in screening tests.¹ Similar observations have been made in other presumably immunized populations in Syracuse, New York;⁴ Cleveland, Ohio;² Houston, Texas;⁵ and in Vellore, India.³ The reasons for this may be technical, or biologic, or due to poor vaccine. The present investigation seeks to quantitate the status of poliomyelitis antibodies in Barbados, including detection of antibody at very low serum levels.

b) Dengue. An age analysis of complement-fixing antibodies to types 1, 2, and 3 dengue virus in Barbados revealed an almost complete absence of antibody in persons under 25 years of age, and a rapidly increasing prevalence rate in those over 25. This result suggested that little or no dengue virus activity has occurred in the past 25 years but that the virus was active in the years prior to that (circa 1948 or before). In the present study neutralization tests will be carried out to confirm the results of the complement-fixation test. In addition, efforts will be made to rebleed a group of persons known to have had antibody in 1958 and whose original sera are available in order to compare antibody titers 16 years apart.

METHOD

a) Poliomyelitis. A microneutralization test will be carried out with Vero monkey kidney cells using serum dilutions of from 1:2 to 1:1024. Overnight incubation of serum/virus mixtures will be made before the addition of cells to enhance the sensitivity of the test to pick up low antibody levels.

b) Dengue. A neutralization test will be employed in newborn mice using an inoculum of 100 LD₅₀, a single dilution of noninactivated serum, intracerebral inoculation, and a 7-day observation period.

RESULTS TO DATE

Poliomyelitis neutralization antibody titers have been quantitatively determined on 28 sera from children under the age of 15 against all three poliomyelitis types. No antibody was detected at a serum dilution of 1:2 for all three poliovirus types in sera from one child; for two types in two children, and for one type in five children. Thus 8 or 28 percent of children tested lacked one or more polio antibodies. Antibody to type 3 was the most commonly absent. The titers in those possessing antibody were generally at a good level.

Dengue antibody determinations have not yet been initiated.

SIGNIFICANCE

If the results of preliminary tests are borne out by further work, there will then be concern about the levels of poliomyelitis antibody in presumably well-immunized populations. This is not due to technical problems with the test. The absence of antibody to at least one type of poliomyelitis in some 25 percent of the groups tested thus far suggests that a sufficient number of susceptible children may accumulate over time to support an outbreak such as that which recently occurred in Trinidad. Whether this is due to inadequate vaccine utilization, poor vaccine, improper storage, ineffective administration, declining antibody levels, or a poor immune response is unknown at the present time. It does point up the need for serologic surveys to monitor the effectiveness of polio immunization campaigns.

PUBLICATIONS

1. Evans, A. S. et al. A health and seroepidemiological survey of a community in Barbados. Int J Epidemiol, in press.
2. Gold, E. et al. Immune status of children one to four years of age as determined by history and antibody measurement. N Engl J Med 289:231-34, 1973.
3. John, T. J. and P. Jayabal. Oral polio vaccination of children in the tropics. 1. The poor seroconversion rates and the absence of viral interference. Am J Epidemiol 96:265-69, 1972.

4. Lamb, G. A. and H. A. Feldman. Rubella and vaccine responses and other viral antibodies in Syracuse children. Am J Dis Child 122:117-21, 1971.
5. Melnick, J. L. et al. Developing gap in immunity to poliomyelitis in an urban area. JAMA 209:1181-185, 1969.

OTHER DATA

Grantee: Dr. Alfred S. Evans, WHO Serum Reference Bank, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut.

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

Timetable: 1973-1974.

PRODUCTION OF IgE ANTIBODIES IN GUINEA PIGS WITH BACTERIAL LIPOPOLYSACCHARIDES AS ADJUVANTS

PROBLEM

That many gram-negative bacteria exert an adjuvant effect is a well known fact. The adjuvant property of these microorganisms is in great part caused by the lipopolysaccharides (LPS) which they contain. The adjuvant effect of purified LPS has been observed in rabbits, mice, and guinea pigs and studied by determining total antibody levels without differentiating between the antibody classes. It was decided to find out how LPS would affect the production of IgE antibodies in guinea pigs.

METHOD

LPS from Bordetella pertussis and Salmonella typhosa was obtained by the method of Westphal and Luderitz. Passive cutaneous anaphylaxis (PCA) was performed according to Ovary's technique. The immunization procedure consisted of administering intraperitoneally 20 µg/kg LPS and 10 µg ovalbumin to guinea pigs. The animals were bled at different times after immunization and the IgE- and IgG1-antibody content of the sera determined by PCA reaction, using a short (24 hours) or long (72 hours) sensitization period.

RESULTS TO DATE

The adjuvant action of lipopolysaccharides on the IgE- and IgG1-antibody production by guinea pigs was studied. LPS was found to induce an early preferential production of IgE antibody. The optimal LPS dose for this effect was of the order of 20 µg/kg. Low amounts of LPS and of antigen elicited the production of IgE antibody whereas high amounts of LPS or antigen induced the production of IgG1 antibody. Later, in the course of immunization, the IgE-antibody content decreased and the IgG1-antibody content increased. Booster injection of antigen brought a considerable and simultaneous increase of both IgE and IgG1 antibodies. Preferential production of IgE antibody was consistently obtained when LPS and antigen were simultaneously injected. Injection of

LPS 24 hours after administration of antigen resulted in a higher production of IgE and IgG1 but in the loss of the preferential IgE-antibody production. Guinea pig IgE antibody persisted in homologous skin for at least 46 days.

SIGNIFICANCE

The research provides information to better understand the mechanisms that lead gram-negative bacteria to preferentially produce anaphylactic antibodies.

PUBLICATIONS

1. Perini, A. and I. Mota. The production of IgE and IgG1 antibodies in guinea pigs immunized with antigen and bacterial lipopolysaccharides. Immunology 25:297, 1973.

OTHER DATA

Grantee: Dr. Adenir Perini and Dr. Ivan Mota. PAHO/WHO Immunology Research and Training Center, Institute Butantan, São Paulo, Brazil.

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

Timetable: 1972-1973.

THE IMPACT OF MALARIA ON ECONOMIC DEVELOPMENT

PROBLEM

For over a decade interest has been growing in the study and quantification of the interaction between health activities and economic development. This interest has been particularly strong in the area of malaria control. Two main difficulties have been encountered in most efforts to study the problem: first, the near impossibility of disentangling the economic effects of the reduction or elimination of malaria from changes in the economy taking place at the same time and stemming from other causes; and second, the lack of adequate economic information on malaria-ridden rural areas of developing countries.

METHOD

To avoid the difficulties of evaluation mentioned above, PAHO/WHO chose a demonstration area where a longitudinal study of a limited number of individual rural families could be carried out to measure the effects of illness from malaria on their microeconomy.

Eight study areas, each consisting of about 40 families, were chosen in Paraguay and each family was repeatedly visited at approximately 15-day intervals between September 1968 and June 1970. Attack-phase measures of the Paraguayan malaria eradication campaign were first applied in these areas beginning in February of 1969, providing months relatively free of malaria in the latter part of the data-collection period.

Following a family census and collection of other basic information, data were periodically obtained on the health of the family members; any population movement; and farm work, including number of hours or days worked, operations performed, the field and crop in which the work was done, and whether it was carried out by adult or child family members, by paid help, or by neighbors. Other relevant information was also collected.

RESULTS TO DATE

The collection of field data was completed in June 1970. Coding, including preliminary checking, was carried out in Paraguay and was completed in 1972.

Processing of coded information was performed in Argentina at the Center for Utilization of Computers in Health Programs, to produce summarized data for statistical analysis. This phase was also terminated in 1972.

Corrections to, and some further refinement of, the basic processing of farm-work data as well as correction of processing errors in harvest data were carried forward during 1973 at PAHO Headquarters in Washington.

Statistical analysis of harvest data was advanced and first steps taken in the evaluation of the corrected farm-work information. Comparisons made to date indicate that the immediate effect of moderately serious malaria morbidity in the family on some economic indicators may be in a reverse direction from that of more grievous malaria incidence. For example, families moderately affected during a given crop year tend to increase the proportion of their crops sold and reduce the proportion consumed, whereas seriously affected families may do the reverse, and decrease the proportion sold. There are also delayed effects, appearing in the latter part of the study period, which may vary according to the different morbidity levels; e.g. planting decisions concerning quantity of land to bring under cultivation and its allocation between cash and subsistence crops. Thus the effect of malaria is not simple but diverse, and affects the economy of the region in many aspects.

SIGNIFICANCE

These studies are providing basic information on the impact of malaria on economic development in rural areas and may serve as a methodologic model for investigations in other areas.

PUBLICATIONS

1. Conly, G. The impact of malaria on economic development. I Interam Symp Malaria Res, San Salvador, November 1971.

2. -----. The impact of malaria on economic development:
a case study. Am J Trop Med 21:668-74, 1972.

OTHER DATA

Grantee: Dr. Gladys N. Conly, Malaria Eradication Program,
Pan American Health Organization, Asunción,
Paraguay.

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

Timetable: 1968-1974.

RESEARCH ON THE EPIDEMIOLOGY OF MALARIA ERADICATION IN PROBLEM AREAS

PROBLEM

Although physiologic resistance to DDT and dieldrin is the most widely recognized problem obstructing progress in malaria eradication in the Americas, several other problems related to vector behavior, parasite strains, and human and vector ecology have been found and might be major contributors to the slow progress of some eradication programs.

The aim of the project is to evaluate the possibility of interrupting malaria transmission in a problem area with the most promising attack measures, to study in depth the various epidemiologic factors that could have brought about the results observed, and to search for the existence of foci of persistent transmission and study its epidemiology.

The project is a continuation, with a broader scope, of the work of the insecticide testing team that has carried out investigations of various insecticides in Central America since 1959.

METHOD

We considered, on the basis of experience from malaria eradication projects and the insecticide testing team's work in Central America, that the fumigant insecticide Propoxur (OMS-33), which is sprayed on selected surfaces in only part of household interiors on a short cycle, might interrupt malaria transmission in a test area.

An area of about 30,000 inhabitants and 500 km² was chosen on the coastal plain of El Salvador (La Paz Department) for study. Its altitude was less than 100 meters, and its annual rainfall was 1,745 mm.

Sixty grams of insecticide were sprayed on the average house of 25 meters in perimeter, normally as two swaths on the inner side of the roof and the angle between the roof and the wall, on a 35-day cycle. Surfaces known to be alkaline or absorbent, i.e., whitewash, plaster, tiles, asbestos, mud, etc., were avoided, and instead good surfaces, i.e., wood, thatch (except rice thatch), etc., were sprayed.

The effects of the attack measures and the epidemiology of malaria in the area were studied by comparing the trial area with two neighboring areas.

RESULTS TO DATE

Entomologic and epidemiologic evaluations of the trial of selective application of Propoxur were obstructed by the appearance of resistance of the vector, Anopheles albimanus to this insecticide. A new trial with another insecticide, OMS-597 (Landrin) was started in March 1973 in its Stages IV and V.

According to the preliminary results of the evaluation, Landrin seems to be effective against the A. albimanus resistant to Propoxur.

The project has made very important contributions to the knowledge of malaria epidemiology in the following fields:

Human ecology: determining the frequency and timing of house renovations and changes in sprayable surfaces and types and distribution of building materials; studying the migration of temporary laborers and the daily habits of the population in relation to their indoor or outdoor permanence;

Mode insecticide action: duration of effect, influence of meteorological conditions, and methodology of bioassay tests;

Vector behavior: determining vectors' cycles and timing of contact with man and of permanence inside houses;

Vector resistance to insecticides and their variations in relation to ecologic conditions and insecticide pressure, as well as the importance of cross-resistance to various insecticides;

Applicability of serologic diagnosis of malaria to the evaluation of malaria transmission in the experimental area, by longitudinal study of a group of high expected vulnerability.

Environmental factors: demonstrating the importance of man:animal ratio in the interpretation of epidemiologic findings, and the need of quantitative studies on the mechanism of the influencing factor.

SIGNIFICANCE

All these studies are fundamental for the finding of a methodology of attack against malaria refractory to conventional attack measures.

Improvements in the methodology of evaluation and supervision will be of assistance to antimalarial campaigns in general.

PUBLICATIONS

1. Lassen, K. et al. The evaluation of Propoxur (OMS-33) in Central America. I Interam Symp Malaria Res, San Salvador, November 1971.
2. Liu, S. Y. and K. Lassen. Notes on a case of OMS-33 tolerance in Anopheles albimanus in El Salvador, Central America, III Cong Centroam y I Mac Microbiología, Guatemala, July 1971.

OTHER DATA

Grantee:	Dr. Knud Lassen, Malaria Eradication Program, Pan American Health Organization, San Salvador, El Salvador.
Funded by:	Pan American Health Organization/World Health Organization.
Timetable:	1970-1973.

SPOROZOITE-INDUCED IMMUNITY IN MAMMALIAN MALARIA

PROBLEM

Our ongoing studies on sporozoite-induced immunity in simian malaria are an extension of our previous and continuing work on rodent malaria immunization and mechanism of immunity. The investigations of the host response to immunization with simian malaria sporozoite serve a double purpose: (1) to verify if the method which we have successfully used to obtain protective immunity to rodent malaria, i.e., immunization with X-irradiated sporozoites, would also produce satisfactory results in a simian malaria system, and (2) to obtain information about similar vaccination attempts in humans, including data on the immunogenicity and possible cross-reactivity of sporozoites of simian and human malaria.

The need to obtain large numbers of sporozoites that are fully immunogenic has led us to investigate: (1) the antigenic maturation of the sporogonic stages of development of Plasmodium cynomolgi, and (2) the methods of purification of the sporogonic stages of this parasite.

We plan to pursue experimental work on immunization with simian malaria parasites by verifying if Rhesus monkeys immunized with X-irradiated sporozoites of P. cynomolgi become resistant to sporozoite challenge, and by investigating the humoral and cellular manifestations of this immune response. We also plan to initiate in-vitro cultivation of sporogonic stages of Plasmodia in an insect tissue culture system.

METHOD

The immunization of the Rhesus monkey will be done with gradient purified sporozoites injected intravenously and accompanied in some instances by the administration of adjuvants.

The presence and titration of anticircumsporozoite (CSP) and neutralizing antibodies will be determined by previously described techniques. Further studies on the strain and species specificity of various simian and human malaria sporozoites will be based on their immunogenicity for rats and the detection of antisporezoite antibodies.

The cellular immunity of malaria-infected or immunized Rhesus monkeys will be evaluated by the blast transformation rate of their lymphocytes. We will also attempt to verify if a delayed-type hypersensitivity can be detected in these animals.

As for the proposed work on insect tissue culture of Plasmodia, this will concentrate on two main approaches: (a) further purification of various sporogonic stages, and (b) attempts to optimize in-vitro conditions for the maintenance and development of Plasmodia.

RESULTS TO DATE

A. Antigenic maturation of sporozoites of simian malaria.

The aim of these experiments was to determine if sporozoite maturation in any given location was time-dependent, or alternatively, if parasite populations from the midguts ever became infective and antigenically mature before moving to the hemocele, or even to the salivary glands.

All sporozoite populations were analyzed by the following three criteria: (1) their infectivity for Rhesus monkeys; (2) their capacity to induce the formation of CSP antibodies; and (3) their capacity to serve as antigen in the CSP reaction.

It was observed that the sporozoite populations varied considerably in their immunogenicity and infectivity according to their location in the mosquito. A prolonged infection period failed to bring about additional sporozoite maturation, unless this was accompanied by migration of the sporozoites toward the salivary glands.

B. Species specificity of CSP antibodies of simian malaria.^{7/}

Antisera produced against salivary gland sporozoites of a number of different simian malarial species have been tested in the circumsporozoite (CSP) reaction. These sera were tested with sporozoites of different species and strains of simian and human malaria, to detect any possible cross-reactions.

The results we obtained indicate that positive reactions occurred only within the homologous system. Even in those instances in which the simian malaria species were believed to be rather closely related, as in the case of the two "ovale type" parasites P. simiovale and P. fieldi, no cross-reactions were observed.

C. Species specificity and strain cross-reactivity of sporozoites of human malaria.¹⁷

Antisera of different specificities were produced by the intravenous immunization of rats with sporozoites of the various types of human malaria. No cross-reactions were observed between sporozoites of *P. vivax* and *P. falciparum*, or any of several simian malaria species. Sporozoite strains consisting of different geographical isolates reacted as strongly with homologous as with heterologous antisera.

D. Immunogenicity and infectivity of gradient purified sporozoites of mammalian malaria.³⁷

Mass isolation of sporozoites through gradient centrifugation was adapted for use in a rodent malaria (*P. berghei*) and simian malaria (*P. cynomolgi*) system, using a linear Renografin/BSA gradient. This procedure made it possible to rapidly process a large number of infected mosquitoes, leading to the recovery of a considerable proportion of sporozoites. Gradient recovered sporozoites (GRS) freed of most bacteria and mosquito tissue contaminants, retained their infectivity and immunogenicity. Mice repeatedly injected intravenously with irradiated GRS of *P. berghei* acquired total protection against an otherwise lethal sporozoite challenge.

E. Immunization of Rhesus monkeys (*Macaca mulatta*) with γ -irradiated sporozoites of *P. cynomolgi*.

In approximately 30 of the immunized animals we have monitored the formation and level of CSP antibodies, and also, in some instances, the sporozoite neutralizing activity of these immune sera. We found that besides a considerable degree of individual variation, CSP antibodies tend to be relatively slow in developing; rarely are they detectable before 2 months' immunization. Furthermore, during the course of immunization, antibody levels were found to fluctuate considerably between boosters.

Only five animals have so far been challenged; two of these remained unaffected upon challenge with an inoculum that produced patent infections in control animals. It was in these two animals that we observed the "highest" titers of CSP antibodies, and also a sporozoite-neutralizing activity.

There appears to be considerable analogy between our results and those obtained by Clyde et al. (Immunity of men against sporozoite-induced falciparum malaria. Am J Med Sci 266:169-77, 1973) in preliminary human trials.

SIGNIFICANCE

Studies on sporozoite-induced immunity in simian malaria are relevant from two different but closely related viewpoints: (a) to attempt to develop a vaccination procedure against human malaria, and (b) to understand the mechanism of host resistance to malarial infections.

It seems certain that much of the background knowledge necessary for the establishment of a successful vaccine against human malaria will have to be derived from further studies of other mammalian malaria systems. This certainly is true for the simian malarias which unquestionably present the greatest analogy with the human host-parasite relationship.

The follow-up studies on the immunogenicity of different developmental stages of the sporozoites of P. cynomolgi, combined with purification procedures, differential absorption, etc., might hopefully in the future lead to the characterization and purification of protective antigens.

From the point of view of immunization with attenuated sporozoites, the successful cultivation of the sporogonic stages of *Plasmodia* would place the prospects of development of a malarial vaccine within the realm of reality.

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Grantee: Dr. Ruth S. Nussenzweig, New York University
Medical School, New York, New York.

Funded by: Pan American Health Organization, World Health
Organization, National Institutes of Health,
U.S. Public Health Service, U.S. Army Medical
Research and Development Command.

Timetable: 1971 - continuing.

STUDIES ON THE NATURAL HISTORY OF TRYPANOSOMA (SCHIZOTRYPANUM)
CRUZI IN AN ENDEMIC AREA OF CHAGAS' DISEASE IN EASTERN BRAZIL

PROBLEM

The objective of this study is to achieve a better understanding of how, when, and why man becomes involved in the natural cycle of T. cruzi transmission and to identify and study the factors that are important in maintaining T. cruzi infection once established in the domestic milieu. The municipality of São Felipe is a rural endemic area of Chagas' disease in which long-term clinical and epidemiologic investigations have been in progress since 1965 by Brazilian workers, under the direction of Prof. A. Prata, University of Brasília. It was thus chosen as a suitable area for complementary biological studies.

METHOD

In order to study the household epidemiology and peridomestic/sylvatic epizootiology of T. cruzi infection among triatomine bug vectors, domestic and wild animals, some 40 bug-infested houses were selected as the basis for an intensive comparative study. These houses were the homes of subsistence-level peasant farmers. The sole domiciliated triatomine vector, Panstrongylus megistus, infested all the houses chosen and household bug populations were studied, with particular regard to intensity of infestation and feeding behavior, and of infection with T. cruzi. Simultaneously, studies were made of the prevalence of T. cruzi infection in domestic, peridomestic, and wild animals in the vicinity of the same houses and a search was made for evidence of a sylvatic T. cruzi transmission cycle in the area. The results of these interdigitating investigations are being correlated to help build a composite picture of the basic ecology of T. cruzi transmission within and near houses.

RESULTS TO DATE

- a) P. megistus in house: infestation density and bug infection rates

About 3,000 P. megistus were collected from the houses studied. Individual household infestation rates varied considerably and for

comparative purposes, were standardized on a bugs/man/hour basis, termed "Apparent Density". Bugs collected were individually examined for T. cruzi infection. The overall rate of infection was 39 percent but infection rates in different houses varied from zero to about 95 percent. Infection rates increased steadily with increased age; from about 6.5 percent among first instar bugs to about 70 percent of adults infected.

b) The Domestic Risk Factor (DRF)

From the figures for bug densities and infection rates, an attempt was made to quantify the risk of T. cruzi infection among different houses and families. This was termed the Domestic Risk Factor (DRF) and is the product of Apparent Density and Infection Rate (divided by 100 for convenience) for each household. The DRF varied from zero to about 35 in the 39 households for which it was calculated. The DRF is a measure of the current level of trypanosome challenge in each household and is essentially predictive of possible future infection rather than an index of current infections, to which it is only loosely related.

c) The feeding behavior of P. megistus

Blood meal squashes were made from the bugs collected in each house studied and later identified by precipitin tests. Of nearly 1,400 feeds identified, 81 percent were of human origin. Avian feeds (chicken) took second place with 13 percent. Feeds from dogs were 2.5 percent; rodent and cat feeds were extremely uncommon--only 1 of each was found. A number of feeds (2.2 percent) could not be identified further than the general category of "mammal". Most of these were from meals classed as weak reactors, but may have included a proportion of feeds from mammals for which specific antisera were not available. Less than 2 percent of meals contained blood of mixed origin from successive feeds: most of these were avian/man mixtures, fewer were avian/dog, one was man/dog, and one avian/pig. Infection rates of bugs containing blood from different sources showed a marked difference: 38 percent of man-fed bugs were infected compared to 19 percent among those bugs whose most recent feed was from chicken, a host solidly insusceptible to infection with T. cruzi.

d) Focalization of household bug infestations and of infected bugs

The systematic demolition of an old, bug-infested house when the owners moved yielded some 500 P. megistus of all stages. These were found to be in two physically separated subpopulations of roughly equal size. One population of bugs was located in the walls of the sleeping quarters: more than 80 percent of these bugs contained human blood

and approximately 50 percent of the bugs were infected with T. cruzi. (Six of the nine family members were also infected with T. cruzi). A second population of bugs was found in the living room walls, against which some 15 chickens roosted at night; 97 percent of these bugs had fed on chicken and only 3 percent on man. The infection rate among the chicken-fed population was 10 percent.

Study of a house before and after the owners ceased to sleep in it showed that while the family occupied the house, 87 percent of bug feeds were from man and that 30 percent of bugs overall were infected with T. cruzi. When this house was demolished 6 months after it was vacated, 171 P. megistus were recovered from it. About 84 percent of these bugs had fed on the single chicken that continued to roost in the house, 1.5 percent (2 bugs) had fed on cat, 7 percent of feeds were in the general "mammal" category, 7 percent contained blood from guinea pig and 1 bug contained mouse blood. The last groups of feeds were probably derived from the caged mice and guinea pigs kept experimentally in the house for 2 1/2 months before its demolition.

e) Parasitization of *P. megistus* eggs by a microhymenopteran

Telonomus fariai, a little-known but geographically widespread tiny wasp, has been found to occur in the area studied. Parasitized eggs and shells from which parasites have emerged are characteristic in appearance. The mean cumulative rate of parasitism among nearly 3,600 eggshells collected in 23 houses and chicken-houses was 19 percent and ranged from 6 percent to 63 percent in 9 houses from which 100 or more shells were collected. From 7 to 12 wasps emerge from each parasitized egg, usually with one male (sometimes 2) and 6-10 females.

f) Animals and *T. cruzi*

Most households had chickens, pigs, and cats; about half had a horse, donkey, or mule but few kept cattle or goats. Several 1969 surveys in the area by Brazilian workers had shown that 6 percent of dogs were infected with T. cruzi; no infections were found among more than 200 pigs.

Among the households in the present study, 1 of 12 dogs was infected (8 percent), 8 of 23 cats (35 percent). No larger animals were found infected. House-mice were caught in 15 of 28 houses where traps were set; 20 mice of the 92 trapped were infected with T. cruzi (22 percent). Many peridomestic and wild animals were caught in the vicinity of the houses studied; 30 percent of opossums (Didelphis azarae) were infected with T. cruzi and 23 percent of Rattus r. frugivorus. A variety of other animals caught were uninfected. Both

D. azarae and R. r. frugivorous are peridomestic animals that frequently visit houses in search of food.

The course of natural and induced infections of T. cruzi in captive D. azarae and Rattus r. frugivorous were followed in the laboratory: infections were always mild, chronic, and of long duration; parasitemia generally subpatent but positive on xenodiagnosis. Human T. cruzi strains behaved similarly in opossums and in an armadillo, without acute onset. Histopathologic changes were slight or absent and no amastigote tissue parasites were seen.

g) Sylvatic bugs and T. cruzi

Psammolestes tertius was common in bird nests in trees but none were infected with T. cruzi. Two other species of sylvatic triatomines were encountered, however; Rhodnius domesticus and Triatoma tibiamaculata. Both species were associated with nests of opossums and other small mammals in bromeliad tree epiphytes not far from human habitations. Both species of bugs harbored trypanosomes considered to be T. cruzi but none of the opossums or other animals associated with these bugs have so far been found infected. The natural vertebrate host or hosts among which these two bug species circulate T. cruzi is thus still unknown.

SIGNIFICANCE

1) Differences in bug infestation density and T. cruzi infection rates in discrete household bug populations led to the concept of the Domestic Risk Factor as an epidemiologic measure.

2) Eighty percent of domiciliated P. megistus feeds are from man: high rates of T. cruzi infection in both imply that each is the major source of infection for the other and that intense transmission occurs in both directions.

3) Dogs are not very common; although some are infected with T. cruzi, insufficient bugs feed upon them to provide an important contribution of T. cruzi to the domestic transmission cycle.

4) Cats and mice in houses have a high T. cruzi infection rate but are fed upon very rarely by P. megistus. Infection in these animals therefore provides an essentially zero input of trypanosomes into the domestic cycle.

5) Opossums and peridomestic rats are also rarely fed upon by bugs although both are frequently infected with T. cruzi.

6) It seems probable that mice and rodents become infected orally by eating bugs. The same may occur with opossums. Cats probably acquire infection largely by eating infected rodents.

7) Chickens, insusceptible to T. cruzi infection, are an important secondary food source for P. megistus. When roosting inside houses they can support largely separate bug populations with a low T. cruzi infection rate.

8) Eggs of P. megistus are subject to a significant degree of parasitism by a minute wasp, Telonomus fariai.

9) Two species of sylvatic bugs, both infected with T. cruzi, occur in an arboreal habitat close to houses. No links have yet been found between the sylvatic and domestic transmission cycles.

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OTHER DATA

Grantee: Dr. D. M. Minter, jointly with Dr. E. Minter-Goedbloed, Dr. M. A. Miles, and Mr. T. V. Barrett, and formerly also with Dr. P. D. Marsden, London School of Hygiene and Tropical Medicine and Chagas' Disease Research Unit, Salvador, Bahia, Brazil.

Funded by: The Wellcome Trust, London.

Timetable: 1971-1975.

INTERRELATIONSHIP OF TRYPANOSOMA CRUZI INFECTION AND CHRONIC CHAGAS' CARDIOMYOPATHY IN A RURAL POPULATION

PROBLEM

The general objective of this study is to determine the causal relationship of T. cruzi infection to morbidity in a defined rural population. The prevalence of T. cruzi infection will be ascertained by selected indicators of infection (e.g., serologic tests for T. cruzi xenodiagnosis, field blood cultures, and microscopic examination of the blood). These indices will be correlated with indicators of disease (e.g., electrocardiogram, history, physical examination, ECG monitoring, and chest X-ray). Field epidemiologic methods reproducible in other endemic areas will be developed.

METHODS

A rural population of 1,000 individuals located 200 km west of Salvador, Bahia, has been selected as the study population. This area is concurrently a test area for a Malathion insecticide study under the direction of the Nucleo de Pesquisas (INERU-FIOCRUZ). A complete census and mapping of the area defined the population base. Subsequently, the field team completed a base-line clinical study in the entire population (10 ml venous blood specimen, filter paper blood specimen by finger prick, stool specimen, blood pressure determination and 5-lead electrocardiogram with a 30-second lead V_1 rhythm strip). All data has been directly recorded on special computer-oriented code sheet for future data analysis.

RESULTS TO DATE

The basic census has been completed and data transferred to IBM cards (October 1973). The clinical study was completed in separate 2-week periods (December 1973 and February 1974). The teams performed all procedures in the rural area. The serum was separated and transported daily to Salvador by bus where it was frozen at -20°C for serologic studies. A 5-lead ECG (I, II, AVL, V_1 , V_5) with a 30-second lead V_1 rhythm strip reduced the time required for a conventional 12-lead ECG. ECG analysis is in progress using a modified Minnesota code with emphasis on the predominant alterations associated with

Chagas' cardiomyopathy, i.e., conduction disturbances and arrhythmias. Substudies on the importance of physical examination, 24-hour electrocardiographic monitoring, and chest roentgenogram are planned.

SIGNIFICANCE

This study provides information on reproducible methods of field evaluation of Chagas' disease. All data will be analyzed by computers readily available in university centers in Brazil. The prevalence of infection indicators will be correlated with the presence of disease indicators. This rural population may serve as a focus for research on quantification of parasitemia, congenital transmission of T. cruzi, and longitudinal study of the natural history of infection with T. cruzi.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Drs. Kenneth E. Mott, J. Stauffer Lehman, Jr., and Richard H. Morrow, Jr., Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts, in collaboration with Drs. Armenio Costa Guimaraes, José Pericles Esteves, Celso Mario de Araujo Pugliese, and Mrs. Celina Marques Bittencourt, Federal University of Bahia, and with Dr. Italo Sherlock, Instituto Nacional de Endemias Rurais (FIOCRUZ).

Funded by: The Wellcome Trust, Pan American Health Organization, Instituto Nacional de Endemias Rurais (FIOCRUZ), Federal University of Bahia, and Harvard School of Public Health.

Timetable: 1973-1975.

NATURAL HISTORY OF SCHISTOSOMIASIS MANSONI IN A RURAL POPULATION OF NORTHEASTERN BRAZIL

PROBLEM

To date few attempts have been made at measuring the impact of schistosomiasis on defined populations. Our broad objective is the creation of a longitudinal study of schistosomiasis morbidity in a highly endemic area of rural Brazil. Quantitative aspects of infection and disease will be measured and correlated, new hypotheses concerning morbid sequelae will be tested, and field epidemiologic methods reproducible in other endemic areas will be developed.

METHOD

A rural population of 1,000 individuals located 200 km west of Salvador, Bahia, has been surveyed by census, qualitative stool examination, and venous blood sample. A substudy of the prevalence of renal disease in 300 individuals included physical examination, urine analysis, urine culture, quantitative urine protein, and quantitative stool egg count (Bell's method). To be tested are the clinical history, the influence of intercurrent infections (including Chagas' disease), and the significance of the quantitative egg output pattern by age as related to morbidity.

RESULTS TO DATE

Greatly differing ecologic situations within the study area contribute to varying prevalences for Schistosoma mansoni infection among contiguous groups (range 25 to 85 percent). A subpopulation with high egg counts and high frequency of hepatic and splenic enlargement has been identified and studied for evidence of renal disease associated with schistosomiasis. One individual with obvious renal disease (heavy proteinuria, abnormal sediment) was found; quantitative urine protein excretion for the entire group is being measured.

SIGNIFICANCE

This collaborative study has developed field methodology and located a suitable rural population for cross-sectional and longitudinal studies of schistosomiasis morbidity. Epidemiologic, clinical, and parasitologic hypotheses may be tested and the influence of multiple variables on schistosomiasis morbidity analyzed by computers within Brazil.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Drs. J. Stauffer Lehman, Jr., Kenneth E. Mott, and Richard H. Morrow, Jr., Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts, in collaboration with the Department of Preventive Medicine, Federal University of Bahia, and the Instituto Nacional de Endemias Rurais (FIOCRUZ).
Funded by:	The Wellcome Trust, Pan American Health Organization, Instituto Nacional de Endemias Rurais (FIOCRUZ), Federal University of Bahia, and Harvard School of Public Health.
Timetable:	1973-1975.

SURVEILLANCE AND RESEARCH ON INFECTIOUS DISEASES ALONG THE TRANS-AMAZON HIGHWAY

PROBLEM

The opening of the Trans-Amazon Highway presented an opportunity to carry out research on the incidence of infectious diseases in a vast area of virgin forest. The highway construction force and settlers are exposed to a variety of ecologic environments, and will almost certainly come into contact with certain zoonotic agents such as arboviruses and Leishmania. In addition, about 80 percent of the workers come from other parts of the country, which creates the possibility that other infectious diseases, e.g., schistosomiasis and their vectors may be introduced into areas of the Amazon valley where they have been previously unknown. The main purposes of this research are to study infectious diseases enzootic in the Highway area that may be of importance to man as well as those that are extraneous to the area but that can be introduced by man.

METHOD

A field team is collecting clinical material from people and trapping animals and arthropods in the Highway area.

Virology. Sera from humans, wild mammals, and birds are tested for Amazon arbovirus groups by hemagglutination-inhibition test. Neutralization tests in mice and tissue culture are made in some cases. Attempts are made to isolate virus in mice and tissue culture using blood from sick persons and wild animals. Hematophagous arthropods are identified, grouped into species pools of approximately 50 individuals, and inoculated into mice. Clinical specimens from sick persons and their contacts are also collected for serologic analysis and attempts to isolate other viruses.

Parasitology. The population is being examined for cases of leishmaniasis, and Leishmania is isolated by inoculation of biopsied material into hamsters. Wild animals are also examined for Leishmania by culturing techniques and hamster inoculation, which also screen Trypanosoma cruzi infections. Sand flies are trapped, using humans and other animals as bait, and samples dissected to pinpoint local vectors of Leishmania. Blood films are examined in suspected cases of malaria, and fecal samples for helminths and protozoa. Searches are continuing for foci of snails that may act as intermediary hosts for Schistosoma.

Bacteriology. Fecal samples from humans are brought to Belém in Stuart's medium and cultured in EMB, SS agar, bismuth sulfite agar, and tetrathionate broth (Kauffmann). After biochemical identification and the maximal serologic analysis possible, strains are sent to an International Reference Center. Material from wild animals is taken from the small and large intestine and examined by the methods described above. Sera of animals are examined for agglutinins against Leptospira by micro-serum-agglutination, and urine cultures are made in Fletcher's medium for isolation of Leptospira.

RESULTS TO DATE

Nineteen strains of arboviruses were isolated from wild animals and mosquitoes. Most isolates--four strains of SLE, three of Turlock, one of Murutucu, and one of An 141106 viruses--came from birds. Two isolates of Phlebotomus group viruses, one (Candiru complex) from a marsupial and the other one (Icoaracy virus) from a rodent, and an untyped strain (An 238758) from Oryzomys sp. were obtained. Three isolates as yet untyped (An 227841, An 228950, and An 238209) were obtained from frogs. In addition, one strain each of the Wyeomyia complex, SLE, Una, and Irituia viruses were isolated from mosquitoes. No isolations were made from humans but 74 serologic conversions to arboviruses were detected by hemagglutination inhibition among 474 colonists with paired sera (January-October 1973). The conversions to the following viruses were detected: Mayaro (12), YF (28 probably caused by the vaccine), Ilheus (2), SLE (2), broad-group B (21), Guaroa (2), Itaporanga (3), Oropouche (1), Catu (2), and Candiru (1). High antibody rates to certain arboviruses have been found among birds (WEE, SLE, and Turlock viruses) and primates (Mayaro, YF, Oropouche, and Phlebotomus viruses). Rodents usually showed high antibody rates only to the Phlebotomus group viruses. In general, high levels of antibodies to groups A, B, and Piriy viruses but low levels to the groups C, Guama, Bunyamwera, Simbu, Phlebotomus, and Anopheles viruses are found among residents. Immunity rate to these viruses is usually low or absent among the immigrants.

A hemorrhagic syndrome has been observed among the colonists of the Altamira area. The illness was first noted in January 1972. To date there have been 92 cases of the disease and 3 deaths. The illness was diagnosed as thrombocytopenic purpura. Familial clustering of cases was found, children being more affected than adults. Epidemiologic and serologic evidence suggests that the syndrome is associated with the presence of the black fly, Simulium, and its bite.

On the parasitologic field, Leishmania mexicana amazonensis was isolated from the skin of 26 of 166 spiny rats (Proechimys guyannensis),

7 of 64 rice rats (Oryzomys capito), 1 of 16 semiaquatic rats (Nectomys squanipes), and 1 of 4 brown opossums (Metachirus nudicaudatus). Parasites resembling Leishmania braziliensis were isolated from the viscera of a single Proechimys guyannensis. Two apparently new species of Leishmania are recorded in the opossum Didelphis marsupialis and the sloth Choloepus didactylus. Two field workers acquired L. b. braziliensis infections during their work along the Trans-Amazon Highway. A number of hemogregarine and piroplasm infections were noted in marsupials (Didelphis marsupialis). Large numbers of phlebotomine sand flies were trapped, using human and animal bait, in attempts to pinpoint local vectors of leishmaniasis. Some new species were encountered and one has been described. Six species of sand flies have been bred in the laboratory for study of immature and mature stages. Other captures were made from day-time resting sites of sand flies on tree trunks and in animal burrows. Search of foci of planorbid snails along the road section between Itaituba and the Anapu river uncovered three foci of Biomphalaria straminea (two in the Altamira and one in the Itaituba counties) and four of Drepanotrema anatinum.

Bacteriologic examination of fecal samples from 1,584 captured animals led to four isolations of Salmonella from mammals; two of them were of the sero-group D, and two of the sero-group C₂. One strain of Arizona was cultured from a specimen of Ameiva (Sauria: Teiidae). Three strains of Salmonella (two sero-group B and one sero-group C₂) and 3 of Shigella (two Sh. dysenteriae and one Sh. flexneri) were isolated from culture of fecal specimens from 448 people living in the study area. Serologic examination of sera for antibodies against Leptospira gave significant titers in 77 of 621 humans and in 20 of 317 animals studied (rodents and marsupials). Attempts were made to isolate Leptospira by inoculating pieces of the renal cortex from 399 animals into Fletcher's medium. Leptospira spp. was isolated in this manner from four Proechimys and two Didelphis.

SIGNIFICANCE

These studies should give information on the following points: (1) diseases and their causative agents introduced by the Highway construction force and colonists coming from other parts of Brazil, and (2) local diseases and infectious agents of the Amazon area that may affect the immigrants.

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Grantee: Dr. M. Martins da Silva (Project Coordinator),
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Evandro Chagas, Fundação Instituto Oswaldo Cruz,
Belém, Pará, Brazil.

Funded by: Pan American Health Organization and the U.S.
Army Medical Research and Development Command.

Timetable: 1971-1975.

SPHERULIN, A NEW DIAGNOSTIC REAGENT IN COCCIDIOIDOMYCOSIS

PROBLEM

Coccidioidomycosis is a pulmonary fungal disease that is widespread throughout the Americas. An estimated total of 55,000 new symptomatic infections occur yearly. But diagnosis is very difficult and the actual number of symptomatic cases may exceed the figure above many times. In numerous instances the course and pathologic features of coccidioidomycosis parallel tuberculosis, and many documented cases of the former have been initially misdiagnosed as the latter.

A most important means for detecting a subject with Coccidioides immitis is the indurated delayed cutaneous response that develops when coccidioidin is administered intradermally. Coccidioidin, which is derived from the mycelial or saprophytic phase of Coccidioides, also plays a prime role in the diagnosis and even in the prognosis of coccidioidal disease. We have previously shown that spherulin, a derivative of the spherule or parasitic growth phase, was a skin-test reagent superior to coccidioidin in mice and guinea pigs. Its efficacy in man was studied in a region of Mexico endemic for coccidioidomycosis.

In the Mexican study (Levine, Gonzalez Ochoa, and Ten Eyck, 1973), we compared the standard dose of coccidioidin (1:100) with a 2.8 μ g dose of spherulin. Spherulin was more efficacious in eliciting delayed dermal hypersensitivity and detected 33 percent more reactors than did coccidioidin. The reactions appeared to be related to an experience with Coccidioides immitis because neither reagent elicited reactions among subjects from nonendemic regions.

The above study did not consider the strong doses of coccidioidin (1:10) or of spherulin (28 μ g). At the higher doses, the reagents could conceivably be comparable. A trial was accordingly carried out in Livingston, California, a zone of coccidioidal endemicity in the San Joaquin Valley, to ascertain the reactivity of both reagents at high concentrations.

METHOD

Spherulin from C. immitis strain 46 was prepared according to previously published methods (Levine, Cobb, and Sclarone, 1969).

Lot 64D4 coccidioidin was tested in the 1:100 and 1:10 dilutions (corresponding to approximately 26 µg and 260 µg, dry wt.), and 2.8 µg and 28 µg (dry wt.) of spherulin were used. Potential cross-reactivity was assessed in selected individuals with histoplasmin (Parke, Davis & Company, Detroit).

Two hundred and ninety-eight volunteers, residing in or near Livingston, California, an area endemic for *C. immitis*, made up the study population. Of these individuals 236 (79 percent) were healthy adult employees of a local factory, who ranged from 18 to 61 years of age. Sixty-two 8-87 year old residents who responded to an appeal for volunteers were also tested at a local community health center. Informed consent in writing was obtained from all subjects.

All volunteers received 0.1 ml of coccidioidin (1:100) in the left forearm flexor surface and 2.8 µg of spherulin in the right. The reactions were recorded at 24 and 48 hours, and expressed as the mean of the longest and shortest axes of the indurated area. Only induration 5 x 5 mm or larger was scored as positive. Subjects with negative reactions to both reagents at 24 and 48 hours were then retested with 10-fold stronger dilutions of each antigen. These tests were also examined at 24 and 48 hours.

As will be described later, a small proportion of subjects were positive to 2.8 µg spherulin and negative to 1:100 coccidioidin. These subjects were retested, 6 weeks later, with 1:100 coccidioidin as well as with 1:10 coccidioidin and histoplasmin. In addition, reactors to only 28 µg of spherulin or 1:10 coccidioidin or both were tested with histoplasmin.

RESULTS TO DATE

Comparison of spherulin and coccidioidin. Two hundred and ninety-eight subjects were completely tested with 2.8 µg spherulin and 1:100 coccidioidin. Forty-nine (16.4 percent) of this group were positive to spherulin and 37 (12.4 percent) to coccidioidin. All subjects who reacted to the latter reagent reacted to the former, but 12 subjects reactive to spherulin were nonreactive to coccidioidin at these dilutions.

Of 207 individuals nonreactive to 1:100 coccidioidin and to 2.8 µg spherulin, 10 (4.8 percent) were positive to 28 µg spherulin and 5 (2.4 percent) to 1:10 coccidioidin. Four individuals reacted to both 1:10 coccidioidin and 28 µg spherulin; 6 were positive to the spherulin only and one to coccidioidin 1:10 only.

Comparison of reaction sizes with spherulin and coccidioidin.

In 41 of 49 subjects, the mean diameter of induration to 2.8 µg spherulin was somewhat larger than that of 1:100 coccidioidin. Only one severe reaction was seen. This was in a 32-year-old woman in good health who developed a 70 x 90 mm-induration reaction to 2.8 µg spherulin and a 60 x 90 mm reaction to 1:100 coccidioidin. There was vesiculation and pain at both sites, but no systemic symptoms.

Reaction ratios as a function of duration of residence. There was an increasing ratio of reactors to 2.8 µg spherulin and 1:100 coccidioidin with increasing duration of residency in the San Joaquin Valley. The greatest increment of change was seen in the 1 to 5 year-residency group (15 percent compared to 4 percent among residents of less than 1 year). After 5 years, further increase was small (4 percent). Some reactions presumably represent sensitivity acquired elsewhere; many subjects came from or visited other areas endemic for C. immitis.

Considerations of cross-reactivity. Ten of 12 subjects who reacted to 2.8 µg spherulin but not to 1:100 coccidioidin were retested with the latter reagent as well as with 1:10 coccidioidin and histoplasmin 6 weeks later. All but one reacted at that time to both dilutions of coccidioidin. Two were also reactive to histoplasmin. Both came from areas of histoplasma endemicity. Two other histoplasmin reactors occurred among eight individuals who were reactive to 2.8 µg spherulin and/or 1:10 coccidioidin. One was from an area endemic for histoplasmosis. Data on the other are unavailable.

SIGNIFICANCE

The present study shows the efficacy of spherulin in detecting sensitivity to C. immitis for epidemiologic purposes. A 2.8 µg dose detected 33 percent more reactors than did 1:100 coccidioidin, and adverse local reactions to both reagents were seen in only one individual. At 10-fold higher concentrations, 28 µg spherulin detected twice as many reactors as did 1:10 coccidioidin among those subjects negative to both initial dilutions. Moreover, the size of the reaction to 2.8 µg spherulin was somewhat larger than that produced by 1:100 coccidioidin, which suggests that spherulin would facilitate detecting weak or borderline reactors.

The reexamination of 10 of 12 subjects in the present study, who were positive to 2.8 µg spherulin but negative to 1:100 coccidioidin showed that such spherulin reactions apparently reflected previous coccidioidal experiences: First, all but one manifested reactivity to both 1:100 and 1:10 coccidioidin when retested 6 weeks later. Previous reaction to 2.8 µg spherulin appears to have boosted the latent sensitivity. This effect has also been described for coccidioidin; strong

doses stimulated responsiveness to the 1:100 dilution. Like coccidioidin, spherulin does not sensitize animals or man in the absence of a previous coccidioidal experience.

A 70-year-old, long-time resident of San Joaquin Valley who had a history of documented coccidioidomycosis volunteered for testing in this study; he proved to be sensitive to 2.8 µg of spherulin but did not respond to either 1:100 or 1:10 coccidioidin.

The strong coccidioidin dose (1:10) is considerably less specific than the 1:100 dilution, especially in subjects with previous histoplasma experiences. To determine if the strong spherulin dose had a similar cross-reacting potential, 7 of 10 subjects who were positive to 28 µg spherulin were tested with histoplasmin. Two were histoplasmin-positive but in these individuals the possibility of dual fungal experiences could not be ruled out. The reactions to 28 µg of spherulin in the other five were clearly not caused by an experience with H. capsulatum. We anticipate investigating further the specificity of spherulin in a large population sample residing in an exclusively H. capsulatum-endemic area. Clinical studies are also in progress to determine the efficacy of this reagent in patients with active coccidioidomycosis. In chronic pulmonary cavitary disease, acute pulmonary disease, and disseminated disease, coccidioidin is frequently non-reactive.

PUBLICATIONS

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2. ----- et al. Dermal sensitivity to Coccidioides immitis. Am Rev Resp Dis 107:379-86, 1973.
3. Stevens, D. et al. Dermal sensitivity to different doses of spherulin and coccidioidin. Chest, 1974, in press.

OTHER DATA

Grantee: Dr. Hillel B. Levine, University of
California, Oakland, California.

Funded by: Pan American Health Organization.

Timetable: 1973.

DETERMINATION OF SERUM ANTIBODIES TO ASPERGILLUS IN THE MARACAIBO POPULATION

PROBLEM

The primary purpose of this study is to complete the research started by our Mycology Section on the high concentration of Aspergillus sp. in the atmospheric air of Maracaibo, and to determine its possible relationship to respiratory problems observed in that area. Results are compared to those obtained with healthy subjects.

METHOD

A team of mycologists and immunologists from the University of Zulia's Microbiology and Tropical Medicine Department, will obtain serum, sputum, and bronchial washings from patients attending the outpatient clinic of a general hospital and from hospitalized subjects.

Specimens will be cultured for Aspergillus and serologic studies--immunodiffusion, precipitin, complement fixation, and indirect fluorescent antibody tests, and immunoelectrophoretic analysis--as well as skin tests will be conducted to investigate the incidence of Aspergillus antibodies.

A suitable control population will also be studied.

Antigen from Aspergillus will be prepared by freeze-thawing procedures and commercial antigens and antigens provided by the U.S. Center for Disease Control in Atlanta will be used as controls.

RESULTS TO DATE

Previous studies in Maracaibo have revealed two important findings:

1. In a survey of atmospheric fungi made by Casas Rincon,^{2,3/} Aspergillus sp. were found to be the commonest molds in the Maracaibo atmosphere totaling 44 percent of all the colonies counted in the survey.

2. Serrano^{4/} found that 71 of 122 patients (58 percent) who had shown positive skin test reactions to mold antigens had skin-sensitizing antibodies to Aspergillus. These 122 patients represented 40 percent of

305 patients studied because of respiratory allergies of different kinds (rhinitis, bronchitis, or bronchial asthma).

SIGNIFICANCE

In Denver, Colorado, Bardana et al.^{1/} found an almost universal presence of serum antibodies to *Aspergillus* among healthy persons patients with collagen diseases, and patients with pulmonary aspergillosis. The authors interpreted the findings as a result of exposure to antigenic determinants shared by *Aspergillus* and other microorganisms ubiquitous in the environment and also as a reflection of the ubiquity of *Aspergillus* spores in the atmosphere, soil, and other organic material.

The full clinical significance of these antibodies in relation to the high incidence of respiratory allergies observed in the Maracaibo area deserves further study.

We would therefore like to: (a) determine whether we can find in our population the same universal antibody response as that of Bardana et al., (b) try to correlate the presence of precipitating antibodies and skin-sensitizing antibodies to clinical findings from patients consulting for respiratory problems, and to the presence or absence of true bronchopulmonary aspergillosis.

PUBLICATIONS

1. Bardana, E. J. et al. The primary interaction of antibody to components of aspergilli. II. Antibodies in sera from normal persons and from patients with aspergillosis. J Allergy Clin Immunol 50:222-34, 1972.
2. Casas Rincón, G. and H. Méndez Romero. Estudio de los hongos atmosféricos de la ciudad de Maracaibo (Study on the atmospheric fungi of the city of Maracaibo). Kasmera 3:89-109, 1969.
3. -----. Contribución al estudio de *Aspergillus* y Aspergillosis (Contribution to the study of *Aspergillus* and Aspergillosis). Kasmera 4:107-83, 1972.
4. Serrano, H. Aeroalergenos y alergias respiratorias en Maracaibo (Aeroallergens and respiratory allergies in Maracaibo). Kasmera 4:107-270, 1973.

OTHER DATA

Grantee: Dr. Guillermo Casas Rincón, Mycology Section of the Microbiology and Tropical Medicine Department, University of Zulia School of Medicine, Maracaibo, Venezuela.

Funded by: Pan American Health Organization.

Timetable: 1974-1976.

A STUDY ON THE CAUSAL AGENTS OF GRANULOMAS AND MYCETOMAS WHICH FORM GREY COLONIES

PROBLEM

The purpose of this study is to identify which and how many fungi among the grey species, are capable of producing granulomatous and mycetomatous lesions in man.

A mycologist who isolates a grey cottonous mold from a black-grain mycetoma, for example, is today almost completely lacking in guidelines to identify it. A rational basis for treatment and for specific identification of the parasite needs thus to be created or consolidated.

The causes of uncertainty and confusion come principally from the existence of two skin species: Madurella grisea (Mackinnon, Ferrada, and Montemayor, 1949) and Pyrenochaeta romeroi (Borelli, 1959). In the opinion of the grantee, other species, producing both mycetomas and granulomas, do exist, however, and must be differentiated from those mentioned above.

METHOD

All available strains of grey molds isolated from human granulomas and mycetomas, are gathered and compared as to their morphologic properties in vivo and in vitro and to their pathogenicity. Special attention is given to the grain structure in vivo and to thermophilic characteristics in vitro. Inoculations into mice and other laboratory animal species are performed.

RESULTS TO DATE

None thus far.

SIGNIFICANCE

Madurella grisea has been isolated from mycetomas in all continents, except Australia; it is the main producer of black-grain mycetomas in the Americas. Pyrenochaeta romeroi has been reported in America, Africa, and Asia. Mycetomas are severe progressive diseases, for which chemotherapy is as yet almost ineffective. Among the grey molds there are also opportunistic infectants, whose frequency is unhappily mounting owing to the introduction and wide-spreading use of antibiotic and immunosuppressive drugs. This study may lead to an easier diagnosis through a clearer identification of pathogens.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Dr. Dante Borelli, Division of Medical Mycology, Institute of Tropical Medicine, Caracas, Venezuela.
Funded by:	Pan American Health Organization, the U.S. Atomic Energy Commission, and the National Science Foundation.
Timetable:	1974-1975.

STUDY OF CROSS-REACTIVITY AMONG FUNGAL SKIN-TEST ANTIGENS

PROBLEM

The primary purpose of this study is to determine the degree of cross-reactivity that may be expected among fungal skin-test antigens derived from Paracoccidioides brasiliensis, Histoplasma capsulatum, and Sporotrichum schenckii in endemic zones of Central and South America, where the respective homologous diseases are prevalent. A secondary objective is to determine the incidence of specific reactivity to each antigen in the different geographic areas included in the study.

METHOD

A large sample of the normal population, comprising all age groups, will be simultaneously skin-tested with three antigenic fractions derived respectively from Paracoccidioides brasiliensis, Histoplasma capsulatum, and Sporotrichum schenckii. The fractions are to be selected on the basis of sensitivity studies previously carried out in patients with active fungal disease. The testing will be done by various collaborating investigators working and domiciled in selected geographic areas of the following countries: Brazil, Colombia, Costa Rica, El Salvador, Guatemala, Panama, and Venezuela. Testing was actually begun in November 1973 and will continue for at least 1 year, or until an adequate and comparable number of individuals have been tested in each geographic area. The data will then be analyzed to determine the degree of cross-reactivity, if any, among the three antigens utilized in the study.

RESULTS TO DATE

None thus far.

SIGNIFICANCE

To assess the true public health importance of mycotic diseases in Central and South America it is necessary first to ascertain the

prevalence and distribution of each disease. This will entail the mapping of endemic areas by means of skin-test surveys. For meaningful interpretations, it is essential that the degree of specificity of the antigens employed in such surveys be known, since an antigen that cross-reacts widely with other fungal diseases prevalent in the surveyed area would have very limited usefulness.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. John D. Schneidau, Jr., Department of
Microbiology and Immunology, Tulane University
School of Medicine, New Orleans, Louisiana.

Funded by: Pan American Health Organization.

Timetable: 1973-1975.

CONTRIBUTION TO THE STUDY OF THE ARMADILLO MODEL

PROBLEM

The main purpose of this study is to experimentally reproduce leprosy lesions with possible development of systemic invasion. If this happens, large numbers of Mycobacterium leprae will be grown (1) to prepare lepromin for epidemiologic and control studies of leprosy; (2) to prepare various types of antigens for immunology, histochemistry, biochemistry, and microbiology research; and (3) to supply material for cultivation attempts.

METHOD

Dasypus sabanicola armadillos are inoculated intradermally and intravenously with various concentrations of M. leprae obtained from untreated lepromatous leprosy patients. The animals are examined every 2 months for macroscopic lesions, with special emphasis on the inoculation sites; a bacteriologic examination of the nasal mucous tissue is also done.

Armadillo lesions produced by the inoculation of M. leprae are photographed in color, then biopsied; the sections are treated with Fite-Faraco and hematoxin-eosin stains. Microphotographs of the stained sections are taken to have a record of the type of granuloma produced.

Fresh smears taken from the biopsy before it is placed in fixative fluid, are treated with pyridine, which we use for identifying M. leprae.

RESULTS TO DATE

Eight months after inoculation of M. leprae, four of seven animals which received the same inocula, started to have lesions at the inoculation site. After that, other animals inoculated with material from different patients, also developed lesions. The lesions are variable in size, ranging from 3-4 mm to 25-30 mm in diameter.

Histopathologic study of these lesions uncovered a macrophagic granuloma whose cells were filled with acid-fast bacteria. The test done with pyridine showed that these bacteria have the same characteristics as M. leprae.

SIGNIFICANCE

This study will permit the production of large numbers of Mycobacterium leprae needed to develop epidemiologic and control programs in leprosy. It will also permit research in various related fields for which a sufficient amount of M. leprae is now lacking for use as antigen or for cultivation and inoculation attempts. Since M. leprae have not up to now been cultured in vitro, we must bear in mind that the only source of leprosy bacilli is the lepromatous patient. This means that only very limited amounts of M. leprae can be obtained.

PUBLICATIONS

1. Convit, J. and M. E. Pinardi. Confirmation of leprosy in the armadillo. Accepted for publication in Science.

OTHER DATA

Grantee: Dr. Jacinto Convit, PAHO International Center for Training and Research in Leprosy and Related Diseases, Caracas, Venezuela.

Funded by: Pan American Health Organization.

Timetable: 1973-1978.

MYCOBACTERIA AND POOR HOUSING IN LEPROGENOUS FOCI

PROBLEM

Leprosy, despite efforts made in many countries to decrease or eradicate it in the world, is still a serious public health problem.

Many publications have noted the problem of poor housing in leprogenous foci, and we have had the opportunity to observe that the prevalence of leprosy tends to decline because of modifications in the conditions of dwelling and a decrease in arthropoda which once abounded in houses. Nevertheless, new cases continue to appear.

Because of this persistence of new cases, it is important to continue ecologic studies in leprogenous foci to compare such places to those where the prevalence of the disease is nil or very low, in order to confirm our hypothesis of the relationship between leprosy and bad housing in special climatologic conditions.

The above problems are closely related to the continuation of studies on the in-vitro culture of Mycobacterium leprae and M. leprae murium and efforts to obtain experimental leprosy in hamsters and armadillos. These aspects are part of this project, which has been divided as follows:

- a) Ecologic aspects of poor housing in leprogenous foci.
- b) Inoculation of experimental animals.
- c) M. leprae and M. leprae murium culture trials.

METHOD

- a) Ecologic aspects of bad housing in leprogenous foci

The material used was obtained by sweeping the rooms superficially, looking directly for arthropoda or organic residues. Cultures are made from this material in order to determine the presence of fungi, green algae, or bacteria. Material is also obtained from areas where the prevalence of leprosy is very low or nil to compare such places to leprogenous foci. In addition, a study of the examined rooms' characteristics is carried out in both areas.

b) Inoculation of hamsters and armadillos

Hamsters were inoculated with BCG or any other autoclaved mycobacteria, simultaneously with, or 10 days or 1 month after, intraperitoneal administration of M. leprae and M. leprae murium. After 4 to 6 months the animals are necropsied, lesions are observed, and cultures and new serial passages are made.

Armadillos were inoculated subcutaneously, intramuscularly, and orally.

c) M. leprae and M. leprae murium culture trials

For the first trials, we started with lepromas and mucus from untreated patients; for the second ones, we used the Pasteur murine leprosy strain which we have maintained in mice since 1954. They are processed under different temperatures, conditions, and culture media.

The author has published several works on the subject before this project.

RESULTS TO DATE

a) Ecologic aspects of bad housing in leprogenous foci

Colombia's main leprogenous foci are mostly in cretaceous areas. The types of vegetation did not cause any difference in the findings, except in dry and very humid tropical areas. The material obtained in both study areas--leprogenous and of low leprosy prevalence--does not show great differences in the presence of common fungi but there is a predominance of candida and yeast-like molds in the first area.

In leprogenous areas, there is a significant amount of enterobacteriae (Klebsiella and Proteus) in the dirt sample cultures obtained from leprosy patients' rooms, which does not appear in the low-leprosy areas.

This calls our attention to the fact that much Proteus was found in nasal mucus samples taken from patients in the area studied; the Proteus from there, isolated in pure culture, agglutinates with leprosy but not with healthy sera.

The presence of arthropoda such as Acarinae (mites), flea larva, and Isopoda is greater in leprogenous than in nonleprogenous areas. It was possible to obtain pure, fast-growing mycobacteria from Isopoda in 100 percent of cases.

There was a predominance of green algae in the study area encompassing San Gil, Mogotes, Socorro, and Charatá, and there is a great quantity of mycobacteria in the bacilloscopies of the floor-hydrated sweepings. The cultures for mycobacteria are under observation.

It is important to note that the prevalence of leprosy has decreased in leprogenous areas. This may have been caused by the modification of floors, the employment of shoes and insecticides, and the use of sulfones in the treatment of ambulatory patients.

b) Inoculation of hamsters and armadillos

Hamsters that received BCG or any other autoclaved mycobacteria before, during, or after M. leprae and M. leprae murium inoculations suffered a constant generalized granulomatosis in the third or fourth month of the observation period, which could be serially passaged.

Positive results with mycobacteria from these granulomatous lesions may be obtained only in hamsters and via all inoculation routes. Likewise, it has been possible to obtain granulomatosis with mashed arthropoda such as lice, Reduviidae (Pitos) and fleas fed to sick animals.

The Armadillos Dasypus sabanicola and Dasypus novemcinctus were used. They were inoculated subcutaneously, intramuscularly, intraperitoneally, and orally. Unfortunately, we were unable to observe them long enough because of Bogotá's altitude (2,671 m).

Since the animals died before they could be observed for any length of time, it was decided to culture them for environmental mycobacteria. Ambient mycobacteria could be isolated from 82.4 percent of the mesenteric ganglia of the 35 animals studied. This work is described in an as yet unpublished paper, "Ambient Mycobacteria in Colombian Armadillos."

c) M. leprae and M. leprae murium culture trials

It has not so far been possible to cultivate indisputably either of the two mycobacteria mentioned above. Nevertheless, the author was able some time ago to obtain cultures from human as well as murine leprosy material which did not have a pathogenic effect on animals and which he considered as saprophytic adaptation phases and not as concomitants.

The author is now able to constantly obtain mycobacteria cultures from hamster granulomatosis produced by M. leprae and M. leprae murium inoculations. These mycobacteria are pathogenic only to hamsters and produce in them a generalized granulomatosis that can be passaged serially. The same mycobacteria are obtained from later passages.

SIGNIFICANCE

Since the information so far obtained seems to confirm our hypothesis that there are real relationships among certain geologic, ecologic, socioeconomic, and epidemiologic conditions in leprogenous foci, the investigations carried out in this project are important because they may eventually explain why leprogenous foci form in certain areas of a country and disappear in others, as was the case in northern Europe, even before the advent of the sulfones.

PUBLICATIONS

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2. ----- . Posibles fenómenos de transformación en micobacterias (Nota Previa). Rev Invest Salud Púb (Mexico), vol. 30.
3. ----- . Notes on the transmission of M. leprae to laboratory animals. VII Iberolatinamerican Dermatology Congress, Caracas, 12-16 December 1971, unpublished document.
4. ----- . Ambient Mycobacteria in Colombian Armadillos. Leprosy Subgroup, National Institute for Special Health Programs, Bogotá, 1973, unpublished document.
5. ----- . Granulomatosis of hamsters inoculated with M. leprae and M. leprae murium, in preparation.

OTHER DATA

Grantee: Dr. Guillermo Muñoz Rivas, Head of the Leprosy Subgroup, National Institute for Special Health Programs, Bogotá, Colombia.

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

Timetable: 1970-1975.

EXPERIMENTAL INFECTION OF THE ARMADILLO WITH MYCOBACTERIUM ULCERANS

PROBLEM

The primary objective of this project is to determine if the nine-banded armadillo (Dasypus novemcinctus, L.) is an ideal animal model for studying the clinical and histopathologic development of Mycobacterium ulcerans infection. The long-range secondary objectives are (1) to determine in Central and South America, the prevalence of M. ulcerans infection, (2) to define and purify the toxic principle of M. ulcerans following which we will try to develop an antitoxin for treatment and prophylaxis, (3) to determine risk factors as have been partially defined in Africa and New Guinea (i.e. increased susceptibility of riverine people, migrant people, and people living in thatched dwellings on or near poorly drained land), and (4) to develop better techniques for screening exposed subjects resulting in earlier treatment essential to preventing the late sequelae of contraction deformity, lymphedema, and amputation.

METHOD

Armadillos, already adapted to laboratory life, have been inoculated at the Gulf South Research Laboratory, New Iberia, LA. These were inoculated at eight different sites on the skin of the abdomen. Starting at the end of 4 weeks postinoculation and continuing every 2 weeks thereafter, a complete inoculation site was removed surgically for histologic and histochemical examination. If ulcers developed, at least one was left untouched to determine the ultimate and natural course of the infection in the armadillo. These serially excised specimens, taken before ulceration and contamination with surface organisms, reveal the spread of M. ulcerans and its effect on the adjacent tissues. When these studies are completed and a thorough knowledge of the infection gained, we will design studies to determine: (1) optimum therapeutic treatment, and (2) practical prophylactic measures. These experiments would include the development and testing of antitoxin and antibodies against M. ulcerans. Blood and serum will be collected with biopsies to determine the armadillos' immunologic response as reflected by cellular and serum-protein profiles during the course of the infection. Lymphoid tissues will be taken and studied to determine the armadillos' cell-mediated response during infection. Histochemical and electron

microscopy studies will also be done along with routine bacteriologic studies.

RESULTS TO DATE

Two of the first four animals inoculated intradermally on the medial side of both thighs developed ulcers at the site of inoculation within 9 weeks. The ulcers in the infected animals were very similar in extent and appearance to naturally occurring human infections. In both, there is extensive necrosis, undermining of the margins of the lesions, and a spreading contiguous necrosis involving full thickness of skin. Fifteen additional armadillos have been inoculated on the abdomen and on the thighs. The infections that are developing appear similar to those obtained with the first group of animals.

Mycobacterium ulcerans in vitro produces an exotoxin that, when inoculated into guinea pig skin, causes inflammation and necrosis and is probably an important factor in the pathogenesis of human infections. The changes in the guinea pig skin resemble those seen in naturally occurring human infections. This toxin is in the culture filtrate, in the cytoplasmic fluid, and in the particulate fraction of the bacilli. It is heat stable and retains activity following ether extraction. These features of the toxin, together with its absence from the cell wall, indicate that it is an exotoxin. Furthermore, the inflammatory lesion it provokes and its physical and chemical properties are also characteristic of an exotoxin. Studies to elucidate its antigenicity and composition are in progress.

SIGNIFICANCE

This research will, (1) create an awareness of this disease in the Americas and lead to a determination of the scope of this infection through more accurate and complete case findings, and (2) in endemic areas in the Americas or elsewhere, lead to a more thorough understanding of the disease and thence to better control, better preventive measures, and to earlier and more effective treatment.

PUBLICATIONS

1. Krieg, R. et al. Toxin of Mycobacterium ulcerans. Production and effects in guinea pig skin. Submitted to Arch Pathol, December 1973.

2. Walsh, G. et al. Mycobacterium ulcerans infection of armadillos (Dasypus novemcinctus, L.). Submitted January 1974 for presentation at the Annual Meeting of the American Society for Microbiology (May 12-17, 1974).

OTHER DATA

Grantee: Dr. Daniel H. Connor, Geographic Pathology
Division, Armed Forces Institute of Pathology,
Washington, D.C.

Funded by: U.S. Army Medical Research and Development
Command and Pan American Health Organization.

Timetable: 1973-1974.

STUDIES OF ARTHROPOD-BORNE VIRUSES

PROBLEM

Arboviruses, including Venezuelan encephalitis and St. Louis encephalitis viruses and others such as dengue, have caused extensive epidemics in the Western Hemisphere in recent years.

The present investigations in Central America have the following purposes: (1) to study the ecology, geographic distribution, and importance to man and domestic animals of Venezuelan encephalitis virus and several other recently discovered arboviruses; (2) to determine whether the equine-virulent strain of Venezuelan encephalitis virus which caused the 1969-1971 Central American-Mexican epidemic-epizootic, has established enzootic cycles in Central America; (3) to determine the factors involved in virus movement from one area to another; and (4) to identify the factors that may affect the virulence of geographic isolates of Venezuelan encephalitis virus.

METHOD

The program involves the collection of sera for detection of antibodies and the gathering of specimens likely to yield arboviruses--such as mosquitoes, birds, and mammals in Central America. Sentinel animals such as Syrian hamsters, are exposed in coarse wire-mesh cages hung under protective roofs in suitable habitats for the detection of virus transmission by flying arthropods. Immunologically susceptible young horses are serially bled to detect antibody conversions. Virus isolations are carried out in suckling mice and in various cell-culture systems. Antibody tests are performed using neutralization, hemagglutination-inhibition, and complement-fixation techniques.

RESULTS TO DATE

In 1971, following the 1969-1970 spread of equine-virulent Venezuelan encephalitis virus in Nicaragua, there was no evidence of disease attributed to that virus. In 1972, however, a second equine epizootic occurred with several hundred deaths. Blood specimens taken from very young horses in August 1972, had high levels

of specific antibody to the epizootic strain of VE virus. However, in 1973 sentinel hamsters failed to reveal enzootic foci of the epizootic strain of VE virus. Sentinel young susceptible horses are now being followed.

At an endemic focus of Venezuelan encephalitis on the Pacific lowlands of Guatemala, 78 percent of wild terrestrial mammals, 62 percent of humans, and 78 percent of dogs have been demonstrated to have antibodies against a Central American strain of Venezuelan encephalitis virus. In contrast, only 3 percent to 15 percent of wild bats were serologically positive. Wild bats may thus be alternate amplifying hosts for VE virus, and extensive field and laboratory studies have been initiated.

Experimental inoculation of four species of bats to date indicate:

(1) Infections with Venezuelan encephalitis virus are not lethal for bats;

(2) Individual species of bats vary in their response to infection with a gradient from high-to-low levels of viremia;

(3) Infection with epidemic-causing strains stimulates higher levels of viremia (although not of longer duration) than does infection with enzootic strains. In the field, banded bats have been recovered up to 8 kilometers from the original site of capture.

The problem of long-distance movement of arboviruses is an unsolved one. This is especially pertinent in explaining the arrival in Central America of the South American epidemic-causing strain of Venezuelan encephalitis virus in 1969. Consequently, antibody levels and longevity of birds in the midst of migration are being studied in properly screened facilities in the field in Guatemala.

SIGNIFICANCE

The research provides information on arboviruses, especially Venezuelan encephalitis virus, in Central America, and it evaluates the role of wild vertebrates 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 agents.

PUBLICATIONS

1. Dickerman, R. W. et al. St. Louis encephalitis virus isolated from a nestling common egret in southeastern Mexico. Bol Of Sanit Panam (English ed.) 6:26-30, 1972.
2. ----- et al. The involvement of dogs in endemic cycles of Venezuelan encephalitis virus. Am J Epidemiol 98:311-14, 1973.
3. ----- et al. Venezuelan equine encephalomyelitis viremia and antibody responses of pigs and cattle. Am J Vet Res 34:357-61, 1973.
4. Jahrling, P. B. and W. F. Scherer. Growth curves and clearance rates of virulent and benign Venezuelan encephalitis viruses in hamsters. Infect Imm 8:456-62, 1973.
5. ----- and ----- . Histopathology and distribution of viral antigens in hamsters infected with virulent and benign Venezuelan encephalitis viruses. Am J Pathol 72:25-38, 1973.
6. ----- and ----- . Homogeneity of Venezuelan encephalitis virion populations of hamster-virulent and benign strains, including the attenuated TC83 vaccine. Infect Imm 7:905-10, 1973.
7. Scherer, W. F. and R. W. Dickerman. Ecologic studies of Venezuelan encephalitis virus in southeastern Mexico. VIII. Correlations and conclusions. Am J Trop Med Hyg 21:86-89, 1972.
8. ----- et al. Observations of equines, humans, and domestic wild vertebrates during the 1969 equine epizootic and epidemic of Venezuelan encephalitis in Guatemala. Am J Epidemiol 95:255-66, 1972.
9. ----- et al. Studies of Patois group arboviruses in Mexico, Guatemala, Honduras, and British Honduras. Am J Trop Med Hyg 21:194-200, 1972.

OTHER DATA

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

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

Timetable: 1961 - continuing.

HEPATITIS B IN THE CARIBBEAN

PROBLEM

The objectives of the study are to determine the prevalence of hepatitis B-antigen carriers among Caribbean residents, the role of hepatitis B infection in liver and other diseases in the residents, and the methods of transmission of hepatitis B virus in the area.

METHOD

Blood specimens will be collected from healthy residents of the islands of the English-speaking Caribbean and tested by counterimmuno-electrophoresis for the presence of hepatitis B antigen. Similar studies will be carried out on patients with liver disease, patients attending venereal disease clinics, and persons whose occupations bring them into contact with blood or blood products or both. Household contacts of hepatitis B cases and carriers will be investigated for evidence of hepatitis B infection. Blood sucking arthropods, collected in the homes of antigen carriers, will also be tested for hepatitis B antigen by radio-immune assay.

RESULTS TO DATE

None thus far.

SIGNIFICANCE

In temperate climates hepatitis B virus infection is associated with acute and chronic liver disease. There is considerable evidence that large numbers of tropical residents are carriers of hepatitis B antigen, which is regarded as evidence of infection with hepatitis B virus. This research project represents an attempt to determine the role played by hepatitis B virus in morbidity and mortality from liver disease in the Caribbean and the methods of virus transmission. It is hoped that the research will make a contribution to the control of viral hepatitis in the Caribbean.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Leslie Spence, Department of Medical Microbiology, University of Toronto, Ontario, Canada; and Dr. Miles C. Williams, Director, Trinidad Regional Virus Laboratory, Port-of-Spain, Trinidad.

Funded by: Pan American Health Organization.

Timetable: 1973-1976.

STUDIES ON RURAL MEDICINE IN BAHIA

PROBLEM

The main purpose of the study is to develop adequate methods of interaction between medical students and rural communities. As a secondary objective, medical students engage in training auxiliary health manpower for immediate investigation of epidemiologic aspects of important health problems in rural communities, such as parasitic diseases, malnutrition, and others.

METHOD

Weekly or quarterly, a team composed of a teaching assistant and five medical students travel to a rural community 80 miles away from the Medical School. A few research scientists interested in the investigation of rural health problems join them. In the rural community of Cruz das Almas, medical students are training auxiliary health personnel, e.g., laboratory technicians. These locally trained auxiliaries will subsequently help in the research efforts of the medical team and will also participate in the necessary preventive or curative measures at the community level.

RESULTS TO DATE

In the second semester of 1973, medical students trained five young laboratory auxiliaries of junior high school level in the performance of stool analysis (helminthoscopy). With the cooperation of the local auxiliaries, an investigation of the level of parasitic infection was carried out on 312 students from six grade schools selected at random in a tobacco and orange plantation area. Tests were positive for Ascaris lumbricoides (76.3 percent), Necator americanus (69.5 percent), Trichuris trichiura (35.3 percent), and for Schistosoma mansoni (69.5 percent). The infested children received treatment, except those with S. mansoni infection because of the toxic effects of existing drugs, and sanitary education.

At the same time, the presence of protein in the urine of 30 schistosoma-positive children and 30 paired controls was

investigated by quantitative methods. There were no statistically significant differences between the two groups.

SIGNIFICANCE

Medical students educated exclusively in modern medical centers usually lack occasions to get in touch with rural communities and their health problems. Medical schools in underdeveloped areas must find opportunities for their students' training in rural health. Our project endeavors to show ways by which medical students could work with rural communities for the investigation of health problems and their solution. These studies could constitute the basis for other research projects on the natural history of important endemic illnesses, such as schistosomiasis, in rural areas.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. José Duarte de Araújo, Department of Preventive Medicine, Federal University of Bahia, Brazil.

Funded by: Pan American Health Organization.

Timetable: 1973-1976.

IDENTIFICATION OF TYPES AND SUBTYPES OF VESICULAR STOMATITIS VIRUS

PROBLEM

Vesicular stomatitis symptoms are similar to those of foot-and-mouth disease. A differential diagnosis of these diseases must therefore be made in the laboratory.

In some areas of the Americas, vesicular stomatitis exists but foot-and-mouth disease does not. Because of this, a prompt diagnosis is necessary to ensure eradication measures in the event of an outbreak of foot-and-mouth disease.

Colombia, Ecuador, Peru, and Venezuela, which are affected both by vesicular stomatitis and by foot-and-mouth disease, have foot-and-mouth disease control programs. In their case, differential diagnosis is doubly necessary.

Recently the Pan American Foot-and-Mouth Disease Center demonstrated the existence of subtypes of the Indiana virus in Argentina and Brazil where the disease had never been diagnosed. This finding points to the need for differential diagnosis, subtyping, and, when necessary, classification of strains.

METHOD

Differential diagnosis, typing and subtyping of vesicular stomatitis virus are achieved by passages in tissue culture and in suckling and adult mice, and by complement fixation. The virus is classified by determination of the serologic relationships and bonds through the 50 percent complement fixation test.

RESULTS TO DATE

Between 1952 and 1973, more than 1,000 specimens of vesicular stomatitis virus from Central and South America were analyzed. During the same period the Pan American Foot-and-Mouth Disease Center demonstrated the existence of subtypes of the Indiana virus.

Subtype Indiana 1 is the strain designated as the Indiana type, which is present jointly with the New Jersey type in the United States, Mexico, Central America, Panama, Venezuela, Colombia, Ecuador, and Peru. Subtype Indiana 2 is represented by the Salto strain, which was diagnosed in Argentina in 1963. Another strain of the same subtype was isolated in Brazil in 1965 and 1966. This subtype also includes the Cocal or Trinidad strain. Subtype Indiana 3 was identified in Alagoas, Brazil, in 1964.

Virus of subtypes Indiana 2 and 3 have not been isolated since 1966. The Salto and Alagoas strains are characterized by the fact that they only affect equines. It has also been observed that subtype Indiana 3 produces smaller plaques than do the New Jersey and the other subtypes of the Indiana type when cultivated in monolayers of BHK cells covered with agar and dextran sulfate or DEAE-dextran.

So far, no subtypes of the New Jersey strain have been identified.

SIGNIFICANCE

Stringent quarantine measures in outbreaks of vesicular diseases, together with a good system for the collection and dispatch of specimens, followed by an efficient diagnosis and rapid communication of results are valuable tools in maintaining areas exclusively affected by vesicular stomatitis free from foot-and-mouth disease. A similar plan is also effective in areas in which the two diseases are present.

PUBLICATIONS

1. Federer, K. E. et al. Vesicular stomatitis virus: the relationship between strains of the Indiana serotype. Bull Off Int Epiz 65:879-80, 1966.
2. ----- et al. Vesicular stomatitis virus: the relationship between some strains of the Indiana serotype. Res Vet Sci 8:103-17, 1967.
3. Garcia Pirazzi, A. J. et al. Estomatitis vesicular. Constatación de la enfermedad y aislamiento del virus. Gac Vet Buenos Aires 22:85-91, 1966.
4. Gomes, I. and A. Vieira. Effect of polyionic compounds on the plating efficiency of some strains of vesicular stomatitis virus. Arch Ges Virusforsch 34:223-31, 1971.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1957 - continuing.

DIAGNOSIS OF FOOT-AND-MOUTH DISEASE AND VESICULAR STOMATITIS BASED ON CATTLE, HORSE, SHEEP, AND HOG SERA

PROBLEM

The diagnosis of Foot-and-Mouth Disease (FMD) or vesicular stomatitis from virus samples contained in sick animals' sera is limited to a short interval of time. For this reason, the laboratory receives a large number of sera from convalescent animals so that a diagnosis of the disease and identification of the virus type involved may be made with them. The suckling mice seroprotection and the tissue culture seroneutralization index methods (SPI and SI) are routinely used at the Pan American Foot-and-Mouth Disease Center, but these procedures are lengthy and costly. Since the diseases of concern to us are produced by various virus types and subtypes, SPI and SI present several diagnostic limitations. It is therefore necessary to investigate inexpensive methods that would permit analysis of a large number of specimens and at the same time provide rapid and reproducible results.

METHOD

The SPI and SI techniques were used. In addition, comparative investigations were made with 50 percent complement fixation (CF 50) hemolysis after 18 hours of incubation at 4°C and 2 hours at 37°C; CF 50 inhibition; double diffusion in Ouchterlony agar gel; and Mancini radial diffusion adapted to foot-and-mouth disease by Cowan and Wagner.

RESULTS TO DATE

Cold CF 50 hemolysis is a highly sensitive and specific method of detecting antibodies in cattle, horses, sheep, and hogs convalescing from vesicular stomatitis.

Inhibition of CF 50 makes it possible to diagnose foot-and-mouth disease and vesicular stomatitis from animal sera collected 1-3 weeks after an attack of the disease.

The double diffusion in Ouchterlony agar gel and the Mancini radial diffusion are very useful in detecting antibodies against foot-and-mouth disease and vesicular stomatitis in the serum of animals that have been in contact with these viruses. These techniques also permit the detection of anti-VIA antibodies in the serum of infected cattle and sheep. The latter technique, however, is more sensitive than agar gel double diffusion for low antibody concentrations.

SIGNIFICANCE

When it is impossible to collect infectious material for the diagnosis of foot-and-mouth disease or vesicular stomatitis, it is possible to diagnose the disease and identify the virus type involved, with serum from convalescent animals, using the cold CF 50, CF 50 inhibition, agar gel double diffusion, and the radial diffusion techniques.

PUBLICATIONS

1. Alonso Fernández, A. et al. Control de vacunas antiaftosas. Relación entre el índice K (Modificado) y los índices de seroprotección y seroneutralización. Bol Cent Panam Fiebre Aftosa, 6:1-16, 1972.
2. Cunha, R. G. et al. El uso de los ratones lactantes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa y su significación inmunológica. Gac Vet 19:243-67, 1957.
3. Gomes, I. Foot-and-mouth disease circulating antibodies in convalescent cattle. Bull Off Int Epiz 77:731-41, 1972.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1957 - continuing.

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

PROBLEM

Vesicular diseases (foot-and-mouth disease, vesicular stomatitis, hog exanthema and hog vesicular disease) are highly contagious and have similar symptoms, which makes rapid laboratory diagnosis essential.

The foot-and-mouth disease virus is very unstable as is shown by the existence of 61 different subtypes. This calls for continuous typing and subtyping of field virus, especially in countries with disease control programs based on preventive immunization, so as to maintain the antigenic or immunologic balance between the field strains and the production and control of vaccines.

The classification of a new subtype involves, in addition to the antigenic study, the determination of the immunologic correlations and bonds between field viruses of epidemiologic significance and those used in vaccine production.

The subtyping and classification of a virus requires appropriate reagents and a standardized methodology to obtain similar and reproducible results. Consequently it entails the standardization of methods and the maintenance of an updated strain bank.

METHOD

Differential diagnosis, typing, subtyping, and serologic classification of the foot-and-mouth disease virus are achieved by a combination of passages of the specimens in cell cultures, suckling, and adult mice and of the 50 percent complement fixation test.

The immunologic bonds are determined by seroprotection in suckling mice, seroneutralization in tissue cultures, index C in guinea pigs, and modified index K in bovines.

RESULTS TO DATE

Between 1952 and 1973, about 10,000 specimens of foot-and-mouth disease field virus from South America were typed and subtyped. During

that period, 20 different subtypes were identified. They may be grouped as follows:

Type O Vallée: Subtypes O_1 and O_8

Type A Vallée: Subtypes A_{10} , A_{13} , A_{16} , A_{17} , A_{18} , A_{19} , A_{24} ,
 A_{25} , A_{26} , A_{27} , A_{29} , A_{30} , A_{31} , and A_{32} .

Type C Waldmann: Subtypes C_2 , C_3 , C_4 , and C_5 .

With the exception of O_1 , A_{10} , and C_2 all were new subtypes in the World Reference Laboratory's international classification.

In 1972 and 1973 subtypes O_1 , A_{24} , A_{26} , A_{27} , A_{32} , C_2 , C_3 , and C_5 were identified in the field in South America, and the countries affected by one or the other of them were Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Guyana, Paraguay, Peru, Uruguay, and Venezuela.

All the South American countries affected by foot-and-mouth disease have national diagnostic laboratories which use identical methods and are equipped to undertake the necessary studies for classifying a new subtype.

SIGNIFICANCE

The standardization of reagents and of differential diagnostic techniques; the typing, subtyping, and serologic and immunologic classification of foot-and-mouth disease viruses; the establishment of a national laboratory in all South American countries affected by foot-and-mouth disease; and the close collaboration of these countries with the Pan American Foot-and-Mouth Disease Center, have made it possible to identify the subtypes present at any time in the field and to take the appropriate preventive measures.

PUBLICATIONS

1. Alonso Fernández, A. et al. Diagnóstico y referencia de la fiebre aftosa. Bol Cent Panam Fiebre Aftosa, in press.

2. ----- et al. Comparación inmunológica de los subtipos del virus aftoso tipo C Waldmann. Bol Cent Panam Fiebre Aftosa 4:9-20, 1971.
3. Federer, K. E. et al. Développement d'un nouveau sous-type du virus de la fièvre aphteuse par passage en série sur bovins partiellement immunisés. International Symposium on Foot-and-Mouth Disease: Variants and Immunity, Lyon 1967. Symp Series Immunol Standard 8:65-72, 1968. (Kerger, Basel, New York).
4. ----- et al. Identificación de un nuevo subtipo "0" del virus de la fiebre aftosa en Sudamérica. Bull Off Epiz 61: 1563-78, 1964.
5. ----- et al. Identification d'un nouveau sous-type A de virus aphteux. Bull Off Int Epiz 57:1171-90, 1962.
6. Lucam, F. et al. Etude immunologique des sous-types du virus aphteux. Principe, methode, application. Bull Off Int Epiz 75:1-20, 1971.
7. Suttmöller, P. et al. Infectivity for cattle and pigs of three strains of foot-and-mouth disease virus isolated from carrier cattle. Am J Vet Res 28:101-05, 1967.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1957 - continuing.

DETECTION OF ANTIBODY TO FOOT-AND-MOUTH DISEASE VIRUS INFECTION ASSOCIATED ANTIGEN (VIA)

1. PURIFICATION AND CONCENTRATION OF VIA ANTIGEN

PROBLEM

The VIA antigen is the enzymatically inactive polymerase (ARN replicase) which appears solely in cells infected with foot-and-mouth disease virus. This polymerase is necessary for the replication of viral ARN and is capable of simulating the formation of antibodies in infected animals. The anti-VIA antibodies are specific to the disease but induce a cross reaction with all the VIA antigens of different virus types. By analysis of these antibodies it is possible to diagnose and to carry out epidemiologic studies on foot-and-mouth disease.

To detect the presence of anti-VIA antibodies in the serum of animals, it is necessary to have concentrated and sufficiently purified VIA antigen to prevent reactions of the sera with other virus antigens, which hinder the interpretation of the results. The concentration and purification of this antigen, by the method developed by Dowan and Graves, calls for equipment which in most cases is not available to the diagnostic laboratories of South American countries. It is therefore necessary to investigate other inexpensive methods to be routinely used by a basically equipped laboratory.

METHOD

VIA antigen was produced from virulent suspensions obtained in BHK cell cultures. These suspensions were treated together with 6 percent p/v of Polyethylene glycol (PG) and with 35 percent p/v of $\text{SO}_4(\text{NH}_4)_2$, after which it was slowly centrifuged.

The antigen produced was investigated by complement fixation (CF), double diffusion in Ouchterlony Agar gel (DDA) and Mancini radial diffusion (RD) adapted to foot-and-mouth disease by Cowan and Wagner. In all the cases the VIA antigen was challenged to sera of animals, which had been previously submitted to various immunization treatments.

RESULTS TO DATE

The virulent suspensions, obtained when the cell monolayer showed clear cytopathic effect, contained VIA and 140 S antigens. Polyethylene glycol added to these suspensions precipitated the 140 S antigen, leaving in suspension the VIA antigen, which was concentrated with $\text{SO}_4(\text{NH}_4)_2$. The behavior of this antigen was compared with that of another produced by the Cowan and Graves method; both antigens gave similar results when used as challenges to cattle, sheep, and guinea pig sera.

SIGNIFICANCE

The VIA antigen, produced from virulent suspensions obtained in BHK cell cultures treated with polyethylene glycol and $\text{SO}_4(\text{NH}_4)_2$ and slowly centrifuged, may be used for diagnosis and epidemiologic studies on foot-and-mouth disease. This method of concentrating and purifying the VIA antigen may be used by basically equipped laboratories.

PUBLICATIONS

1. Alonso Fernández, A. and M. S. Söndahl. Purificación y concentración del antígeno asociado a la infección del virus de la fiebre aftosa (VIA). Bol Cent Panam Fiebre Aftosa, in preparation.
2. ----- et al. The detection of antibodies to the foot-and-mouth disease virus infection associated antigen in field research. Am J Epidemiol, in preparation.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1973 - continuing.

DEVELOPMENT OF A PASSIVE HEMAGGLUTINATION MICROTEST AND ITS USE AS A SIMPLE METHOD FOR THE DIAGNOSIS OF FOOT-AND-MOUTH DISEASE IN THE FIELD

PROBLEM

Veterinarians frequently lack a reliable method for rapidly diagnosing Foot-and-Mouth Disease (FMD) and establishing the degree of immunity developed in convalescent cattle. Although at the present time a passive hemagglutination test is available for titrating FMD antibodies, the field veterinarian still faces the problem of having to examine a large number of specimens and especially of having to prepare antigen-sensitized cells. A greater simplification of the method is therefore necessary. The following studies have been undertaken to: (1) develop a simple, rapid, passive hemagglutination microtest that may be used in the field as a diagnostic method, and (2) evaluate the antigenic stability of the virus conjugated with sheep erythrocytes when the complexes are maintained at -70°C and 4°C for varying periods of time.

METHOD

The A₂₄ Cruzeiro virus was selected for the preliminary tests. The source of antibodies was sera from immunized and hyperimmunized guinea pigs and convalescent cattle. Sheep erythrocytes were sensitized with the virus in a concentration of 40 $\mu\text{g}/\text{ml}$ according to the slightly modified Tokuda and Warrington technique. Immediately after preparation, the sensitized erythrocytes were suspended in a pH 7.6 phosphate-glycocol buffer solution with a 5 percent concentration, divided into 1-ml aliquots and rapidly frozen in a dry-acetone ice bath, and subsequently stored at -70°C . These aliquots were thawed at weekly intervals and their antigenicity compared with that of an aliquot stored at 4°C and of recently prepared conjugates. They were titrated with the Takatsy microtitrator.

RESULTS TO DATE

Sensitized erythrocytes do not lose their antigenicity for at least 6 months when they are maintained at -70°C or at 4°C . The titers are very reproducible and both the hemagglutination standards

and the negatives are clear. Titers of up to 65,000 have been found in hyperimmunized guinea pigs while values of up to 4,000 are commonly obtained with sera from animals immunized twice with water-in-oil vaccines. Titers varying from 320 up to 1,280 have been found in the sera of convalescent cattle.

SIGNIFICANCE

The results obtained to date show that the virus-erythrocyte conjugates are rather stable, especially as regards the antigenic properties of the virus. This will undoubtedly make it possible to prepare large stocks of sensitized erythrocytes for subsequent dispatch to distant regions, in dried ice, for use in diagnosis.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1973 - continuing.

MECHANISM OF ACTION OF MODIFIED LIVE-VIRUS VACCINES IN REVACCINATION

PROBLEM

To study the mechanism of action of modified live virus in revaccination in the presence of circulating antibodies in order to determine whether the virus is replicated or whether it simply acts like an inactivated vaccine.

METHOD

A selected group of young, under 1-year-old cattle were vaccinated several times at two-month intervals with an inactivated vaccine (AEI) Frenkel type, absorbed in aluminum hydroxide. The cattle were bled and their sera analyzed to ascertain the seroneutralization index in test tubes of BHK-21, clone 13, cells. In addition the anti-VIA antibody was studied. Subsequently the animals were revaccinated with modified live virus vaccines and 14 days thereafter they were bled on several occasions and their sera was examined in the presence of anti-VIA antibodies.

RESULTS TO DATE

The replication of the modified live virus in the presence of antibodies was demonstrated through the presence of the anti-VIA antibody after revaccination with modified live virus vaccine for several weeks.

SIGNIFICANCE

The importance of this study lies in the verification of the replication of modified live virus by the study of the anti-VIA antibody in animals revaccinated with modified live-virus vaccine.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1973 - continuing.

METHOD FOR THE RAPID MODIFICATION OF FOOT-AND-MOUTH DISEASE VIRUS. USE OF CLONES AS MODIFIED LIVE-VIRUS VACCINE

PROBLEM

Virus multiplication in the organism usually induces sound and lasting immunity. The use of a virus as a vaccine is contingent on the absence of pathogenicity and the existence of multiplication capacity. We propose to isolate clones from a few passages of O-Vallée, A-Vallée, and C-Waldmann viruses in embryonated chick eggs to study the clones' possible use as modified live-virus vaccine for cattle.

These clones are studied in susceptible animals from the standpoint of the presence or absence of viremia, specific antibody response, and the appearance of postvaccinal reactions (pathogenicity).

METHOD

We studied clones from strains already modified by a specified number of passages in 14-day-old embryonated eggs and suckling mice and from strains modified by a limited number of passages in embryonated eggs. In some cases low temperatures (28°C) were used to study cold mutants. The clones to be studied were inoculated by the intradermolingual route in susceptible cattle to investigate in the animal the presence or absence of viremia, the circulating antibody response measured by neutralization in cell cultures ("S" index) and manifestations of pathogenicity (postvaccinal reactions).

RESULTS TO DATE

- a) Cloning resulted in a substantial reduction in pathogenicity.
- b) It is possible to obtain more homogenous and stable virus populations.
- c) This study has made it possible to isolate clones with great multiplication capacity (viremia); on the other hand, there are clones that do not produce viremia but induce a very pronounced immunologic response by causing the appearance of a substantial number of specific antibodies, and other clones that produce very poor antibody

responses. It is therefore possible to avoid the large number of passages in embryonated eggs, suckling mice, and cell cultures, which was previously done to gradually reduce their pathogenicity, but which also comprised their immunizing potential.

SIGNIFICANCE

The study of clones from a limited number of passages in embryonated eggs, as modified live-virus vaccine for cattle, has made it possible to reduce the number of passages, to obtain more antigenic vaccines with more homogeneous and stable virus populations, and to develop a method for rapid modification.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1972 - continuing.

INFLUENCE OF THE AMOUNT OF ANTIGEN ON THE IMMUNIZATION OF ANIMALS UNDER 1 YEAR OF AGE WITH MODIFIED LIVE-VIRUS VACCINE

PROBLEM

One aspect of research on modified live-virus vaccines is to determine the amount of antigen necessary to effectively immunize animals under 1 year of age which we know to have been unsatisfactorily immunized.

METHOD

To carry out this experiment, three monovalent modified live-virus vaccines type O, A, and C suspended in a 50 percent glycerine phosphate solution were prepared. Young animals under 1 year of age were used, their ages ranging from 4 to 12 months. They were divided into three vaccinated groups, and one unvaccinated control group. The doses of each monovalent vaccine were 1 ml, 5 ml, and 25 ml and there was a 2-month interval between the administration of each type of vaccine. At the end of 3 months the animals were revaccinated with the same vaccine. The animals were bled before vaccination and thereafter monthly in order to study the antibody curve measured by the seroprotection test in suckling mice.

RESULTS TO DATE

(a) When the amount of antigen administered was large (25 ml) the antigenic response measured by the antibody study was more evident;

(b) Cruzeiro virus A₂₄ showed the same tendency but, because it is an excellent antigen, 5 ml of it was sufficient to produce the same response as 25 ml of the others;

(c) The use of an adjuvant together with larger amounts of antigen (25 ml) could prolong the duration of immunity.

SIGNIFICANCE

This study, to ascertain the amount of antigen necessary to obtain an effective immunity in animals under 1 year of age, will contribute to the control of foot-and-mouth disease in South America.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1973 - continuing.

LOCAL (NASAL AND BUCCAL) IMMUNOLOGICAL RESPONSE INDUCED
IN NEWBORN CALVES AND CATTLE IMMUNIZED WITH
MODIFIED LIVE-VIRUS VACCINE

Biologic significance of secretory immunoglobulins
in the foot-and-mouth disease defense mechanism

PROBLEM

The purposes of this study are:

- 1) To investigate the local immunologic response induced by a modified live virus in newborn calves fed on colostrum; the local defenses developed in the buccal and nasal membranes; and the protection conferred against reinfection with modified virus or against field virus, as compared to the protection developed in immunized animals with similar characteristics but fed on soy milk.
- 2) To investigate the role of secretory immunoglobulins, fundamentally IgA, in protection against foot-and-mouth disease virus in locally vaccinated animals.

METHOD

In this study strain A₂₄ Cruzeiro foot-and-mouth disease virus will be used.

- 1) A group of cows-in-calf will be immunized during the last month of pregnancy. The calves will be separated into two groups at birth. One group will be fed on colostrum and the other on normal or on soy milk. All the calves will be immunized with modified live virus 48 hours after birth. Blood samples will be taken before and after each of these steps. Three months later the animals will be tested by means of the intranasal application of field virus in the form of aerosol. The presence and concentration of IgA in the nasal secretions will be investigated 48 hours after this treatment. The duration of the local immunity and of the booster effect of repeated applications of the virus in the form of aerosol will be studied.
- 2) A group of young cattle, which have not been in contact with foot-and-mouth disease virus, will be immunized with modified live virus

applied in the form of aerosol by the intranasal route. Forty-eight hours later nasal secretions will be collected with a view to identifying the various immunoglobulins and establishing the IgG:IgA ratio. The "booster" effect of monthly inoculations over a period of 3 months will be studied. After that period, the animals will be bled and the degree of seroprotection developed will be established.

3) The various immunoglobulins will be investigated by immunodiffusion, radial immunodiffusion, immunoelectrophoresis, electrophoresis, and disk-electrophoresis.

RESULTS TO DATE

None thus far.

SIGNIFICANCE

Cattle immunization against foot-and-mouth disease consists now of parenteral inoculations of virus propagated in a water-and-oil emulsion or in aluminum hydroxide suspensions. The defense against foot-and-mouth disease virus conferred by local immunity in these animals appears, however, to be rather important. This is a fact to which so far sufficient attention has not been given. It is assumed that the development of a good immunologic defense at the oro-nasal level will efficiently protect most of the animals. In addition it must be borne in mind that salivary IgA levels rise rapidly and reach adult concentrations more rapidly than do those of the serum. Newborn calves, passively immunized by colostrum, react feebly to active immunization. They may, however, be immunized intranasally with aerosol to provide a local immunity capable of protecting the young animal until its general immunologic system reaches a higher degree of maturity.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1973 - continuing.

ROLE PLAYED BY OIL AND ALUMINUM HYDROXIDE ADJUVANTS IN MODIFIED LIVE-VIRUS VACCINE

PROBLEM

The purpose of this study is to determine the role played by adjuvants such as oil (Arlacel-Markol 52) and aluminum hydroxide in modified live-virus vaccines. To this effect vaccines prepared in adjuvants are compared with vaccine routinely prepared in glycerine phosphate.

METHOD

Three trivalent vaccines--O, A, and C--were prepared using (a) an oil adjuvant (Arlacel: 1 part; Markol 52:9 parts) in the first; (b) aluminum hydroxide in the second; and (c) 50 percent glycerine phosphate in the third. The vaccines were inoculated subcutaneously to young cattle under 1 year of age. The animals were bled before and after vaccine inoculations and again every 2 months for 6 months. At that time, the group was revaccinated to study the antibodies measured by the BHK₂₁ cell tube seroneutralization method ("S" index).

RESULTS TO DATE

Oil adjuvant added to O, A, and C vaccines brought a much higher circulating antibody index for longer periods than did aluminum hydroxide or 50 percent glycerine phosphate.

SIGNIFICANCE

Adjuvants (oil or aluminum hydroxide type) play an important role in the antigenic behavior of the vaccines and in the absence of post-vaccinal reactions (pathogenicity).

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1970 - continuing.

ADJUVANT ACTION OF BACTERIAL TOXINS AND THEIR POTENTIAL USE TO INCREASE THE IMMUNOLOGIC RESPONSE TO FOOT-AND-MOUTH DISEASE IN CATTLE

PROBLEM

The immunologic response against antigens inoculated parenterally depends primarily on the nature of the antigens and the quantity of the antigenic substance inoculated. This immunologic response, however, may be increased or regulated by proper use of immunoadjuvants. These act by stimulating phagocytosis; providing a vehicle for the transport of the antigen towards distant points; slowly releasing the localized antigen as an insoluble deposit; and by increasing the number of antibody-forming cells in the spleen and lymph nodes.

No adjuvant of the endotoxin type which acts on the antibody-forming cells by eliciting an increase in their synthesis, is at present used in vaccination against foot-and-mouth disease (FMD). The possibility of using bacterial lipopolysaccharides is therefore postulated for improving the immunologic response to this virus. It is speculated that the synergic effect between the adjuvants that operate by promoting phagocytosis and slowly releasing the localized antigens as an insoluble deposit and the adjuvants that increase the number of antibody-forming cells, would increase the intensity and duration of the immunologic response.

METHOD

The synergic effect between Freund incomplete adjuvant and endotoxins is being studied in guinea pigs. The information obtained from these tests will be used in future studies involving cattle and hogs.

Seven-month-old guinea pigs with an average weight of 750g were divided into five groups and immunized intramuscularly as follows: (1) with oil-adjuvant vaccine; (2) with oil-adjuvant vaccine and endotoxins (Difco Lipopolysaccharide B. E. Coli 055:B5); (3) with virus in saline solution; (4) with virus in saline solution and endotoxins; (5) with endotoxins alone. In all cases 20 µg of inactivated virus was injected. Endotoxins were injected simultaneously but at a different site from that of the virus. The animals were bled periodically and the immunologic response studied by passive hemagglutination, complement fixation, seroneutralization, and mouse seroprotection tests. In addition the quantitative and qualitative variations in the immunoglobulins of the immune sera were studied by disk-electrophoresis and immunoelectrophoresis.

RESULTS TO DATE

The guinea pigs have been immunized 3 times and bled 10 times including the bleeding before immunization. Approximately 100 sera have been studied by passive hemagglutination. From the results obtained to date, it may be inferred that endotoxins increase the immunologic response against FMD, not only when they are administered simultaneously with the virus transported in a water-in-oil emulsion but also when antigen in a buffered physiologic solution is inoculated. The best results were, however, obtained with oil-adjuvant vaccines accompanied by endotoxins.

SIGNIFICANCE

The weak immunologic response obtained in cattle immunized with FMD virus and its short duration is an obstacle to the operation of a large-scale program for the eradication of the disease in cattle. Any success in the search for means to increase the degree of immunity in them will be extremely valuable to the economies of the countries affected by this 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.
Timetable:	1973.

STUDY IN CATTLE OF THE IMMUNOLOGIC BEHAVIOR OF AN ANTI- FOOT-AND-MOUTH DISEASE VACCINE WITH OIL-TYPE ADJUVANT

PROBLEM

Nearly all national campaigns against foot-and-mouth disease now under way in South American countries use inactivated vaccines for systematic vaccination of the cattle herd every 4 months. Results of recent laboratory studies made at Plum Island and the Pan American Foot-and-Mouth Disease Center on vaccine inactivated with acetylethyleneimine (AEI) emulsified with oil, have indicated that a satisfactory immunity was conferred to cattle for a period of more than 6 months. So far, there is no information on the behavior of this vaccine in the field (open environment), considering the following aspects: (1) immunologic response, (2) secondary effects, (3) handling of vaccine, (4) routes and period of inoculation, and (5) industrial production. It is proposed to develop this project in three stages.

In the first, the immunizing and secondary effects of the vaccine on a cattle population will be studied.

In the second, problems of vaccine administration, such as handling and inoculation routes, will be covered.

The third stage will be concerned with the feasibility of industrial production of this oil vaccine.

The first stage's objectives are: (1) evaluation of the immunologic response of cattle when a 6-month vaccination schedule is used, and (2) evaluation, during the period covered by the study, of secondary effects in the cattle, as well as in their carcasses after the cattle are slaughtered.

METHOD

The cattle used are 5- to 7-month-old at the start of the study. These animals are crossbred $5/8$ Angus and $3/8$ Zebu beef cattle, with approximately the same number of males and females, and belong to the "Cinco Cruzes" Experimental Station in Bagé, Rio Grande do Sul.

The study was conducted in two parts: A. Inductive study, and B. Comparative study.

A. Inductive study

Since the main object is to estimate the percentage of cattle protected, with a confidence interval, sampling has been chosen as means of operation. The response variable to be considered will be the immunity measured by the level of circulating antibodies, using the serum-protection index (SPI) in suckling mice.

B. Comparative study

This study ascertains the possible differences in immunity between groups treated with an oil-adjuvant vaccine and with an aluminum hydroxide vaccine, and a control group, the only variable element being the adjuvant.

RESULTS TO DATE

The preliminary results obtained to date with the oil-adjuvant vaccine, using a 6-month schedule, point to a satisfactory immunologic response in cattle without the appearance of any undesirable secondary effects.

SIGNIFICANCE

The results of the present studies will contribute greatly to the control of foot-and-mouth disease and directly to the costs of the national campaigns.

PUBLICATION

1. Callis, J. J. et al. Foot-and-mouth disease virus immunization. Proc World Vet Cong, Mexico City, August 1971.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1972-1973.

TESTS ON THE POSSIBLE COLLATERAL EFFECTS OF MERTHIOLATE USED AS A PRESERVATIVE IN INACTIVATED FOOT-AND-MOUTH DISEASE VACCINES

PROBLEM

Merthiolate is widely used by industrial producers as a preservative in foot-and-mouth disease vaccines.

Experiments with poliomyelitis inactivated vaccines have shown that merthiolate degradation products may affect the virus antigen during vaccine storage. The harmful effect of these degradation byproducts may be offset by treatment with a chelating agent, EDTA (soltetrasodium). This agent apparently does not affect the bacteriostatic or the fungistatic properties of merthiolate.

The purpose of this study is to find out whether the use of merthiolate as a preservative in inactivated foot-and-mouth disease vaccine has any harmful effect on the storage of the vaccines. This is of particular importance, especially in view of the fact that, in order to have vaccines in the quantity required by foot-and-mouth disease control campaigns, it is necessary to store vaccine for relatively long periods.

METHOD

A batch of trivalent inactivated foot-and-mouth disease vaccine will be divided into four fractions as follows:

- 1) Normal vaccine, without addition of merthiolate.
- 2) " " , with addition of merthiolate at 1:30,000.
- 3) " " , with addition of merthiolate at 1:10,000.
- 4) " " , with addition of merthiolate at 1:10,000 plus EDTA (10 mol EDTA per 1 mol merthiolate).

Each of the four vaccines will be administered to four different groups of cattle that have never been vaccinated. Vaccination will be made with newly prepared vaccines and with vaccines stored for 6 and 12 months respectively in a cold store.

The immunogenic capacity will be evaluated by measuring the circulating antibodies.

RESULTS TO DATE

Preliminary experiments with monovalent vaccines have not shown differences in the four vaccines produced under the conditions described above, 27 and 90 days after preparation.

SIGNIFICANCE

These experiments are important because a large part of the industrial production of vaccines in South America is carried out under conditions such that the use of merthiolate is virtually essential to control the contamination of the vaccine by spores and bacteria.

PUBLICATIONS

None thus far.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1973 - continuing.

QUALITY CONTROL OF VACCINES AGAINST FOOT-AND-MOUTH DISEASE

I. IMMUNITY CONTROL

PROBLEM

Countries intent on controlling Foot-and-Mouth Disease (FMD) by the preventive immunization of the susceptible population must have a sufficient supply of vaccines, which must exceed a minimum immunogenic potential and not provoke serious disorders in the animals vaccinated. This presupposes the quality control of all the vaccines produced.

In quality control the study of immunity is all-important. In this regard it must be noted that direct methods are very expensive. It is therefore not possible to control the quality of all the production when only direct methods are used. The use of direct methods is even more limited in identifying highly susceptible cattle since in many affected countries there are no disease-free areas.

Immunity control of all the vaccines produced involves the use, by countries with FMD, of cattle from enzootic areas and also when indicated, of indirect control methods correlated with the direct ones and adapted to the possibilities of each country.

METHOD

Unvaccinated cattle from enzootic areas, which are free of anti-FMD circulating antibodies, and have not been in contact with the virus, are vaccinated and tested according to the modified K index method (modified IK).

The vaccinated cattle's serum is controlled in suckling mice by seroprotection and in tissue cultures by seroneutralization indexes.

Depigmented guinea pigs aged four months or more and weighing more than 500g are vaccinated and tested by the guinea pig index method.

RESULTS TO DATE

Analysis of the modified IK has shown that if a vaccine is to provide 75 percent or more protection against pedal generalization,

the protection at the point of inoculation (IK) must be equal to or greater than 1.5. When IK is equal to or greater than 0.7 but less than 1.5, protection against pedal generalization may be greater or less than 75 percent. When IK is less than 0.7, protection against pedal generalization is less than 75 percent.

Cattle which give a seroprotection or a seroneutralization index in the serum equal to or greater than 2 or 1.5 respectively, would be protected against pedal generalization. Vaccines tested by the guinea pig index method and which are equal to or greater than 2, would have an IK equal to or greater than 1.5 in cattle.

SIGNIFICANCE

It is possible to control all the anti-FMD vaccines produced in South America by using a combination of the guinea pig index, seroprotection, seroneutralization, and modified IK methods.

PUBLICATIONS

1. Alonso Fernández, A. et al. Control de vacunas antiaftosas. Relación entre el índice K (Modificado) los índices de seroprotección y seroneutralización. Bol Centro Panam Fiebre Aftosa, 6:1-16, 1972.
2. ----- et al. Comparación inmunológica y serológica de dos subtipos del virus aftoso tipo C Waldmann. Bol Centro Panam Fiebre Aftosa, 4:9-20, 1971.
3. Cunha, R. G. et al. El uso de los ratones lactantes en la evaluación de los anticuerpos contra el virus de la fiebre aftosa y su significación. Gac Vet Buenos Aires, 19:243-67, 1957.
4. Lucam, F. et al. Etude immunologique de sous-types du virus aphteux. Bull Off Int Epiz 75:1-20, 1971.

OTHER DATA

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

Funded by: Pan American Health Organization.

Timetable: 1969 - continuing.

STUDIES ON PERSISTENT VIRAL INFECTIONS IN BOVINES INFECTED WITH FOOT-AND-MOUTH DISEASE VIRUS (FMDV)

PROBLEM

Persistent viral infections in bovines, which occur as a sequela to infection with FMDV, have been well documented. The mechanism(s) of such infections in convalescent animals remains, however, poorly understood in the presence of adequate neutralizing antibody.

A possible explanation for this continuing infection might be a virus-induced alteration of cellular function of the lymphocytes, thereby subverting protective cellular immune process of the host.

The objective of these studies is to determine (a) the response of peripheral blood lymphocytes in vitro to mitogenic influence of phytohemagglutinin (PHA), and (b) to establish a correlation with the concurrent ability to isolate virus from the pharyngeal region of animals and the functional impairment of the lymphocytic response to PHA.

METHOD

Lymphocytes cultured in vitro with PHA can undergo transformation to blast-type cells. This blastogenic transformation is primarily a measure of thymus-dependent lymphocyte (T-cell) function. It is also one of the means of studying the responsiveness and competence of lymphoid cells.

Lymphocytes from normal and chronically infected bovines will be evaluated for blastogenesis in the presence and absence of PHA. Cell smears will be stained by the Giemsa's method and morphologically examined under the microscope. Transformation will be expressed by the percentage of transformation of small lymphocytes to blast-type cells.

RESULTS TO DATE

No preliminary results are available at this time.

SIGNIFICANCE

These studies may help us to understand the inability of these chronically infected animals to eliminate the virus from their systems. Cellular immunity is an important host defense to some virus infections and therefore alteration of cellular function may contribute to the persistent shedding of virus in chronically infected animals. In addition, this alteration of lymphocyte responsiveness might also prove a valuable factor in the detection of viruses that cannot be identified by presently available methods.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Pan American Foot-and-Mouth Disease Center, Rio de Janeiro, Brazil.
Funded by:	Pan American Health Organization.
Timetable:	1974 - continuing.

STUDIES ON THE BIOCHEMISTRY AND METABOLISM OF LARVAL ECHINOCOCCUS GRANULOSUS

PROBLEM

Knowledge on the permeability characteristics and the transport control mechanisms of the hydatid cyst membranes is of the utmost importance in the rational design of chemotherapeutic studies.

One of the first questions is whether a substance of known capacity to interfere with important metabolic functions is able to act on the parasite itself or on the cyst membranes. Although it has been implied that a damaging action can be exerted from the external side of the cysts, it is generally agreed that the substance must penetrate into the cyst to exert its action.

Therefore it is relevant to know what are the regulatory mechanisms that control the permeability of the cystic membranes, in both directions, to water, electrolytes, and nonelectrolytes; and the possible effects of charged groups and side chains that on modifying the structure, change the permeability of the selected chemical substances. This information could be used in introducing slight structural changes in known parasitocides to facilitate entrance into the cyst without losing its desired effects.

METHOD

Several techniques were devised to determine flux kinetics under steady state conditions of titrated water, Cl^{36} and Na^{24} .

Cysts deriving from experimental secondary infection in mice of a known age of infection were chosen for influx and efflux studies. In the case of titrated water the effect of the antidiuretic hormone was also studied.

RESULTS TO DATE

The hydatid cyst wall (laminated plus germinative membrane) has very low Na^+ and Cl^- permeabilities and a very high diffusional permeability to water that increases in the presence of the antidiuretic hormone after an incubation period.

SIGNIFICANCE

Although the cyst is very permeable to water, the results for ion permeability indicate that the membrane does not behave like a collander but instead has regulatory mechanisms that limit the influx and efflux of charged small ions.

Although the mechanisms of this transport have not been elucidated and more knowledge is needed in this direction, the possibility to alter them through chemotherapeutic agents opens a more rational approach to treatment.

PUBLICATIONS

1. Perez-Esandi, M. V. et al. The chemical composition of hydatid cyst fluid Echinococcus granulosus: relationship to studies in chemotherapy, in preparation.
2. Rotunno, A. et al. Studies on the permeability to water, sodium, and chloride of the hydatid cysts of Echinococcus granulosus, in preparation.

OTHER DATA

Grantee:	Dr. William S. Kammerer and Mr. M. V. Perez-Esandi, Lic. Chem., Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.
Timetable:	1972-1973.

A STUDY OF HOST-PARASITE RELATIONSHIPS FOLLOWING
PRIMARY ORAL INFECTION OF LABORATORY ANIMALS
WITH ECHINOCOCCUS GRANULOSUS

PROBLEM

Common livestock species, the natural intermediate hosts of E. granulosus, are not usually practical for experimental studies because of their large size and maintenance cost, and the slow development of the parasite in these hosts. A major advance in the development of laboratory animal models of this disease was the finding that white mice and some other animals were susceptible to the intraperitoneal inoculation of protoscolices. In this way the parasite could be maintained by serial passage of protoscolices without allowing the completion of the parasite's cycle to the infective adult stage, thus obviating the concomitant dangers to technicians and animal caretakers. Although this method permits the prolonged laboratory maintenance of the parasite, it is not suitable for all studies because it does not allow the full natural development of the parasite in the intermediate host from egg to larval cyst. Furthermore, some workers had experienced difficulties in avoiding the incidental production of peritonitis in inoculated animals. It had been previously shown in the Pan American Zoonoses Center's laboratories that white mice and jirds (Meriones unguiculatus) were susceptible to the intraperitoneal inoculation of eggs with the involvement of a high proportion of the embryos. This is useful but again presents the problem of peritoneal bacterial infection incidental to inoculation and does not permit the parasite to follow its normal infective route, i.e., by penetration of the intestinal mucosa. It was believed that primary oral infection of white mice and jirds, if successful, would provide an excellent laboratory animal system while avoiding the inconveniences mentioned above.

METHOD

The oral infectivity of E. granulosus eggs to white mice and jirds is assayed, evaluating the effect of egg dosage and host age, sex, and genetic strain.

Experimental animals are sacrificed at periodic intervals to determine cyst growth and the host's immunologic and histopathologic responses.

RESULTS TO DATE

Both mice and jirds were determined to be highly susceptible to the oral administration of 1,000 eggs of E. granulosus. In jirds the majority of cysts were localized in the lungs, while in white mice most cysts were located in the liver.

In both mice and jirds, a direct relationship was observed between the number of eggs ingested and the magnitude of the resulting cyst burdens; immature animals were consistently more susceptible than older animals but no effect of host sex on susceptibility was noted. Certain strains of mice were markedly more susceptible than others.

SIGNIFICANCE

Primary oral infection in white mice and jirds could prove very useful for immunologic and chemotherapeutic studies of larval E. granulosus in laboratories prepared to work with infective eggs.

Information about the host's serologic response and histopathologic reaction is providing new information useful for the interpretation of immunodiagnostic tests in other species.

PUBLICATIONS

1. Colli, C. and P. M. Schantz. Growth and development of Echinococcus granulosus oncospheres in mice. J Parasitol 59: December, 1973.
2. ----- and ----- . Estudio y desarrollo de modelos experimentales de infección con huevos de Echinococcus granulosus en roedores de laboratorio. Arch Int Hídat 26: in press.
3. ----- and ----- . Influence of dose, age, and sex in the susceptibility of white mice and jirds (Meriones unguiculatus) to primary Echinococcus granulosus infection, in preparation.

OTHER DATA

Grantee: Dr. Peter M. Schantz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1970-1974.

STUDIES OF ECHINOCOCCOSIS IN ARGENTINE SYLVATIC ANIMALS

PROBLEM

Three species of fox from Argentina had previously been reported infected with adult Echinococcus spp. The role of these animals in the dissemination and transmission of E. granulosus to man and domestic animals was never clarified and the species identification of the parasites remained controversial.

Because of the potential effect of these factors on the control of E. granulosus infection, a study was initiated to determine the prevalence and species identification of Echinococcus spp. in sylvatic definitive and intermediate hosts as well as the significance of these findings.

METHOD

A wide variety of sylvatic animals, both potential definitive and intermediate hosts, are captured and examined by necropsy in search of adult or larval Echinococcus parasites. Recovered parasites are used for studies of experimental transmission to domestic and laboratory animal species or stained for taxonomic studies. These parasites are compared morphologically with E. granulosus recovered from experimental infections of dogs and Argentine sheep.

The susceptibility of sylvatic animals to larval and adult E. granulosus is determined by experimental infection of animals maintained in captivity with protoscolices or eggs of E. granulosus.

RESULTS TO DATE

A survey carried out in the Argentine province of Neuquen revealed adult-stage infection in 30 percent of 25 South American red foxes (Dusicyon culpaeus) and larval infection in 4 of 71 European hares. No other infections were observed among four other carnivorous species and six other potential intermediate-host species. This was the first report of naturally acquired Echinococcus infection in Lepus europaeus and the first association in South America of infection in foxes with larval infection in sylvatic animals.

Experimental infection of Dusicyon culpaeus with protoscolices of ovine origin showed that species to be susceptible to E. granulosus with the development of many gravid worms 80 days after infection.

D. gymnocercus and D. griseus were also susceptible but markedly less so than D. culpaeus. The parasites in foxes, although fully developed and containing embryophores that were infective to experimental animals, were slightly different morphologically as compared with worms from control dogs. This host-induced morphologic variation may account for the previous taxonomy confusion caused by the presence of Echinococcus worms in foxes.

The finding of Echinococcus oligarthrus (Diesing 1863) in Geoffroy's cats (Felis geoffroyi) captured in La Pampa province confirmed the presence in Argentina of more than one species of Echinococcus.

SIGNIFICANCE

The role of sylvatic animals in the epidemiology of E. granulosus has potential implications for the possible control of this infection. If foxes, for example, may serve as a source of infection to domestic intermediate hosts, it is clear that control measures that successfully interrupt the cycle of transmission between dogs and sheep will not necessarily achieve complete control of the disease.

On the other hand, some or all species of domestic animals locally present, as well as man, may be resistant to the parasite from foxes, and the sylvatic cycle may thus have little bearing on a control program. It is important, therefore, to determine these factors in any area where control programs are being contemplated.

PUBLICATIONS

1. Schantz, P. M. et al. Echinococcus infection in South American red fox (Dusicyon culpaeus) and the European hare (Lepus europaeus) in the Province of Neuquen, Argentina. Ann Trop Med Parasitol 66:479-85, 1972.
2. ----- and C. Colli. Echinococcus oligarthrus (Diesing 1863) from Geoffroy's cats (Felis geoffroyi) in Argentina. J Parasitol 59:December, 1973.

3. ----- et al. On the morphology and biology of adult-stage Echinococcus granulosus (Batsch, 1786) from Argentine domestic and sylvatic animal hosts, in preparation.
4. ----- et al. Susceptibility of Argentine sylvatic carnivores to Echinococcus granulosus (Batsch, 1786),
5. -----, Echinococcus spp. (Cestoda: Taeniidae) y su biología. Proc First Argent Congr Parasitol, in press.

OTHER DATA

Grantee: Dr. Peter M. Schantz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1971-1974.

EPIDEMIOLOGIC STUDIES ON HYDATID DISEASE IN THE AMERICAS

PROBLEM

The lack of available data on the incidence, prevalence, and economic and public health significance of hydatid disease in the Americas is a barrier to the effective evaluation of control measures. Investigative efforts need to be initiated in each of the severely affected countries of Latin America, and prophylactic steps must be taken to prevent introduction of the disease into those areas now free of infection.

METHOD

Present data about the prevalence and distribution of hydatid infection in man and lower animals are collated for the countries of the Americas. This information is regularly updated as new studies are performed.

Field visits are made to countries about which no information is available or about which additional information is required to perform necessary surveys. In other cases retrospective hospital surveys or diagnostic surveys of dogs or livestock are carried out in collaboration with national authorities.

Advice and material assistance is provided for the preparation of research projects and pilot control programs.

RESULTS TO DATE

An account and discussion of the actual state of hydatidosis in the Americas has been published, based on information in scientific publications and government reports. It was pointed out that the major problem areas include Argentina, Chile, southern Brazil, the mountainous portion of Peru, and Uruguay, where, aside from the magnitude of the problem in humans, large economic losses are suffered because of the existence of the disease in livestock.

A comprehensive study carried out in an endemic focus in Argentine Patagonia demonstrated a very high rate of infection in humans and identified a variety of socioeconomic and cultural factors associated with parasite transmission.

A survey of infection in humans, dogs, and livestock in Uruguay for the period of 1962-1971 was completed in collaboration with the Uruguayan National Hydatid Control Commission. It was learned that on a nationwide basis, human prevalence was higher in Uruguay than in any other country from which information has been reported.

Visits to Peru and Bolivia have revealed new information about the disease, and for the latter country, verified the existence of the infection in man, dogs, and livestock in the altiplano.

Assistance and advice were given in Argentina and Uruguay about the progress of pilot control programs, and a report was made to the Government of Peru concerning the implementation of control measures in the Central Sierra.

SIGNIFICANCE

A realistic assessment of the present situation is essential for effective future evaluation of control efforts against hydatidosis. The research under way should provide much of the data needed.

PUBLICATIONS

1. Purriel, P. et al. Human hydatidosis in Uruguay: indices of morbidity and mortality, 1962-1971. Bull WHO, in press.
2. Schantz, P. M. Magnitud del problema y perspectivas de control. Bol Of Sanit Panam 73:187-97, 1972.
3. ----- . Localización de la hidatidosis en el sistema nervioso central. Bol Of Sanit Panam 73:198-202, 1972.
4. ----- et al. The epidemiology of hydatid disease in southern Argentina. Comparison of morbidity indices, evaluation of immunodiagnostic tests and factors affecting transmission. Am J Trop Med Hyg 22:629-41, 1973.

5. ----- . La vigilancia epidemiológica de la hidatidosis. El Tórax (Montevideo), in press.
6. ----- . La hidatidosis en el Peru. Situación actual y recomendaciones para el control, in preparation.
7. ----- and S. Clivio. Hallazgo de Taenia ovis Cobbold, 1860, en un perro del Departamento de Flores, Uruguay. El Tórax (Montevideo), in press.
8. Williams, J. F. et al. An account of the current incidence and distribution of hydatidosis, with special reference to the Americas. Am J Trop Med Hyg 20:224-36, 1971.

OTHER DATA

Grantee: Dr. Peter M. Schantz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1969-1974.

IMMUNOLOGIC STUDIES OF OVINE HYDATIDOSIS

PROBLEM

No methods are currently available for the diagnosis of ovine hydatidosis in vivo. An immunologic approach appears to be most promising and of great potential use in studies of the infection's prevalence in large animal populations. Sheep have been shown to respond immunologically to infection with Echinococcus granulosus, but the specificity of the serologic reactions studied is extremely low. The spectrum of immunologic responses in sheep has not been investigated.

METHOD

The diagnostic value of an intradermal test and of several serologic techniques is evaluated, using sheep naturally and experimentally infected with E. granulosus.

The intradermal test is evaluated at different skin sites and with different methods of interpretation. The serologic techniques employed include indirect hemagglutination, latex agglutination, and immunoelectrophoresis. Immunodiagnostic test results are associated with the stage and degree of infection, as determined at autopsy. Identification of certain other parasites is made to establish the possibility of cross-reactions.

An attempt is made to determine the class or classes of immunoglobulins participating in these reactions, including the homocytotropic antibody detectable by perchloric acid (PCA) reactions.

RESULTS TO DATE

Immunodiagnostic test results have been found to be quite variable among animals grazed in Azul, Argentina. Detectable immunologic responses are found in some but not all infected animals, and many positive results are found in animals with no apparent cestode infection as well as those with the closely related cestode Taenia hydatigena.

Homocytotropic antibody specific to hydatid antigens was detectable by PCA reactions in sheep, although positive animals were not always infected with E. granulosus.

SIGNIFICANCE

Knowledge of the significance of immunologic responses in sheep, the sequential appearance of such responses, and the role of allergic mechanisms in the host-parasite relationship should be gained from this research. Results obtained from herds living in an environment with a high risk natural exposure will be of doubtful value, however.

Studies of the anaphylactic response of sheep to hydatid fluid antigens may have special relevance, since anaphylatic responses are a common feature of human hydatidosis. In addition, the possibility exists of developing a rapid field test, based on immediate skin reactions, for hydatidosis in sheep.

PUBLICATIONS

1. Schantz, P. M. Immunodiagnostic tests with Echinococcus antigens in sheep with homologous and heterologous larval cestode infections. Rev Inst Med Trop São Paulo 15:179-94, 1973.
2. ----- Homocytotropic antibody to Echinococcus antigen in sheep with homologous and heterologous larval cestode infection. Am J Vet Res 34:1179-81, 1973.

OTHER DATA

Grantee:	Dr. Peter M. Schantz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Government of Argentina.
Timetable:	1969-1973.

EVALUATION AND STANDARDIZATION OF THE TECHNICAL VARIANTS OF THE IMMUNOELECTROPHORESIS TEST FOR THE DIAGNOSIS OF HUMAN HYDATID DISEASE

PROBLEM

The value of the immunoelectrophoresis (IEP) test for the diagnosis of human hydatidosis has been studied by several investigators in different areas of the world. Various laboratories perform this test under different conditions: supporting medium (agar or agarose); voltage and timing of run; antigens (whether whole or purified hydatid fluid); their concentration and the way it is determined; their characteristics, whether immunologically defined or based on its host source); the size and shape of the antigen well; basis on which the diagnosis is made. In view of the foregoing, the need to evaluate these variants and standardize the test seems evident. This evaluation is also necessary to recommend the test (with the variants of choice) to hydatidosis laboratory workers.

The present study deals with the comparison of the above-mentioned technical variants to select the optimal conditions for performing the IEP test under standard criteria.

METHOD

Antigen: study of the frequency of antigens for the E. granulosus specific "arc 5" of Capron et al. in hydatid fluid (HF) pools collected from individual sheep and in large pools of fluid collected from many animals.

Comparison of antigens from ovine and bovine material.

The same rabbit antisera to HF and serum from hydatidosis patients, and the same antigen lot were used to study the effects on the morphology, mobility, and detectability of the diagnostic "arc 5" of the antigen concentrations, size, and shape of the antigen well, voltage, and time of run used by different authors. These variants were examined with agarose as supporting medium and also with agar and the purified antigen of Oriol et al.

RESULTS TO DATE

The number of precipitation bands and the presence of the E. granulosus-specific "arc 5" are the diagnostic criteria used by different authors. The diagnostic specificity of the latter makes it the criterion of choice. The most favorable conditions were with agarose as the supporting medium, a rectangular antigen well, 200 mg/ml antigen (lyophilized dry weight) run for 1 1/2 hours at a potential difference of 20V per slide. Addition of brom-phenol blue at the level of the antigen and electrophoresing it until it had moved 3.5 cm was a satisfactory reference for antigen migration. The diagnostic "arc 5" was found in individual and pooled HF from sheep. The optimal conditions for using the purified HF antigen were the same as those for the standardized whole HF antigen except that a concentration of 30 mg/ml was found to be preferable.

SIGNIFICANCE

Pools of sheep HF were found to contain the antigen(s) for the E. granulosus-specific "arc 5"; HF prepared in the manner described above is therefore an adequate source of large volumes of antigen that can be standardized and used in the IEP test.

The technical variants of the IEP test were compared. A standardized antigen and a unified technique may now be proposed that will allow the comparison of results obtained in different laboratories.

PUBLICATIONS

Manuscript in preparation.

OTHER DATA

Grantee: Dr. Victor M. Varela-Díaz, Pan American
Zoonoses Center, Ramos Mejía, Buenos Aires
Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1972 - 1973.

EVALUATION OF IMMUNODIAGNOSTIC TESTS IN EPIDEMIOLOGIC SCREENING FOR HYDATID DISEASE

PROBLEM

The most commonly used index of human hydatid infection is the annual incidence of surgical cases. This is at best only a crude estimate of infection rates because of the long period in which the patient may remain asymptomatic (in some cases this may be his lifetime). The value of this rate as an index for comparison with other countries or regions is reduced because reporting rates and the availability of medical facilities vary widely between countries and regions. The results of mass miniature radiographic surveys provide useful prevalence information but only detect intrathoracic cysts. Furthermore, this type of survey is expensive and is being discarded in many areas as a traditional tuberculosis control measure.

Immunodiagnostic tests have not yet been evaluated as epidemiologic screening tools for hydatid disease, although they would appear to offer potential advantage because of their relatively low cost and the fact that indices thus obtained would not be affected by administrative and political factors.

METHOD

The general modus operandi of this study is to screen population samples in different geographic localities with the intradermal and a variety of serologic tests (where possible, this screening is done in conjunction with mobile chest X-ray units, thereby giving thoracic radiographs of each person tested). Serum samples are obtained and studied in the indirect hemagglutination, latex agglutination, and immunoelectrophoresis serologic tests for hydatid disease. Serologically positive persons are examined further by clinical methods (e.g. liver screening, cholangiography, and others) to determine, as far as possible, the presence or absence of liver cysts.

These studies are carried out on people of all ages and both sexes to determine age or sex differences in susceptibility. They are also performed in populations with different prevalences of other parasitic infections to determine the possibility that the other infections may cause cross-reactions to hydatid antigens.

RESULTS TO DATE

Seroepidemiologic screening has been carried out in populations in Buenos Aires, Río Negro, and Neuquén Provinces, Argentina, and in Uruguay, Bolivia, and Peru. The Río Negro findings have been published and the results in other populations are being prepared for publication. The Casoni intradermal test has been shown to be highly sensitive but relatively nonspecific in comparison with the serologic tests. Non-specificity was a particular problem in Peru where infection by Hymenolepis, Fasciola, and taeniids have been shown to give nonspecific hypersensitivity reactions to Echinococcus antigens.

The serologic procedures have proved effective for the detection of hydatid cyst carriers, though not all infected persons have a detectable serologic response. Blood drops eluted from filter papers has been shown to be a practical and efficient method for large-scale serologic screening using the indirect hemagglutination test.

SIGNIFICANCE

Immunodiagnostic tests, if properly standardized and evaluated, are a potentially useful and economic tool to obtain human prevalence data on hydatid infection in different endemic populations and would allow the obtention of important epidemiologic information.

PUBLICATIONS

1. Purriel, P. and P. M. Schantz. A seroepidemiological study of hydatid disease in the Department of Flores, Uruguay, in preparation.
2. Schantz, P. M. et al. The epidemiology of hydatid disease in southern Argentina. Comparison of morbidity indices, evaluation of immunodiagnostic tests and factors affecting transmission in southern Río Negro Province. Am J Trop Med Hyg 22:629-41, 1973.
3. -----, Algunas técnicas para determinar la prevalencia de la hidatidosis. Arch Inter Hidatid 26: in press.

4. ----- and L. A. Yarzábal. Comparative sensitivity and specificity of the intradermal and immunoelectrophoresis tests for hydatid disease, in preparation.
5. ----- et al. Reacciones falso-positivas en la reacción de Casoni para la hidatidosis en personas con otras enfermedades parasitarias, in preparation.

OTHER DATA

Grantee: Dr. Peter M. Schantz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1970-1974.

EVALUATION OF THE IMMUNOELECTROPHORESIS TEST USING WHOLE AND PURIFIED HYDATID FLUID ANTIGENS FOR THE DIAGNOSIS OF HUMAN HYDATIDOSIS

PROBLEM

The immunoelectrophoresis (IEP) test is reported to be more sensitive using antigens for the "arc 5" than using the purified sheep hydatid fluid antigen. The comparative sensitivity of both antigens and their respective diagnostic criteria cannot be ascertained because the conditions under which they were evaluated differ in the techniques and sera used.

METHOD

To determine the sensitivity and specificity of the two antigens, the IEP test for hydatid disease is performed under standard conditions and whole and purified hydatid antigens are used simultaneously on the sera of the same patients. The optimal conditions for each test were selected in the preceding project.

RESULTS TO DATE

Results are being tabulated. Data are not presently available because the sera were coded before testing.

SIGNIFICANCE

Knowledge of the relative merit and limitations of each antigen will provide us with a rational basis for recommending their use to workers in the field.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Victor M. Varela-Díaz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1972-1974.

PASSAGE OF HOST IMMUNOGLOBULINS INTO HYDATID CYSTS

PROBLEM

Previous work in our laboratory demonstrated the presence of the species-specific antigenic determinants of host immunoglobulins in hydatid cyst fluid from different host species, suggesting that these molecules entered the parasite from the surrounding host tissue. The possibility of a parasite origin of these proteins was discarded. Evidence of the actual penetration of these immunoglobulins into the cysts is needed to support this conclusion.

METHOD

Hydatid cysts from mice were transplanted into the peritoneal cavity of guinea pigs. Three months after cyst transplantation the recipient animals were necropsied, the cysts (viable) removed, and the fluid collected, frozen at -50° and lyophilized.

The fluid pools were reconstituted to 1/20th of their original volume and analyzed by Ouchterlony tests using species-specific antisera to mouse and guinea pig IgG.

RESULTS TO DATE

The species-specific antigenic determinants of both donor's and recipient's immunoglobulins were found in the fluid of mouse hydatid cysts transplanted into guinea pigs 3 months previously.

SIGNIFICANCE

The present findings showing that host immunoglobulins penetrate healthy hydatid cysts are important because of the possibilities for immunologic or chemotherapeutic elimination or sterilization of the parasite. Further studies along these lines are the subject of interest in our laboratory. These observations lend support to the suggestion^{1/}

that host immunoglobulins reach the hydatid fluid by diffusion through the cyst membranes from the surrounding host tissues. The hypotheses that the laminated membrane acts as a macromolecular filter and that these immunoglobulins are formed by the parasite through host induction or evolution are shown not to be applicable to the present observations.

PUBLICATIONS

1. Varela-Díaz, V. M. and E. A. Coltorti. Further evidence of the passage of host immunoglobulins into hydatid cysts. J Parasitol 58:1015-16, 1972.

OTHER DATA

Grantee:	Dr. Victor M. Varela-Díaz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Government of Argentina.
Timetable:	1972-1973.

THE PRESENCE OF HOST IMMUNOGLOBULINS IN THE HYDATID CYST MEMBRANE

PROBLEM

Several hypotheses have been put forth to account for the prolonged survival of parasites in immunologically competent hosts. These are based either upon antigenic sharing between host and parasite as a result of host induction, evolution, and phenotypic adaptation, or upon bound host antibody. The need to test these hypotheses is of interest to the understanding of the mechanisms involved in cyst survival.

The present study was designed to determine if host immunoglobulins or other components are detectable in the membranes of Echinococcus granulosus cysts.

The results will prove or disprove the above hypotheses and those of studies on cestode protein structure and hydatid serology found in the literature.

METHOD

Healthy, viable hydatid cysts were collected from sheep and mice. Mouse cysts were also collected 3 months after their transplantation into recipient guinea pigs.

Membranes were thoroughly washed and ground in a vortex and antisera to each membrane homogenate were prepared in rabbits. Antisera to the supernatant water were prepared using a sample collected at the end of the washing process.

Host sera were separated electrophoretically and precipitation patterns developed with the antisera to the cyst membranes from the corresponding host species. Antisera were absorbed with normal serum from the homologous and heterologous hosts to determine whether they recognized host species-specific serum antigens. Antisera to the supernatant water was used as a control on the effectivity of the washing process.

RESULTS TO DATE

Immunoelectrophoretic analyses revealed that the antigenic determinants for the IgG immunoglobulins of the host were found in the membranes of hydatid cysts from a natural (sheep) and an unnatural (mouse) host and that those of both donor and recipient immunoglobulins were found after transplantation of mouse cysts into guinea pigs. The exquisite host specificity of these immunoglobulins suggests that these "shared antigens" are not synthesized by the parasite during the course of evolution. Since a large part of the parasite genome would be necessary to code such fine antigenic variations, host induction may be equally disregarded in the explanation of these findings. A host origin of these immunoglobulins seems more likely.

SIGNIFICANCE

It is possible that the immunoglobulins detected in the cyst membranes represent host molecules trapped while diffusing to the interior of the cyst bladder and aggregated during the washing process. The greater solubility of albumin in water may account for their absence. These immunoglobulins may also represent host contaminants adsorbed to the parasite (phenotypic adaptation) or alternatively antibodies bound to parasite antigens.

Further studies are in progress to examine these alternatives.

The presence of host IgG in the hydatid cyst membrane indicates that in the published reports on the chemical analysis of the structural proteins and amino acids of metacestodes (and possibly of other helminths), the differentiation of parasite components from host immunoglobulins (and possibly other host protein contaminants) may be difficult to assess.

False-positive reactions in nonhydatid patients with serologic tests for hydatid disease have been attributed to the host serum components in hydatid fluid antigens. The present findings indicate that the same problem may arise when antigens prepared from cyst membranes, are used in hydatid serology.

PUBLICATIONS

1. Varela-Díaz, V. M. and E. A. Coltorti. The presence of host immunoglobulins in the hydatid cyst membranes. J Parasitol 59:484-88, 1973.

OTHER DATA

Grantee: Dr. Victor M. Varela-Díaz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1973.

LOCALIZATION OF HOST IMMUNOGLOBULINS ON THE PARASITE MEMBRANES OF ECHINOCOCCUS GRANULOSUS CYSTS

PROBLEM

Host immunoglobulins of the IgG class were found in the parasite membranes of hydatid cysts from several host species. It is not known whether these immunoglobulins are found in the laminated or the germinal layer of the cyst, or both.

The present study was designed to determine the localization and distribution pattern of these immunoglobulins on the parasite membranes of healthy, viable Echinococcus granulosus cysts.

METHOD

Mouse, human, and sheep hydatid cysts, as well as mouse cysts transplanted into gerbils were used. Cryostat sections of the mouse cysts and paraffin-embedded sections of the other cysts were made and examined by the indirect fluorescent antibody test (IFAT) using rabbit-antihost IgG and a goat-antirabbit IgG conjugated serum and the corresponding controls.

Sheep cyst sections were also examined by IFAT using rabbit-antisheep albumin.

RESULTS TO DATE

These studies revealed the presence of host IgG and albumin on the laminated membrane of hydatid cysts. Neither IgG nor albumin were found on the germinal membrane.

SIGNIFICANCE

The presence of host IgG and albumin on the laminated membrane indicates that these molecules are trapped while in transit through the parasite membranes. The finding of both donor and recipient-

specific immunoglobulin determinants in transplanted cysts further supports this conclusion. Whether some of these immunoglobulins are antibodies directed against the laminated layer antigens or represent host contaminants with survival value to the parasite remains to be determined.

The finding of these immunoglobulins suggests that if cytotoxic antibodies to germinal layer or scolex antigens could be induced, these could penetrate through the laminated layer to exert their action on the germinal layer or scoleses.

PUBLICATIONS

1. Coltorti, E. A. and V. M. Varela-Díaz. Localization of host immunoglobulins on the parasite membranes of Echinococcus granulosus cysts. Exp Parasitol, 1974, in press.

OTHER DATA

Grantee:	Dr. Victor M. Varela-Díaz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Government of Argentina.
Timetable:	1973-1974.

EXPERIMENTAL CHEMOTHERAPY OF LARVAL ECHINOCOCCUS GRANULOSUS INFECTION IN LABORATORY ANIMALS

PROBLEM

Hydatid disease is one of the major parasitic diseases of man and domestic animals in parts of Latin America, for which no effective chemotherapy exists. For humans, surgery is the only recourse, with all the risks and economic burden that it involves.

Until successful control is achieved an effective chemotherapy would find immediate application in human disease and possibly in control programs.

METHOD

Experimentally infected laboratory rodents such as albino random CF₁ mice from Carworth Lab. Cages Inc., and jirds (Meriones unguiculatus) inoculated intraperitoneally with 1,000 viable sheep protoscolices 2 days to 8 months before their inclusion in chemotherapy trials, were used.

Several drugs were selected on the basis of their known interference with important metabolic functions in bacteria, fungi, and helminths, and others because of their reported efficacy against infection in humans.

RESULTS TO DATE

Trials made with eight compounds in mice and jirds gave negative results. There was no statistically significant difference between treated and control groups with respect to number or size of cysts.

SIGNIFICANCE

Although the group of selected drugs were not effective in reducing significantly the size or number of cysts with respect to the controls, under the experimental conditions used, the importance

of this disease from the human and economic point of view, calls for a continuous effort in this direction.

PUBLICATIONS

1. Kammerer, W. S. and M. V. Perez-Esandi. The current status of chemotherapy in hydatid disease. Bol Of Sanit Panam 7:47-52, 1973.
2. ----- and ----- . Chemotherapy in experimental E. granulosus infection, ready for publication.

OTHER DATA

Grantee: Dr. William S. Kammerer and Miss Miguella Perez-Esandi, Lic. Chem., Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1972-1973.

STUDIES OF THE SUSCEPTIBILITY OF ECHINOCOCCUS GRANULOSUS EGGS TO SELECTED CHEMICAL AND PHYSICAL AGENTS

PROBLEM

Although the microanatomy of the eggs and embryos of taeniid tapeworms has been thoroughly documented, the physiologic characteristics of these organisms are not well defined. Establishment of the conditions required for continued survival and infectivity of E. granulosus eggs, in the field and the laboratory, could be of immediate importance to control and investigational programs. The susceptibility of eggs to extremes of temperature, desiccation, ionizing radiation, chemical compounds, and various natural microclimates still requires precise determination.

METHODS

Eggs of E. granulosus are obtained from gravid proglottids of the adult tapeworm recovered from dogs. Samples of eggs are then subjected to the following conditions: extremes of temperature, ultraviolet and X-irradiation, taenicial drugs and other chemicals that might have ovicidal properties, and exposure to a series of controlled, artificial microenvironments.

The infectivity of treated eggs and of those maintained under control conditions is then determined by oral and intraperitoneal inoculation into white mice or jirds.

RESULTS TO DATE

The effects of extremes of temperature and X-irradiation upon the embryospheres have been determined and the results published.

Among a variety of chemicals tested, the anthelmintic drug bunamidine hydrochloride had a marked in-vitro ovicidal effect when contained in proglottids or loose in solution. Eggs produced by parasites that had survived previous treatment with bunamidine were infective to mice.

SIGNIFICANCE

There now exists no known chemical compound that, at practical concentration, effectively destroys E. granulosus eggs and thus permits the sterilization of laboratory surfaces and instruments. The results of these studies are expected to yield information that will remedy this deficiency.

The finding of the ovicidal properties of bunamidine hydrochloride may have important consequences in regard to the value of this drug in the treatment of infected dogs.

The findings of the effects of X-irradiation upon embryos may permit the development of effective immunization procedures using eggs attenuated by X-irradiation.

PUBLICATIONS

1. Colli, C. W. and J. F. Williams. Influence of temperature on the infectivity of eggs of Echinococcus granulosus in laboratory rodents. J Parasitol 58:422-26, 1972.
2. Williams, J. F. and C. W. Colli. Influence of ionizing irradiation on infectivity of eggs of Echinococcus granulosus in laboratory rodents. J Parasitol 58:427-30, 1972.
3. ----- and ----- . Influence of bunamidine hydrochloride on the infectivity of eggs taeniid tapeworms. J Parasitol, in press.

OTHER DATA

Grantee: Dr. Peter M. Schantz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1969-1973.

STUDIES ON ANTIHELMINTHICS ACTIVE AGAINST ECHINOCOCCUS GRANULOSUS IN DOGS

PROBLEM

An effective chemotherapeutic agent against the intestinal phase of Echinococcus granulosus would find immediate application in control programs in many countries. The drugs tried so far have all had drawbacks: low or unsatisfactory level of activity (arecoline), prohibitive cost (Yomesan), or toxicity (hexachlorophene).

Bunamidine hydrochloride has shown promise in the treatment of canine echinococcosis; it requires, however, three successive treatments to insure the elimination of all worms and at the recommended dosage, is lethal to some dogs. Other drugs are worthy of attention, although the lack of basic knowledge on the host-parasite relationship in canine infection renders drug trials both expensive and difficult to interpret.

It has also been consistently observed that bunamidine is less effective against immature than mature Echinococcus in the dog. The reasons for this are not clear, but one hypothesis suggests that there is less contact between parasite and drug in the immature form because of their smaller size and the protective effect of intestinal mucus.

METHOD

New drugs having known taenicial activity are being screened in preliminary trials involving small groups of dogs. Those worthy of further investigation are examined in large-scale trials, and their activity is critically evaluated.

Some of the factors influencing the evolution of infection with E. granulosus in dogs are being examined, including immune responses, background level of infection in the natural population, and histopathologic responses of the intestine during infection. Studies will also be done on the standardization of methods for determining infective doses.

To increase contact between bunamidine and the immature Echinococcus, various measures are contemplated, including the use of organic solvents, antiperistaltics, and topical anesthetics.

RESULTS TO DATE

Preliminary analysis of results in 60 dogs, comparing bunamidine alone with bunamidine given with the organic solvent dimethyl sulfoxide indicated no specific advantage to the combination. Two other promising compounds are being studied in critical trials that are not yet completed.

SIGNIFICANCE

The major drawback to the use of bunamidine is its lack of effectiveness against immature stages of Echinococcus. Until a better drug is found, ways to increase its effectiveness against these stages should be investigated.

PUBLICATIONS

1. Kammerer, W. S. Treatment of Echinococcus infection in dogs. Proc First Argent Cong Parasitol, in press.
2. Schantz, P. M. Guía para el empleo del bromhidrato de arecolina para la diagnosis de la infección por Echinococcus granulosus en el perro. Bol Chil Parasitol 28: in press.

OTHER DATA

Grantee: Dr. Peter M. Schantz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1960-1973.

ANTIBIOTIC RESISTANCE PATTERNS AND RESISTANCE TRANSFER
FACTOR OF ENTEROBACTERIACEAE ISOLATED FROM ANIMALS,
FOODS OF ANIMAL ORIGIN, AND FOOD HANDLERS

PROBLEM

The increasing number of antibiotic-resistant Enterobacteriaceae capable of transferring such resistance to other microorganisms, which has been evidenced in recent years in isolates from animals and foods of animal origin, seems to be due to the indiscriminate use of antibiotics for nontherapeutic purposes. The impact of this phenomenon on public health is obvious, since among other reasons it makes the treatment of human diseases caused by these organisms more difficult.

The purpose of this project was to study the antibiotic resistance patterns and the presence of resistance transfer factors (RTF) in Enterobacteriaceae isolated from horses and cattle destined for human consumption that had not received any antibiotic either in their feeding or for prophylactic purposes. Enterobacteriaceae (Escherichia coli) and Salmonellae) isolated from the meat of these animals and from meat handlers were also studied.

This project is part of a cooperative study with the U.S. Food and Drug Administration.

METHOD

E. coli and Salmonella were isolated in fecal samples from horses, cattle, and meat handlers, and in swabs taken from the carcasses of the animals. The sensitivity tests to antibiotics (ampicillin, tetracycline, chloramphenicol, streptomycin, kanamycin, and nalidixic acid) were performed by the Bauer and Kirby technique. Multiresistant strains (resistant to two or more drugs) were studied for the presence of RTF using the method described by Schroeder et al.

RESULTS TO DATE

Enterobacteriaceae (E. coli and Salmonella) isolated from feces of 40 horses, 40 cattle, and 160 meat handlers and in surface swabs of 40 horse and 40 cattle carcasses were studied. In total, 385 E. coli

strains from food of animal origin and 380 strains of human origin were isolated, together with 8 Salmonella strains of animal origin and 27 of human origin.

None of the Salmonella strains proved to be multiresistant to antibiotics assayed.

Thirty nine strains of E. coli showed multiresistance. All of them had been isolated from human beings. Of these 39, 26 were capable of transferring their resistance.

SIGNIFICANCE

The results obtained demonstrate the absence of antibiotic-resistant organisms in the animals and their carcasses that were studied. Such results were to be expected as the drugs had not been administered to them for either prophylactic or nutritional purposes. The percentage of resistant and RTF-positive isolates of human origin is not surprising since the use and abuse of antibiotics is more frequent in man.

PUBLICATIONS

1. Michanié, Silva et al. Transferable drug resistance among E. coli and Salmonella isolated from horses and horse meat handlers. Rev Lat Am Microbiol 14:137-40, 1972.
2. ----- et al. Transferable drug resistance among E. coli isolated from cattle and meat handlers, in preparation.

OTHER DATA

Grantee: Dr. Fernando Quevedo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1970-1971.

IMPROVEMENT OF LABORATORY METHODS FOR RABIES DIAGNOSIS

PROBLEM

Laboratory methods for rabies diagnosis are not completely efficient. The lack of sensitivity of the Seller's stain and the time required for the performance of the immunofluorescence (IF) and mouse inoculation tests justify studies to correct some of these drawbacks.

In spite of the fact that several fixatives, times of fixation, and time and temperature of incubation have been reported for IF, the standard technique calls for air drying of the impressions for 30 minutes, fixation in acetone for 4 hours and incubation at 37°C for 30 minutes--a total of approximately 5 1/2 hours for the whole test. Other antigens do not require fixation for the IF test and it is known that the initial combination between antigen and antibody takes only a few seconds.

The direct and indirect immunoenzyme methods have been employed for rabies but they have not been evaluated in a large number of samples. Furthermore, it has been reported that antirabies nucleocapside serum could be more specific for the detection of rabies antigen than serum prepared with whole virus particles.

METHOD

Impressions obtained from brain samples received for rabies diagnosis were fixed in acetone at different intervals while some were kept unfixed. All the impressions were stained in accordance with the standard IF technique for rabies, and the results evaluated.

In the second phase of this study, impressions were fixed with the method that appeared fastest in phase 1. These impressions were stained at different temperatures and times. These results were also evaluated by comparison with those obtained from the classical rabies IF test.

In a third phase of this study, rabies immunoperoxidase will be prepared as described. The immunoenzyme staining technique will be compared to IF and mouse inoculation for rabies diagnosis. On the other hand, IF conjugates prepared with antirabies nucleocapside serum

will be compared with the regular rabies conjugate for the same purpose.

RESULTS TO DATE

A total of 204 samples, 105 positive and 99 negative, were studied in phase 1. The results showed that unfixed impressions were as good as fixed ones for rabies IF.

One hundred fourteen brain samples, 56 positive and 58 negative, were studied in phase 2. The results showed that the IF test could be incubated at room temperature for 10 minutes with no influence on the results.

IgG of rabbits immunized with high purified rabies virus of tissue culture origin was conjugated with peroxidase. This reagent had a titer of 1:40. The fluorescein conjugated antinucleocapside serum gave a titer of 1:90. So far no difference in sensitivity has been found between these new techniques and the classical IF method.

SIGNIFICANCE

The improvement of rabies diagnostic tests would enable the laboratory to report the results more quickly and accurately to the physician considering rabies treatment of bitten persons.

An immunoperoxidase test would have an advantage over the conventional IF test because it could be performed at any diagnostic laboratory without the resource of a fluorescent microscope, if sensitivity is shown to be equal to IF by further research.

PUBLICATIONS

1. Larghi, O. P. and E. Jiménez Ch. Métodos para acelerar el diagnóstico de la rabia. II Jornadas Argentinas de Microbiología, November 1970.
2. ----- and ----- . Methods for accelerating the fluorescent-antibody test for rabies diagnosis. Appl Microbiol 21:611-13, 1971.

3. ----- and ----- . Método para acelerar la técnica de inmunofluorescencia para el diagnóstico de la rabia. Bol Of Sanit Panam 71:36-40, 1971.
4. ----- . Avances en el diagnóstico de la rabia. Conferencias, Simposios y Plenarios. V Congreso Latinoam Microbiol. Punta del Este, Uruguay, 1971, pp. 339-43.

OTHER DATA

Grantee: Dr. O. P. Larghi, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1970 - continuing.

NONFATAL RABIES IN DOMESTIC ANIMALS

PROBLEM

Occurrence of nonfatal rabies in animals naturally infected has been postulated and experimentally tested.

With the work of Andral and Serié some doubts have arisen on the concept of fatal rabies in dogs and the necessity to systematically investigate in enzootic areas the occurrence of abortive disease in canine populations.

Bell et al. carried out serologic, cytologic, and virologic studies in 550 dogs of the city of Buenos Aires and the Gran Buenos Aires area but were unable to detect nonfatal disease. Taking into account that the number of dogs studied only represented 0.5 percent of the total canine population exposed, it would be very interesting to increase the number of animals studied.

The presence of rabies-neutralizing antibodies has been demonstrated in brains from experimentally infected animals that showed symptoms of the disease and survived. No such antibodies could be demonstrated in the brains of vaccinated animals. Thus the determination of antibodies in brain samples from sacrificed or dead animals from the same enzootic area can constitute a reliable indicator of abortive rabies.

The purpose of this project is to determine whether asymptomatic rabies is present in animals from an enzootic rabies area.

METHOD

Brains from animals whose previous behavior offered interesting speculation as to their rabies status, but were rabies negative in routine laboratory tests, are used in this study.

Identification of animals: All specimens were identified in the laboratory.

Serum-neutralization test: An aliquot of the unheated supernatant of a 20 percent suspension of each brain was mixed with an equal volume of a CVS virus suspension containing 10-100 mouse LD₅₀ per 0.03 ml.

Suspensions that neutralized in the screen test were subjected to titration and neutralization index tests.

RESULTS TO DATE

Two of 1,015 dog brains that were rabies negative by laboratory tests, presented neutralizing indices of 199 and 251.

One of 144 cat brains that were rabies negative by laboratory test, showed a neutralization index of 200.

SIGNIFICANCE

According to these results, the prevalence of this atypical form of rabies in the studied dog population of the area was calculated to be 0.2 percent (2/1015) with a 95 percent degree of confidence and with a range of 0.02 percent to 0.7 percent.

Prevalence in cats in the same area, would be 0.7 percent (1/144) with a 0.02-3.9 percent interval of confidence.

PUBLICATIONS

1. Díaz, A. M. et al. Nonfatal rabies in domestic animals, manuscript in preparation.

OTHER DATA

Grantee:	Dr. E. Fuenzalida, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.
Timetable:	1971-1972.

ECOLOGY, VIROLOGY, AND SEROLOGY OF BAT AND OTHER SYLVATIC RABIES

PROBLEM

The main purpose of these studies is to determine how the rabies virus maintains itself in rural and urban ecosystems and to investigate the possible transmission of virus within sylvatic and between sylvatic and domestic species. Other objectives are (1) to evaluate the effectiveness and possible hazards of the methods to control bat rabies through bat population control presently being applied by several countries, and (2) to study the relationship of vampire bat abundance to natural ecosystems.

METHOD

Vampire and other bat populations are studied in relation to rabies outbreaks in rural and urban areas. The relationships of terrestrial carnivores and other mammals to vampire and other bats are likewise investigated. Brain, saliva, salivary gland, and interscapular fat are tested for rabies virus and sera for rabies-neutralizing antibody. Bat populations are censused and relative abundance is assessed in relation to ecosystems and rabies outbreaks in cattle. Behavior as it bears on virus transmission of both captive and natural colonies of vampire bats is under study. The anticoagulant and other methods of vampire bat control are being evaluated both for effectiveness and possible detrimental effect on other sectors of the ecosystem. Relations between rabies in urban bat colonies and urban rabies is being investigated.

RESULTS TO DATE

A project to control bovine rabies through population control of vampire bats in an area of northern Argentina was successful and the results are being published. Similar observations on the epizootiology of rabies virus in vampire bat populations before, during, and following outbreaks of bovine rabies are in manuscript form ready for publication. Findings of another study showing the appearance of rabies-neutralizing antibody in terrestrial carnivores (foxes and skunks) following an outbreak of vampire-bat-transmitted bovine

rabies will soon be printed. An index to the abundance of bat populations is summarized in a manuscript to be published.

Briefly, removal of more than 90 percent of the population of vampire bats from an area 30 x 50 km resulted in the protection of that area from an outbreak of bovine rabies. Examination of sera from more than 1,000 vampire bats bled before, during, and following outbreaks of bovine rabies showed little antibody before the outbreaks, antibody and virus isolations from tissues of some bats during outbreaks, and following outbreaks, antibody rates in some populations reached 58 percent with no virus isolations.

Rabies-neutralizing antibody was found in sera of four of eight foxes and two of two skunks bled several months following the passage of a bovine rabies outbreak. Antibody was also disclosed from the vectors, the vampire bats in that area. These terrestrial carnivores, some of them recently born, were possibly exposed to rabies virus by eating dead or moribund vampire bats. Summary of 66 indices developed as an assay to the relative abundance of bats indicate the method to be useful for the low-flying species such as the vampire bats, fruit-eating bats, and some others, but not for the high-flying Molossidae. A positive relation was observed between vampire bat abundance and the ecotone of forest and prairie in northeastern Argentina.

SIGNIFICANCE

The successful control of bovine rabies through the control of a vampire bat population indicates that this method may be used in addition to vaccination of cattle to check the disease. The method, when strategically applied, may also be useful in eliminating rabies outbreaks in vampire bat populations. This is even more apparent when one considers findings on the vampire rabies epizootiology. These findings indicate that rabies in vampire bats exists as a moving epizootic, coming from a neighboring population and infecting a proportion of the bats, some of which die and others survive presenting rabies antibody. Then, when the density of susceptibles has been reduced below the threshold of optimal transmission, the disease dies out or moves on to neighboring susceptible populations. The finding of antibody in terrestrial carnivores following an outbreak of vampire bovine rabies indicates a possible route of interspecific transmission of virus. Finally, the association of denser vampire populations with certain ecotones may indicate methods for refining population control of vampire bats with an end to controlling bovine rabies. A possible correlation, if found, between rabies in urban bat colonies and urban rabies would mean consideration of another area to further control of this problem.

PUBLICATIONS

1. Delpietro, H. et al. Extensión de la distribución del vampiro de alas blancas (Diaemus youngi). Physis 84:224, 1973.
2. Fornes, A. et al. Control of bovine rabies through vampire bat control. J Wildlife Dis, in press.
3. Lord, R. D. et al. Vampiros que se alimentan de murciélagos. Physis 84:225, 1973.
4. ----- et al. Observations on the epizootiology of vampire bat rabies. Manuscript ready for publication.
5. ----- et al. Presence of rabies-neutralizing antibodies in wild carnivores following an outbreak of bovine rabies. Manuscript ready for publication.
6. ----- et al. An index for comparing South American bat populations. Manuscript ready for publication.

OTHER DATA

Grantee: Dr. R. D. Lord, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1968-1978.

THE USE OF HAMSTERS AS SENTINELS TO DETECT RABIES VIRUS AEROSOLS

PROBLEM

The airborne transmission of rabies infection became an important epidemiologic problem when two persons died of rabies after being exposed to rabies-infected bat environment in Frio Cave, Texas. Later, the transmission of the disease by breathing contaminated air in the same area was demonstrated for foxes and coyotes. Other investigators also proved the transmission of fixed and street rabies virus to laboratory animals by aerosols.

In 1972 the first laboratory-acquired rabies infection by fixed virus was reported; a microbiologist died 20 days after preparing rabies vaccine with a homogenizer known to produce aerosols.

The purpose of this project is to determine whether or not vaccinated or nonvaccinated hamsters will react to the presence of rabies virus aerosols and to investigate the possibility of using these hamsters as sentinels.

METHOD

Vaccinated and nonvaccinated hamsters will be exposed to aerosols produced with several rabies virus strains. Some of the hamsters will be maintained under observation to detect rabies infection while others will be bled periodically and their sera tested by the rabies sero-neutralization test. Vaccinated and nonvaccinated hamsters will be kept as nonexposed controls.

RESULTS TO DATE

Hamsters, whether vaccinated or not, did not become infected when exposed to aerosols.

Vaccinated hamsters exposed to aerosols maintained the rabies neutralizing titer better than did nonexposed animals. The difference was statistically significant.

SIGNIFICANCE

A method using vaccinated hamsters to detect rabies aerosols would be more reliable, inexpensive, and easier to control than the present one using foxes and coyotes.

PUBLICATIONS

1. Savy, V. and O. P. Larghi. El uso de hamsters como centinelas para detectar la presencia de aerosoles de virus rábico. III Jornadas Argentinas de Microbiología. Tucumán, Argentina. September 1973.

OTHER DATA

Grantee: Dr. O. P. Larghi, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1973-1975.

RABIES VACCINE OF TISSUE CULTURE ORIGIN

PROBLEM

The lack of enough dog vaccine is one of the main problems encountered in the control of rabies in Latin America. Although suckling mouse brain and chick embryo vaccines are very effective to control rabies, the countries of the Region have been unable to produce all the vaccine they need. Tissue culture vaccines could be the solution for this problem if a technology were developed and provided to the countries.

Several cell culture vaccines have been produced, as attenuated or inactivated virus preparations. Most of these vaccines protected all the dogs challenged 1 year after vaccination; but none protected the animals 3 years after vaccination. Attenuated vaccines protected better than inactivated ones.

Rabies virus propagates in a great variety of tissue culture systems, but the best viral yield is obtained in BHK₂₁ C 13 cells. The production of attenuated vaccine with these cells has, however, raised objections because of their carcinogenic properties for hamsters and, by inference, for human beings and other animals. Inactivation might eliminate the carcinogenic properties. Inactivated foot-and-mouth disease vaccine produced in BHK cells are of worldwide use.

The purpose of this project is to develop a cell-culture inactivated vaccine with an antigenic value of 3 by the NIH potency test, which is supposed to protect dogs for 3 years.

METHOD

Studies will include:

- 1) The growth kinetic on BHK cells of several rabies virus strains to determine the best growth conditions.
- 2) Several inactivating agents to choose the one producing the best results.
- 3) The development of an effective stabilizer as well as the use of cells capable of growing in media simpler than that used for BHK cells.

- 4) Duration of immunity in different animal species.

RESULTS TO DATE

The growth kinetic for seven rabies virus strains and their inactivation kinetic for three chemical agents have been obtained. Fifteen lots of vaccine have been prepared using these strains and agents, and all of them but one have passed the NIH potency test with antigenic values ranging from 0.76 to 5.0.

SIGNIFICANCE

The development of a vaccine that could be produced on a large scale without the limitations of the currently used methods would help to overcome the shortage of this vaccine for canine rabies control programs in Latin America. Furthermore, it could possibly help to develop the "know-how" of a tissue culture vaccine for human use.

PUBLICATION

1. Larghi, O. P. et al. Desarrollo de una vacuna antirrábica en cultivos celulares. Resultados preliminares. III Jornadas Argentinas de Microbiología. Tucumán, Argentina, September 1973.

OTHER DATA

Grantee: Dr. O. P. Larghi, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1973-1978.

IMMUNOGENICITY AND SAFETY OF ERA AND WIRAB (ERA-BHK)
RABIES VACCINES FOR CATTLE

PROBLEM

The present production of rabies vaccines is not sufficient for the control of cattle rabies in Latin America. Tissue culture vaccines can be produced in large quantities and may offer the solution to this problem.

The commercial ERA-strain vaccine has shown to be very effective in protecting cattle in experimental trials (1,2) and in extensive field vaccination campaigns (3). This virus strain has been cloned and propagated in BHK cells for the production of the WIRAB vaccine. Conclusive knowledge about the immunogenic capacity and safety of this vaccine for cattle is not yet available, however.

The purpose of this project is to compare the immunogenicity of ERA and WIRAB vaccines for cattle.

METHOD

1) Four groups of 16 1-year-old cattle each will be vaccinated as follows:

- Group A: 2 ml ERA commercial vaccine
- Group B: 2 ml ERA commercial vaccine diluted 1:100
- Group C: 2 ml WIRAB vaccine
- Group D: 2 ml WIRAB vaccine diluted 1:100
- Group E: 16 animals will be kept as controls

2) Serum samples will be collected from groups A, B, C, and D on day 7, 30, 100, 240, and 365 after vaccination. A serum sample will be taken from Group E before challenge.

3) Challenge: One year after vaccination, seven animals per group will be selected at random and will be challenged with two injections of DR-19 virus (2.5 ml each) in both cervical muscles. The challenge virus dilution will be determined by previous titration in susceptible cattle.

4) Serum samples: will be collected from all groups including controls 10 days after challenge, and from nonchallenged animals 18 and 24 months after vaccination.

RESULTS TO DATE

Results of the challenge tests, a year after vaccination did not show significant differences in the protection conferred by both vaccines. Mortality: Group A: 28.5 percent, Group B: 0 percent, Group C: 14.2 percent, Group D: 28.5 percent, Group E (control group): 100 percent.

The remainder of animals from all the vaccinated groups and of the corresponding control group will be challenged after 3 years of vaccination.

SIGNIFICANCE

Both vaccines protected cattle 1 year after vaccination while a mortality of 100 percent was observed in the control group.

Vaccines diluted to 1:100 protected 70 percent-100 percent of the cattle while 100 percent of deaths occurred in the controls.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Dr. C. Sanmartín, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.
Timetable:	1971-1974.

LABORATORY INVESTIGATIONS ON THE ENCEPHALITOGENIC ACTIVITY OF ANTIRABIES VACCINES

PROBLEM

The feasibility of inducing experimental allergic encephalitis (EAE) in laboratory animals with myelin and nervous tissue of murine origin is of interest in the investigation of postvaccinal neuroparalytic accidents in antirabies postexposure immunization with suckling mouse brain (SMB) vaccine. The likelihood of a common underlying immunopathogenetic mechanism for the natural and experimental diseases requires laboratory evidence. The ontogeny of encephalitogenic activity in the suckling mouse is not known. The possibility of detecting and removing myelin contaminants from vaccine preparations requires investigation.

METHOD

The feasibility of producing EAE is being studied using guinea pigs and rabbits, mouse brain homogenates, and purified myelin preparations from the two tissues. Central nervous system and peripheral nerve dissections were performed on all experimental animals for subsequent histopathologic analysis. Skin tests were performed with the different encephalitogenic preparations and clinical data were obtained from all experimental animals.

Different centrifugation schemes were evaluated for their ability to remove myelin from SMB homogenates. The presence of myelin was assessed in all these studies and in brain preparations from 9-day-old mice by electronmicroscopy and by tests for encephalitogenicity and histopathologic lesions in guinea pigs.

RESULTS TO DATE

It was possible using mouse myelin to produce EAE in guinea pigs and rabbits ranging in severity from clinically severe to mild and inapparent. Animals were found to exhibit a positive delayed hypersensitivity reactions a week before the onset of symptoms. The results underlined the dependence of the encephalitogenicity of a preparation

on the concentration and state of the sensitizing dose; the relationship between skin test reactivity and the onset of symptoms of the disease; the role of the nonmyelin components in the skin reaction; and the role of the type of adjuvant used in eliciting the disease.

Myelin was isolated from the brains of adult mice by differential centrifugation in two layers of sucrose, characterized by electron-microscopy, and found to induce EAE in guinea pigs and rabbits.

Myelin sheaths were also detected by electronmicroscopy in similar preparations obtained from 9-day-old mouse brains and moderate histopathologic lesions compatible with EAE were observed in guinea pigs sensitized with this material.

SIGNIFICANCE

These results indicate that SMB preparations are potentially encephalitogenic when inoculated into experimental animals and presumably, also in man. The wide spectrum in the incidence and degree of severity of EAE observed in this and other studies suggests that both in the natural and experimental situations, individuals differ in their susceptibility or response, or both, to a given dose of encephalitogenic antigens. The small amounts of myelin detected in 9-day old mouse brains as compared to the yield from adults, may account for the lower rate of neuromuscular complications following SMB rabies vaccination in man. Previous results by other workers on the lack of clinical and histopathologic evidence of neurologic disease following inoculation of brain material from immature animals may be explained in terms of a low concentration of encephalitogenic antigens in the preparations rather than on their absence.

PUBLICATIONS

1. Trejos, A. et al. Laboratory investigations of neuromuscular accidents associated with suckling mouse brain rabies vaccines. I. Encephalitogenic and virological studies, submitted for publication.
2. Varela-Díaz, V. M. et al. Estudios preliminares sobre la inducción de encefalitis alérgica experimental usando tejido de origen murino. VII Cong Nac Alergia Inmunol, Salta, Argentina, August-September, 1971.

3. ----- et al. Laboratory investigations of neuro-paralytic accidents associated with suckling mouse brain rabies vaccine. II. Encephalitogenicity of murine brain myelin preparations, submitted for publication.

OTHER DATA

Grantee: Dr. Victor M. Varela-Díaz, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1971 - 1972.

COMPARISON OF TUBERCULIN REACTIONS TO DIFFERENT PURIFIED PROTEIN
DERIVATIVES (PPD) IN GUINEA PIGS SENSITIZED BY "ATYPICAL"
MYCOBACTERIA ISOLATED FROM ANIMAL SAMPLES

PROBLEM

It is well known that mycobacteria other than M. bovis and M. tuberculosis sensitize cattle to mammalian tuberculosis, although some of them produce weak and unstable reactions. This is the principal reason for the use of comparative test with avian and mammalian PPDs. Infections produced by M. avium, M. johnei and by "Runyon Group III" mycobacteria, cause larger reactions to avian than to mammalian PPD.

Other mycobacterial species, groups III and IV of the Runyon classification, that are isolated with regular frequency from samples of animal origin, show a weak cross-reaction with avian PPD. In previous studies conducted at the Zoonoses Center, mycobacterial strains of Runyon groups II and IV were frequently isolated in lymph nodes from swine with apparent tuberculous lesions and in raw milk samples, all from the Province of Buenos Aires, Argentina. These mycobacterial strains might possibly be the origin of paraspecific sensitization on cattle in that region.

Purified protein derivative prepared from M. tuberculosis is used in some countries in place of PPD of M. bovis origin, for diagnostic purposes in cattle, because of the better yields of tuberculo-protein produced. The problem is whether or not specificity of human PPD is similar to that of bovine PPD, with regard to sensitization produced by these "atypical" mycobacteria.

The purposes of this project are to determine (1) the antigenic relationship between some mycobacterial species frequently isolated from animal samples in a particular region, and M. bovis, M. tuberculosis, and M. avium, and (2) which of human PPD or bovine PPD is the most specific in this type of sensitization. Additionally, it is of interest to compare results of similar and different concentrations of mammalian and avian PPD in relation to specificity.

METHOD

Four strains, two of them previously isolated from pigs and two of them from raw milk, were used. They have been selected as representative

strains of a large number of isolations with the same characteristics. Standard strains D₄, AN5, and DT were also employed.

Guinea pigs weighing 400-600g served as laboratory animals.

Purified protein derivatives were prepared from each of the strains by trichloroacetic acid precipitation of culture filtrates.

Different groups of guinea pigs were sensitized with each strain. Groups of 12 animals were sensitized using paraffin oil suspensions of killed and dry bacilli, containing 4 mg/ml, injected by intramuscular route.

RESULTS TO DATE

Reactions on guinea pigs previously sensitized with the following "atypical" mycobacteria were compared:

Strain 411: Rapid grower, scotochromogenic, rose pigmented, identified as M. lactis (Kantor and Tacquet, Ann Inst Pasteur 122:31-41, 1972).

Strain 326: Rapid grower, not chromogenic, identified as M. fortuitum.

Strain 318: Rapid grower, not chromogenic.

Two biologic assays were made on each group. In the first one, 20 and 200 IU of human PPD, and concentrations equivalent to 10 and 100 IU of the homologous PPD were used respectively. The second assay was made with 10 and 100 IU of each PPD.

It was observed that human PPD gave larger reactions than did avian PPD in the first assay with three groups of "atypical" sensitized animals, and that the largest reactions were obtained with avian PPD when equal concentrations were used as shown by the second assay. In both experiences bovine PPD gave the smallest reactions.

SIGNIFICANCE

Results to date clearly demonstrate the convenience of employing similar concentrations in comparative tests and also the superiority of bovine PPD as compared to human PPD. Bovine PPD showed a higher specificity than did human PPD in cases of animals sensitized by some atypical mycobacteria.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. I. N. de Kantor, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1973-1974.

THE SPECIFICITY OF PURIFIED PROTEIN DERIVATIVES (PPD) OF
MAMMALIAN TUBERCULIN PREPARED FROM MYCOBACTERIUM
TUBERCULOSIS AND M. BOVIS

PROBLEM

In many countries where tuberculin testing in cattle is carried out, a mammalian tuberculin prepared from strains of Mycobacterium tuberculosis is used. The original reason for using human strains of tubercle bacilli for the production of mammalian tuberculin for animals was that the organisms grew on liquid culture media more readily than did bovine strains, and the yield of tuberculoprotein was higher. With improved production techniques, these practical disadvantages of bovine strains can now be largely overcome.

Theoretically, it seems reasonable to suppose that the homologous tuberculin would be preferable to the heterologous for the diagnosis of bovine tuberculosis. In a pilot trial comparing some different techniques of tuberculin-testing cattle in one area of Argentina, Weybridge bovine PPD gave better discrimination between tuberculous and non-tuberculous cattle than did Weybridge human PPD. Moreover, the countries of the European Economic Community have adopted a batch of bovine PPD, produced in Rotterdam from the Weybridge strain AN5, as their reference preparation of mammalian tuberculin PPD for veterinary use. Further investigations are therefore required to compare the biologic activity of human and bovine PPD, in guinea pigs, men, and in naturally sensitized cattle in the field.

METHOD

A. Essay in guinea pigs

Three tuberculins have been used in these comparisons: (1) Weybridge standard mammalian tuberculin, PPD 278 (prepared from M. tuberculosis strains C, DT, and PN); (2) Weybridge bovine PPD, Batch B1/66 (prepared from M. bovis strain AN5); and (3) Rotterdam bovine PPD, Batch B 60-12 (prepared from M. bovis strain AN5). All three preparations were adjusted to a concentration of 2 mg of PPD per ml.

To assay the potency and specificity of the three tuberculins, they were compared for biologic activity both in homologously and in heterologously sensitized guinea pigs. Assays were carried out in four groups of nine guinea pigs, each group sensitized 30 days

previously with a different species of Mycobacterium, as follows: (1) M. tuberculosis, strains C, DT, and PN (mixed); (2) M. bovis, strains AN5; (3) M. avium, strain D4; (4) M. paratuberculosis (also called M. johnei), strain 316F.

Three fivefold dilutions in isotonic phosphate buffered saline of each PPD were injected intradermally in randomly allocated sites, four on each side of every guinea pig, using a balanced incomplete block design. Tests were read by measuring the diameter of reactions 24 hours after injection.

Further comparisons will be carried out in naturally sensitized cattle in the field, followed by postmortem examinations at a slaughterhouse.

B. Essay in human beings

Weybridge human and bovine PPDs as described above were used. Two separate concentrations of each PPD were used in the test, labeled (a) and (b) for the human PPD containing 0.02 µg/0.1 ml and 0.2 µg/0.1 ml, and labeled (c) and (d) for the bovine PPD with the above concentrations. In the case of human PPD, 1 IU and 10 IU were employed.

The comparative tuberculin test was carried out on 10 tuberculous patients infected by M. bovis and in a control group infected by M. tuberculosis.

RESULTS TO DATE

Essay in guinea pigs

Potencies (expressed as percentages) of each of the preparations of bovine PPD relative to the human PPD are given below:

Sensitization	Weybridge bovine PPD pct.	Rotterdam bovine PPD pct.
<u>M. tuberculosis</u>	47.1 (38.5 - 57.6)	59.0 (48.4 - 71.8)
<u>M. bovis</u>	137 (107 - 175)	149 (117 - 192)
<u>M. avium</u>	36.2 (27.0 - 48.5)	61.2 (46.3 - 80.8)
<u>M. paratuberculosis</u>	54.1 (41.1 - 71.2)	73.6 (56.2 - 96.4)

At equal doses by weight, both of the preparations of bovine PPD were significantly more potent than the human PPD in the bovine-sensitized groups of guinea pigs. In the other sensitized groups, including the human sensitization, however, both bovine PPDs were significantly less potent than the human PPD, indicating that they have greater specificity than the human PPD for an M. bovis sensitization.

Essay in human beings

Reactions obtained with the homologous PPD were always bigger than with the heterologous.

The tuberculin reactions obtained indicated a higher level of sensitivity in the groups infected with M. bovis.

At equal concentrations of protein, bovine PPD gave more specific results than did human PPD.

SIGNIFICANCE

These results demonstrate marked differences in the specific potency of human and bovine PPDs under experimental and natural conditions. The specificity of a tuberculin preparation differs with the animal species and the type of heterologous sensitization, and may therefore vary with the type of test and the environmental factors involved. The ultimate trial in the assessment of a tuberculin is that carried out in the field in humans or animals with sensitizations naturally acquired and in the particular environment in which the test is to be used.

The diagnostic value of bovine tuberculin PPD in both animals and men deserves further study under field conditions.

PUBLICATIONS

1. Kantor, I. N. and I. W. Lesslie. Respuesta al PPD en pacientes tuberculosos infectados por M. tuberculosis o por M. bovis. Actas XIV Congreso Argentino de Fisiología y Neumonología, in press.
2. Lesslie, I. W. et al. Response to PPD of tuberculous patients infected with M. tuberculosis and M. bovis, submitted for publication to Bull WHO.

OTHER DATA

Grantee: Dr. J. D. Roswurm, Pan American Zoonoses Center,
Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health
Organization, United Nations Development Program,
and the Governments of the Americas.

Timetable: 1970-1973.

COMPARISON BETWEEN CULTURE AND GUINEA PIG-INOCULATION METHODS FOR THE ISOLATION OF MYCOBACTERIUM BOVIS

PROBLEM

Guinea pig inoculation has traditionally been considered the only sure method to isolate Mycobacterium bovis from different materials. There are now, however, media with pyruvate that allow an eugonic development of bovine bacilli.

The purpose of this investigation was to compare the value of guinea pig inoculation with culture methods on Stonebrink media, for the isolation of M. bovis.

METHODS

A comparison was made between:

a) Bacillary suspensions of homogenates from the spleens of guinea pigs inoculated 30 days before with bovine strains. Appropriate dilutions of these suspensions were administered intramuscularly to two guinea pigs and sown into Stonebrink media tubes.

b) Bacillary suspensions from cultures in Stonebrink media, containing approximately 1 mg/ml of bacilli. Dilutions of the order of 10^{-6} , 10^{-7} were injected to guinea pigs and sown into Stonebrink media tubes.

c) Suspensions of lymph nodes from tuberculous swine, administered intramuscularly to guinea pigs and sown into Stonebrink media tubes as in a) and b).

All animals inoculated were tested with 100IU of mammalian purified protein derivative (PPD) after 3 weeks, and sacrificed 60 days later. At the end of that period the corresponding cultures were examined.

RESULTS TO DATE

Guinea pig inoculation gave a higher number of isolations than did the culture technique, except when diluted bacillary suspensions from M. bovis culture were used. The total positive results obtained from both methods was 127 (100 percent); by inoculation there were 122 (96 percent), and by culture 113 positive results (89 percent).

SIGNIFICANCE

In the evaluation of techniques used by veterinary diagnostic laboratories, costs must be taken into consideration. These are always higher for inoculation than for culture, though a difference of 7 percent was encountered in favor of the former procedure.

Moreover, the existence in animal samples, of other mycobacteria different from the bovine bacilli and not pathogenic for the guinea pig, must be also taken into account.

It is concluded that for investigation of the bovine bacilli, diagnostic laboratories may do without guinea pig inoculation and use, instead, culture media containing pyruvate. Future improvement of culture media will offer a possibility of reducing the differences now observed.

PUBLICATIONS

1. Kantor, I. N. and I. W. Lesslie. Comparación del cultivo y de la inoculación al cobayo como método para el diagnóstico bacteriológico de la tuberculosis bovina. Bol Of Sanit Panam, in press.
2. ----- and -----, El cultivo y la inoculación al cobayo como método para el aislamiento del Mycobacterium bovis. Actas III Jornadas Argentinas de Microbiología. Tucumán, Argentina, 16-21 September 1973.

OTHER DATA

Grantee: Dr. Isabel N. de Kantor, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1972-1973.

TUBERCULOSIS OF SWINE

PROBLEM

A successful program to eradicate tuberculosis must include an attack against the disease wherever it occurs. For that reason, it is necessary to know the prevalence and distribution of tuberculosis in swine and in birds. The classification of mycobacteria from these animals and animal production in different areas should be carried out whenever possible.

The purpose of this program has been to investigate the species distribution of mycobacteria in pigs in Argentina.

METHOD

Lymph nodes from 715 pigs commercially slaughtered in Buenos Aires, have been tested in the laboratory for the isolation of tubercle bacilli. The samples of tissues were randomly selected from pigs with macroscopic lesions, suspected of being tuberculous at regular-kill meat inspection.

RESULTS TO DATE

Five hundred and seventy three mycobacteria strains were isolated. Of these, 509 (88.6 percent) were M. bovis and 43 (7.5 percent) M. avium. Both M. bovis and M. avium were simultaneously present in one sample.

Thirteen strains (2.2 percent) of M. scrofulaceum and M. fortuitum were also isolated. Other atypical mycobacteria were found together with M. avium or M. bovis in 8 (5 percent) of the cases. Serotype II Schaefer was found to be predominant in a lot of M. avium strains subjected to immunoagglutination tests.

M. intracellulare was not isolated from any of the samples.

SIGNIFICANCE

These results show the high incidence of bovine-bacillus infection in a region where tuberculosis in cattle occurs. In the same region, pigs are usually infected through the digestive route, when fed with residues of dairy by-products.

PUBLICATIONS

1. Kantor, I. N. and I. W. Lesslie. Estudio bacteriológico de ganglios de cerdos tuberculosos en Argentina. Bol Of Sanit Panam. Submitted for publication.
2. ----- and ----- . Micobacterias aisladas de ganglios de cerdos tuberculosos. Actas de III Jornadas Argentinas de Microbiología. Tucumán, Argentina, 16-21 September 1973.

OTHER DATA

Grantee:	Dr. Isabel N. de Kantor, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.
Timetable:	1972-1973.

STUDY OF BIOLOGICAL PROPERTIES OF M. TUBERCULOSIS AND M. BOVIS STRAINS RESISTANT TO RIFAMPICIN

PROBLEM

It is very well known that strains of M. tuberculosis resistant to Isoniazid lose both their virulence for guinea pigs and their catalase activity. A certain reduction of virulence for these animals was observed in M. tuberculosis strains resistant to Rifampicin. In the case of M. bovis, studies have not been made on resistant mutants isolated from wild strains.

METHOD

Suspensions of M. tuberculosis and M. bovis sensitive strains and resistant mutants isolated in a glutamate-egg medium and in Dubos' medium containing high concentrations of Rifampicin or Isoniazid were inoculated into guinea pigs by the intramuscular route.

Necropsies were performed at 6 and 12 weeks after inoculation. An index of virulence was determined and cultures were made from organs to confirm the persistence of viable bacilli and of the initial resistance to the drug.

RESULTS TO DATE

A first assay was carried out using animals inoculated with bacillary suspensions from egg medium containing 30 and 50 µg/ml of Rifampicin or 0.2 and 0.5 µg/ml of Isoniazid (the latter used as control for the loss of virulence). Only a small reduction of virulence, compared with the sensitive strain, was observed in mutants resistant to 50 µg/ml of Rifampicin.

SIGNIFICANCE

The purpose of this study is to obtain more knowledge on M. bovis drug resistance and on the action of antituberculous drugs on its biologic behavior.

PUBLICATIONS

1. Kantor, I. N. and I. W. Lesslie. Virulencia para el cobayo de M. bovis y de M. tuberculosis resistentes a Rifampicine o a Isoniacida. Actas XIV Congreso Argentino de Tisiología Neumonología. October 1973, in press.

OTHER DATA

Grantee: Dr. James D. Roswurm, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1973-1974.

ISOLATION OF MUTANTS RESISTANT TO DIFFERENT ANTITUBERCULOUS DRUGS AMONG WILD STRAINS OF MYCOBACTERIUM BOVIS AND M. TUBERCULOSIS

PROBLEM

The rate of Mycobacterium bovis infection in man varies in different regions according to the importance of the tuberculous infection in cattle. To correctly treat patients with tuberculosis produced by M. bovis, it is necessary to know the sensitivity of the bacillus to antituberculous drugs and, for this, to determine the proportion of spontaneous resistant mutants in wild strains. Media employed at present for M. bovis cultures contain piruvate that inactivates some antituberculous drugs, precluding the determination of mycobacterial sensitivity to these drugs.

METHOD

The medium used is composed of eggs and sodium glutamate which permits a good development of both mycobacteria species, without inactivating the drugs added to the medium.

The drugs and concentrations used were as follows:

<u>Drug</u>	<u>Medium Concentrations</u>
Isoniazid	0.2-0.5 µg/ml
Rifampicin	10-20 µg/ml
Ethambutol	1-2 µg/ml
Cycloserine	20-30 µg/ml

The drugs were always added to the medium before coagulation.

One milliliter of culture suspension was sown in 10 tubes of medium with drugs, and at the same time, dilutions of 10^{-4} , 10^{-5} , and 10^{-6} were sown in medium without drugs, to determine the number of bacilli in the concentrated suspension.

The count of the colonies was done after 40 days of incubation at 37°C.

RESULTS TO DATE

Considering the number of initial mutants resistant to isoniazid, both M. tuberculosis and M. bovis demonstrate similar composition. With Rifampicin, Ethambutol, and Cycloserine, M. tuberculosis had higher proportions of spontaneous resistant mutants than did M. bovis strains.

It was not possible to isolate any mutants resistant to Cycloserine from a high percentage of M. bovis strains, even when using bacillary suspensions containing 10^7 - 10^8 bacilli/ml.

SIGNIFICANCE

The results should be considered in relation to the possible emergence of resistance to these drugs during patient treatment. No difference between species was observed for isoniazid but bacilli existing in tuberculous lesions must be more numerous in bovine than in human strains, to contain some mutants resistant to Rifampicin or Ethambutol.

An enormous bacillary population (approximately 1,000 million bacilli) would appear necessary to contain mutants resistant to Cycloserine. According to these results, the possibility of acquiring resistance to Ethambutol, Rifampicin, and especially to Cycloserine is smaller in M. bovis-infected patients, than in patients infected with human bacilli, when other conditions are similar.

PUBLICATIONS

1. Kantor, I. N. and I. W. Lesslie. Aislamiento de mutantes resistentes a drogas antituberculosas en cepas salvajes de M. bovis. Medicina, in press.

OTHER DATA

Grantee: Dr. Isabel N. de Kantor, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1972-1973.

STANDARDIZATION OF THE CONGLUTINATING COMPLEMENT TEST AND ITS ADAPTATION TO MICROTEST FOR THE DIAGNOSIS OF BRUCELLOSIS

PROBLEM

None of the serologic techniques routinely used for the diagnosis of brucellosis is sensitive enough to detect all individuals infected. A highly specific and sensitive method will be an asset for the diagnosis and control of this disease.

The objective of the project is to establish a standardization of the technique to be used routinely on microdilution plates and to assess its advantages and disadvantages as compared with other serologic tests.

METHOD

Trials to determine the optimal concentration of red blood cells and the appropriate volumes of reagents for the microtiter test were performed.

Fresh horse sera of different origins were titrated to assess their conglutinating complement content.

The content of antibody to sheep red blood cells and the conglutinin will be titrated in 100 normal bovine sera.

Technical details will be given, together with a summary of advantages and disadvantages, as compared with the hemolytic complement fixation test and other techniques.

RESULTS TO DATE

The conglutinating complement test was adapted to microtest and the optimal concentrations of the different reagents were determined.

The conglutinating complement test, the hemolytic complement fixation test, and the serum agglutination test for the detection of specific Brucella antibodies are being comparatively studied using

sera of normal, infected, and vaccinated animals of different species. Promising results have been obtained, so far, as to sensitivity and early detection of antibodies.

SIGNIFICANCE

A method that would detect antibodies at an early stage of infection can be of great importance for the treatment of human infection or for the control of animal brucellosis.

The application of the agglutinating complement test to micro-technique will save much time and reagents.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Dr. Casimiro García Carrillo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.
Timetable:	1970-1974.

A SIMPLE METHOD FOR ISOLATING BRUCELLA FROM HEAVILY CONTAMINATED MATERIAL

PROBLEM

Isolation of Brucella strains from contaminated materials is a common problem, especially in the veterinary field. The addition of antibiotics and other bacteriostatic substances to the basal media inhibits to some extent the growth of contaminants. Elberg et al., Felsenfeld et al., Kuzdas and Morse, Banmgartner, and Mair have reported on the use of media with antibiotics or bacteriostatic substances. Some Brucella strains are nevertheless sensitive to antibiotics and other bacteriostatic substances, limiting the value of selective media to the isolation of only resistant cultures. The number of Brucella organisms encountered in a specimen is frequently low and any inhibiting effect by antimicrobial agents might further reduce the possibilities of their isolation.

METHOD

We used: two basal media, Brucella agar Albimi (Pfizer) and trypticase soy agar (BBL); two enriched media, one containing 10 percent serum and the other one 0.5 percent Tween 40; and two selective media each containing bacitracin, polymixin B sulfate, and cycloheximide incorporated into the above basal media in the amount used by Kuzdas-Morse. Albimi serum with antibiotics and Albimi Tween with antibiotics were also used. Most of the cultures used were standard strains of the known Brucella species and their biotypes.

Method of decontamination: Specimens of lymph nodes from swine, spleens of armadillos, testes of rams, lymph nodes and organs of guinea pigs artificially infected with different Brucella species were used. Organs from the guinea pigs were allowed to become contaminated by their own intestinal fluid. Five milliliters of N hydrochloric acid and two drops of a 1/5,000 phenol red solution were pipetted into a wide mouthed, air-tight 25-ml bottle. Small sections of organs were put into acid for 15 minutes at room temperature. Following neutralization with 5 ml of NaOH solution N, the tissues were cut and a sample of the inner area spread on the agar media. All the samples were divided into two halves: one part was directly inoculated on Albimi and Kuzdas-Morse media and the other was acid treated and inoculated on the same media.

RESULTS TO DATE

When the number of expected colonies was less than 10, trypticase soy agar only allowed the isolation of some Brucella biotypes. Trypticase with antibiotics completely inhibited most strains of Brucella or showed very small colonies.

The batch of Albimi agar used in this study permitted the isolation of all Brucella strains with the exception of those requiring serum. Albimi agar with rabbit serum enrichment was the only medium that supported the growth of all the strains studied. Antibiotics incorporated into Albimi reduced the number of colonies recovered from several biotypes. This effect, although lower, was also apparent in the presence of serum. The addition of Tween 40 increased the number of isolations and the size of the colonies of most strains with the exception of B. abortus, biotype 2, B. ovis, B. canis, and B. suis, biotype 2. The two strains, B. ovis and B. canis, were very sensitive to antibiotics.

The cultures of spleens from guinea pigs previously contaminated with intestinal fluid were completely invaded by contaminants. After acid treatment, however, mostly pure Brucella cultures were obtained. No contamination was observed on the plates of Kuzdas-Morse inoculated after treatment but the number of colonies and their sizes were consistently lower than the ones obtained in Albimi medium.

Of the 203 samples plated on all media, 131 strains were recovered after acid treatment and only 10 Brucella cultures were obtained by conventional methods. Only one of the 203 treated samples were contaminated yet most of the nontreated were heavily infected.

SIGNIFICANCE

In Latin American countries where B. abortus, biotype 2 has been isolated, routine use of antibiotics or antimicrobial agents would not permit assessing the actual brucellosis problem. In addition to the proposed acid treatment, some media containing serum should be used when attempting isolation.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Casimiro García-Carrillo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1972-1973.

EVALUATION OF THE CARD TEST (BUFFERED BRUCELLA ANTIGEN) WITH NONDISPOSABLE MATERIAL

PROBLEM

The purpose of this study is to make the card test available for general use in Latin American countries. This test has proved to be most reliable for the diagnosis of animal brucellosis. Its use in Latin America has been limited, however, because the disposable material required for the test is imported, and its cost therefore high.

METHOD

a) Production and standardization of the buffered Brucella antigen according to the U.S. Department of Agriculture (USDA) standard.

b) Performance of the test on bovine and other species sera, comparing the results obtained using disposable commercial kit materials with those obtained using nondisposable plates, pipettes, and droppers.

RESULTS TO DATE

Sera of vaccinated and nonvaccinated cattle have been processed. The results obtained using disposable and nondisposable materials were similar. Antigens from USDA and the Pan American Zoonoses Center were found to be of equal sensitivity.

SIGNIFICANCE

Promotion of the use of this test and its application in brucellosis control and eradication programs in Latin America.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Raúl Casas Olascoaga, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1973-1974.

PATHOGENESIS OF RAM EPIDIDYMITIS CAUSED BY BRUCELLA OVIS. EXPERIMENTAL STUDIES IN LABORATORY ANIMALS

PROBLEM

Ram epididymitis is an infection of sheep caused by Brucella ovis. During the course of the disease microorganisms may be isolated from liver, kidney, spleen, lymph nodes, epididymis, testicle, and semen. Clinically, the infection is predominantly characterized by its primary epididymal localization and secondary testicular involvement (epididymitis and orchitis).

The object of this project is to study the pathologic and immunologic factor responsible for the selective localization of the microorganisms in the male genital tract.

METHOD

The study consists of three stages:

1) Experimental inoculation of several laboratory animals species with a B. ovis isolate from ram semen to determine the species most susceptible to the infection that may be a useful experimental model of the natural disease.

2) The host species indicated above will be studied in terms of the lesions produced and the localization of the microorganisms and its antigens. These studies will be performed using light, fluorescent, and electron microscopy.

3) Immunopathologic studies of the experimental infection in the laboratory model of the disease will be performed.

RESULTS TO DATE

The first stage has been terminated and results published. The following laboratory animal species were inoculated: mouse, gerbil, Lewis and Wistar rats, guinea pig, hamster, and Flander's rabbit. An animal from each species was necropsied every 30 days until day 150 post-

inoculation. The complement fixation and agar-gel diffusion tests were performed with the sera. Spleen, liver, and testicles were cultured to isolate the organism. Histopathologic studies on epididymis, testicle, seminal vesicle, prostate, bladder, kidney, spleen, lymph node, pancreas, heart, thymus, and lung were carried out. Serologic data and the frequency of epididymal and testicular lesions revealed that the gerbil is the species most susceptible to B. ovis.

The second stage of this project has been completed. The experimental model chosen for the previous stage was again used. A total of 64 gerbils were inoculated. Every 30 days, four animals previously inoculated intraperitoneally and four animals inoculated intratesticularly with a recently isolated strain of B. ovis, were sacrificed. The last group of animals were sacrificed at 8 months postinoculation. Serologic studies were carried out in all animals, using complement fixation and diffusion in agar gel. At autopsy, samples were taken of liver, spleen, testicle, epididymis, seminal vesicle, prostate, mesenteric lymph gland, pancreas, and kidney for histologic studies; samples of epididymis were also taken for examination by electron microscopic techniques. Attempts to detect antigens using immunofluorescent techniques did not provide satisfactory results. Samples of spleen, liver, testicle, and epididymis were taken for bacterial isolation.

Conventional histologic studies have been completed. Studies using the electron microscope continue.

SIGNIFICANCE

Knowledge of the pathogenesis of experimental B. ovis epididymitis may be of importance in the eventual development of disease prevention in rams. Data on the damage to other organs by this organism is equally important. This information may also be of value in understanding the immunopathologic aspects of similar diseases caused in man and animals by other etiologic agents.

PUBLICATION

1. Cuba-Caparó, A. and D. M. Myers. Pathogenesis of epididymitis caused by Brucella ovis in laboratory animals. Am J Vet Res 34:1077-85, 1973.

OTHER DATA

Grantee: Dr. Alberto Cuba-Caparó, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1971-1974.

EVALUATION OF VACCINES AGAINST SWINE BRUCELLOSIS

PROBLEM

The INTA vaccine against swine brucellosis was developed in Argentina some years ago, but it has not found acceptance outside the country where it originated. A definitive evaluation of this vaccine is required and, in addition, a comparison against the most promising of the other methods that have been put forward experimentally for immunization against swine brucellosis would be desirable.

METHOD

In a previous experiment, the 50 percent infecting dose (ID_{50}) of Brucella suis challenge strain was determined for sows.

It has been suggested that the Rev. 1 B. melitensis vaccine might be suitable to vaccinate pigs against B. suis infection, and a preliminary trial to assess the duration of infection in swine was conducted (unpublished data).

The second phase of the evaluation involves guinea pigs and sows.

The guinea pig experiment was performed with 377 animals. Groups of guinea pigs were vaccinated using the INTA immunizing procedure (live attenuated B. abortus and killed B. suis culture) followed by a booster of glucid-lipidic extract of B. suis, 28 days later; another group of guinea pigs received an inactivated B. abortus vaccine in an oil adjuvant and the extract of B. suis, 28 days later. A third group was vaccinated twice at a 4-week interval with B. suis inactivated vaccine in an oil adjuvant and a fourth group was vaccinated with a single dose of Rev. 1 B. melitensis vaccine (1/10 of the normal dose). After 8 weeks, all the vaccinated animals and the nonvaccinated controls were challenged with graded doses of B. suis virulent culture.

Eight weeks after the challenge all animals were sacrificed. Blood samples for serologic tests and lymph nodes and spleen for bacteriologic examination were taken.

In the evaluation of the INTA procedure, 45 sows are vaccinated with a B. suis inactivated vaccine in oil adjuvant and 25 sows are being kept as nonvaccinated controls. Blood samples are taken periodically to study the evolution of antibodies by conventional and supplementary tests. Mating of the experimental animals will take place at the age of 7 to 8 months. The animals will be challenged at 40 days of pregnancy (6 1/2 months after vaccination). Fifteen days after farrowing all sows will be sacrificed and lymph nodes and organs removed for bacteriologic examination.

RESULTS TO DATE

From a statistical evaluation of the results obtained up to 31 December 1973 with guinea pigs, it is possible to conclude that:

- 1) there is a highly significant difference in the response to challenge infection between vaccinated animals and nonvaccinated controls;
- 2) within the vaccinated groups of animals the only immunizing agent that produced a significant protection was the Rev. 1 B. melitensis vaccine.

SIGNIFICANCE

There are efficient vaccines against brucellosis for cattle, sheep, and goats but not for swine. This study aims at evaluating the immunogenic values of different antigens proposed for swine immunization.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Casimiro García-Carrillo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina and Dr. V.C.F. Cedro, Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Buenos Aires Province, Argentina.

STUDY OF INACTIVATED BRUCELLA VACCINES

PROBLEM

The purpose of the project is to evaluate the immunogenic properties that inactivated vaccines may have in the control of animal brucellosis.

Live attenuated vaccines, especially B. abortus strain 19 and B. melitensis Rev. 1 are generally recognized as the best immunogenic agents against bovine and caprine brucellosis. These vaccines have, however, some disadvantages such as persistence of agglutination titers. For this reason the use of young animals is recommended.

In countries where the prevalence of brucellosis is low or where the last stage of an eradication campaign has been achieved, it is advisable either to discontinue vaccination completely or else to use vaccines that would not cause persistent agglutination.

In 1957 Renoux announced very promising results, obtained in laboratory animals by using B. melitensis H38 vaccine inactivated with formol and suspended in an oil adjuvant. Further work by the same author and his colleagues would indicate that vaccine H38 is more efficient than strain 19 vaccine for immunizing goats and cattle though this vaccine is also agglutinogenic. Little confirmatory information exists, however, on H38 vaccine.

There are conflicting opinions on the nonagglutinogenic 45/20 vaccine, and many Latin American countries are requesting advice on the use of some of the inactivated and oil-adjuvant 45/20 vaccines.

Pilet and Bonneau (1969) have developed a nonagglutinogenic vaccine by means of saturation with specific agglutinins of the agglutinogens of strain 19. This vaccine has been produced on a commercial scale without sufficient testing of its immunogenic quality.

METHOD

The vaccines will be tested in two phases: a) in laboratory animals and, b) in domestic animals. The vaccines will be tried in large animals, only if they have satisfactorily passed the laboratory animal tests.

Local reactions, immediate and mediate serologic responses, and the potency of the vaccines will be studied.

First stage: PB 19 nonagglutinogenic and H38 agglutinogenic inactivated vaccines were compared with live strain 19 and B. melitensis Rev. 1 vaccines in guinea pigs.

Live vaccines were inoculated in a single dose and inactivated vaccines were administered to two groups, one receiving a single dose, the other receiving two doses at a 20-day interval. All vaccinated and control animals were challenged 8 weeks after vaccination with 1,500 cells of virulent Brucella abortus 2308 and sacrificed 8 weeks after challenge.

Animals containing any number of *Brucella* cells in precrural or cervical lymph nodes or spleens were considered infected.

A complementary study in mice was performed using PB 19 vaccine and 45/20 vaccine in an oil adjuvant. The animals were vaccinated with 0.5 ml of PB 19 and 0.25 ml of 45/20. The nonvaccinated controls and the vaccinated mice were challenged 8 weeks after vaccination using B. abortus strain 2308 at a challenge dose of 10^3 viable cells. The animals were sacrificed 8 weeks after challenge and their organs cultured.

RESULTS TO DATE

Under the conditions of the experiment, the PB 19 vaccine tested in guinea pigs offered very poor protection with one as well as with two doses. Only three of 43 and 3 of 30 animals vaccinated once and twice respectively with PB 19 vaccine yielded cultures negative for Brucella abortus. In the infected animals macroscopic lesions were comparable to those observed in the control group.

The protection conferred by all other vaccines was highly significant. The percentages of protected guinea pigs with the different vaccines were as follows: H38 (1 dose): 73.2; H38 (2 doses): 92.9; strain 19: 70; and Rev. 1: 86.1.

The PB 19 vaccine tested in mice afforded 80 percent and the 45/20 vaccine, 91.49 percent protection. All the nonvaccinated mice were infected.

SIGNIFICANCE

The knowledge of the immunogenic value of different inactivated vaccines is essential for control purposes.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Dr. Casimiro García-Carrillo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.
Timetable:	1972 - continuing.

NUTRITIONAL REQUIREMENTS AND BEHAVIOR OF THE REV 1 VACCINE
STRAINS OF BRUCELLA MELITENSIS CULTIVATED UNDER CONDITIONS
OF CONTROLLED AERATION, OXIDATION-REDUCTION
POTENTIAL, AND pH STABILIZATION

PROBLEM

Development of a large-scale but simple method for cultivating the Rev 1 strain of B. melitensis using a rotor fermentor. This study aims at learning more about the nutritional requirements of smooth Rev 1 cells under conditions of large-scale growth for vaccine production.

METHOD

Up to the present we have sought a combination of equipment and procedures to carry out the proposed research. We have now settled upon and assembled a very complex apparatus, which is being checked for operational efficiency and safety. Dissolved oxygen concentration monitoring, pH recorder-controller, temperature and oxidation-reduction potential recorder, and controller devices have been incorporated into the unit along with the usual antifoam agents that are under test for activity and toxicity to Brucella organisms.

Techniques: Optical density or viable counts or both will be used to monitor growth. In addition, population stability (absence of S-R mutation-selection) will be ascertained by appropriate microbiologic techniques.

Batch submerged culture experiments: The liquid medium will be investigated, starting with "Trypticase Soy Broth," and making any changes or additions that seem beneficial (e.g. erythritol). The effect of several environmental parameters will be studied independently. These factors might include pH, temperature, aeration/dissolved oxygen tension, redox potential, and the size and age of inoculum. Some attempts may be made to determine the nature and extent of growth inhibition and mutation-selection caused by the accumulation of metabolic end products. This may involve the use of dialysis techniques or other methods as indicated.

Continuous culture experiments: The behavior of Brucella melitensis Rev 1 with respect to dilution rate will be studied, and the optimum dilution rates for vaccine production will be determined.

Based on the batch culture results, certain environmental factors may be reexamined in continuous culture, especially dissolved oxygen and metabolic end product inhibition.

Associated aspects of vaccine production: Concurrently with some of the growth experiments, investigations would be conducted on methods of harvesting and preservation of vaccine organisms produced by submerged culture. This may include a study of the amount of cell "washing" required for a satisfactory vaccine, and techniques of harvesting, such as flocculation with carboxy-methyl cellulose. Preservation will focus mainly on the freeze-drying, storage, and viability characteristics of the vaccine.

RESULTS TO DATE

The equipment described under Methods has been assembled.

SIGNIFICANCE

The vaccine lot prepared by such a method is expected to lead to the production of bacilli cell populations in the smooth form, thereby avoiding the occurrence of rough forms of bacilli that seem to complicate vaccine production in certain areas. Cells with maximal immunizing antigen will be available for the project.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Sanford S. Elberg, Graduate Division, School of Public Health, University of California at Berkeley.

Funded by: Pan American Health Organization.

Timetable: 1973-1975.

IMMUNITY IN GUINEA PIGS CONFERRED BY STRAIN 19 VACCINE AGAINST DIFFERENT BIOTYPES OF B. abortus PREVALENT IN LATIN AMERICA

PROBLEM

It is well recognized that strain 19 vaccine protects cattle challenged with virulent strains of B. abortus. Taran et al. have demonstrated that interspecies cross-immunity exists, and that strain 19 and Rev. 1 vaccines have protective action not only against homologous infection but also against heterologous species.

Immunity produced by strain 19 challenged with different biotypes of B. abortus has not been sufficiently studied. Some Latin American countries have requested information from the Center about the degree of immunity produced by strain 19 vaccine against B. abortus biotypes different from biotype 1.

So far, biotypes 1, 2, and 4 of B. abortus have been found to be present in Latin America.

The principal objective of this program is to study the immunity conferred by strain 19 vaccines against native strains of B. abortus, biotypes 1, 2, and 4 isolated in Latin America.

METHOD

Latin American strains of the different biotypes with a known history were chosen and their virulence in guinea pig titrated.

Guinea pigs were vaccinated with lyophilized B. abortus strain 19 vaccine at 1/10 of the cattle dose. The vaccinated guinea pigs and the control animals were challenged after 8 weeks of vaccination with each strain previously titrated and maintained in liquid nitrogen.

Control tests of the vaccine were conducted previous to and simultaneously with the vaccination of the guinea pigs.

In a preliminary assay 180 guinea pigs were vaccinated with 1/10 dose of B. abortus strain 19. Groups of previously vaccinated animals were challenged with 10-100 infective doses of each of the biotypes. Each of the three control groups received a 100-, 10-, and 1-infective challenge dose. Eight weeks after challenge, all animals

were slaughtered, blood samples collected, and lymph nodes and spleen removed for serologic and bacteriologic examinations.

A second assay was performed using 468 guinea pigs; 12 groups of 18 guinea pigs were vaccinated with strain 19, and 14 groups of 18 guinea pigs were left as nonvaccinated controls. After 8 weeks the groups of vaccinated animals were challenged with 10^3 , 10^4 , 10^5 , 10^6 virulent viable organisms of each one of the biotypes. Nonvaccinated controls received 5^1 , 5^2 , 5^3 , 5^4 , and 5^5 virulent viable cells of each one of the biotypes. After 8 weeks of challenge, all the guinea pigs were sacrificed; blood was taken for serologic tests and lymph nodes and spleen removed for bacteriologic examinations.

RESULTS TO DATE

Results of the first experiment were as follows:

Strain 19 vaccinated guinea pigs, challenged with 10^5 and 10^4 viable cells of the different biotypes, were protected against biotype 1 at 18 percent and 65 percent respectively; against biotype 2 at 45 percent and 67 percent respectively; against biotype 4 at 57 percent and 75 percent respectively.

The controls were exposed to doses ranging from 10^3 to 10^5 viable cells of the three biotypes, and the infection rate varied from 95 percent to 100 percent.

The second experiment in guinea pigs is under evaluation.

SIGNIFICANCE

It is important to learn if strain 19 vaccine, presently in use in the majority of the Latin American countries, protects as well against the different biotypes of B. abortus as it does against biotype 1. The results in guinea pigs will indicate the suitability of further research.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Casimiro García-Carrillo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1972-1974.

FACTORS WHICH AFFECT THE STORAGE OF LYOPHILIZED VACCINES

PROBLEM

Strain 19 B. abortus vaccine is considered an effective immunogen to prevent bovine brucellosis. Rev. 1 B. melitensis vaccine is being used in control programs in several areas to prevent B. melitensis infections in goats. From the literature available there appears to be some divergence of opinion regarding the criteria concerned in the stability of lyophilized vaccines when stored at different temperatures. Published reports state that Strain 19 vaccine loses its potency a few days after it has been held at a temperature of 37°C. Nevertheless, sufficient studies have not yet been made to determine if this loss is caused exclusively by temperature, or if other factors such as residual humidity, integrity of vacuum, or composition of stabilizing agents play a significant role.

METHOD

- a) Preparation of a lot of Strain 19 and Rev. 1 vaccines.
- b) Lyophilization using stabilizing agents of different composition.
- c) Division of the lyophilized vaccines into:
 1. Intact vacuum
 2. Ampoules in which 8 percent of the vacuum is replaced by sterile air
 3. Ampoules in which 80 percent of the vacuum is replaced by sterile air.
- d) Storage of ampoules from each group in incubator (37°C), room temperature (22°C), and in refrigeration (4°C).
- e) Viability and dissociation tests are carried out weekly, at the beginning of this project and at several intervals.
- f) Determination of residual humidity.

RESULTS TO DATE

Testing was done on a lot of B. melitensis Rev. 1 vaccine suspended in a standard stabilizing vehicle kept at different temperatures and at varying degrees of vacuum.

One hundred thirty days after starting the assays, tests showed viability losses in all vaccines with the exception of the vaccine kept at 4°C with complete vacuum.

SIGNIFICANCE

The determination of optimal conditions for the production and preservation of lyophilized vaccines is of great importance for the operation of control programs everywhere, especially in tropical countries.

The research is also important to improve the quality of immunogens most commonly used in brucellosis control in Latin America.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Dr. Casimiro García Carrillo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program and the Governments of the Americas.
Timetable:	1973-1975.

PURIFICATION, GROWTH ENHANCEMENT, AND ISOLATION OF LEPTOSPIRAS BY INCORPORATION OF NEOMYCIN TO CULTURE MEDIA

PROBLEM

One of the problems involved in isolation of leptospires from the tissues and body fluids of men, animals, or contaminated cultures is the concomitant presence of other microorganisms in the inoculum. The methods routinely used in attempting to obtain pure leptospiral cultures from these materials include inoculations of laboratory animals and filtration and inoculations of culture tubes with serial dilutions of the sample. The pyrimidine analogue, 5-fluorouracil, was found to control the growth of contaminants; it must, however, be used while the contaminating organisms are in the lag phase of growth. This bacteriostatic agent is not effective for all bacterial species and may have some inhibiting action for certain leptospiral serotypes. It was therefore deemed worthwhile to search for other antibiotics with the ability to selectively isolate leptospires in vitro by inhibiting contaminants in the sample studied. In evaluating 27 commercially available antibiotics, neomycin sulfate was found to be the most promising antimicrobial agent for further study.

METHOD

Cultures of leptospires were contaminated with cultures of Escherichia coli. They were then inoculated into various tubes of the different leptospiral media containing varying amounts of neomycin to determine the optimal concentrations required. The tubes were incubated and examined for the presence of unrestricted growth of the leptospires and absence of E. coli growth. Thirteen Leptospira serotypes were grown in media containing these varying concentrations of antibiotic for tolerance studies.

Pure culture isolations of leptospires were attempted from hamsters previously inoculated with a contaminated Leptospira culture and compared with results obtained with media containing neomycin, 5-fluorouracil, and media without antibiotics. Swine kidneys were collected from a local abattoir to determine the effectiveness of neomycin in the isolation of leptospires from clinical material.

RESULTS TO DATE

The incorporation of neomycin to Leptospira culture media was found to be effective in inhibiting contaminants without interfering with the growth of leptospires. The antibiotic was also found to enhance growth without altering the antigenic identity of the serotypes studied. Culture media with neomycin yielded leptospires from the blood of infected hamsters and from contaminated swine kidney material collected in an abattoir. During the weekly culture examinations of clinical material, an earlier growth was detected in the media containing neomycin which had a higher number of positive cultures than did media containing 5-fluorouracil and media without antibiotics. Contaminated cultures growing in media containing 5-fluorouracil were purified by subculturing into neomycin-containing media. Further studies of the growth promoting effect of Leptospira by neomycin are in progress.

SIGNIFICANCE

Results indicate that diagnosis and epidemiologic research can be improved if neomycin is added to the culture media. This is especially true when contaminated clinical and pathological specimens have to be handled, as is often the case in leptospirosis. Additional advantages include the possibility of purification of contaminated cultures in the laboratory, and the availability of neomycin in Latin American countries where 5-fluorouracil is difficult to obtain.

PUBLICATIONS

1. Myers, D. M. and V. M. Varela-Díaz. Selective isolation of leptospires from contaminated material by incorporation of neomycin to culture media. Appl Microbiol 25:781-86, 1973.

OTHER DATA

Grantee: Mr. Donald M. Myers, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1972-1974.

SEROLOGIC SURVEY OF BOVINE LEPTOSPIROSIS AND ATTEMPTS
AT LEPTOSPIRA ISOLATION FROM KIDNEY MATERIAL
COLLECTED IN ARGENTINE ABATTOIRS

PROBLEM

Bovine leptospirosis is of economic importance to countries involved in dairy and cattle raising. The same leptospires that cause bovine leptospirosis also produce human disease. Although the mortality in individual outbreaks may not be high, losses in milk yield of affected cows and in weight among beef cattle, and the losses of calves are considerable. The occurrence of inapparent infection in herds presents a serious problem in the recognition and control of bovine leptospirosis. A major problem associated with clinically inapparent infection is that infected animals remain undetected without laboratory aid and can spread the disease as renal carriers. The main objectives are:

(1) To evaluate the rate of bovine leptospirosis in Argentina on the basis of serologic evidence of infection from a statistically significant sample in the provinces comprising the major bovine population in the country. Previous testing of bovine sera in Argentina have consistently shown a high percentage of reactors to the hebdomadis serogroup although a serotype belonging to this serogroup has never been isolated.

(2) To attempt to isolate leptospires from bovine kidney material obtained from abattoirs in Buenos Aires.

METHOD

Sera collected for the serologic survey were tested using 15 leptospiral antigens in the microscopic-agglutination test. The kidneys obtained from apparently normal animals were collected in groups of 10 to 20 during repeated visits to the abattoirs. Each kidney was transported to the laboratory in individual plastic bags and using improved techniques, cultured into both Fletcher semisolid and modified Ellinghausen media. In addition, neomycin sulfate was added to the cultures as an aid in controlling contaminants and enhancing the growth of the Leptospira. At weekly intervals cultures were examined for the presence of leptospires by darkfield microscopy and positive isolations subcultured into liquid media for use as antigens. Isolates were identified by cross-agglutination patterns

using rabbit prepared antisera of each against reference Leptospira strains. Definitive classification is being carried out using cross-agglutination-absorption procedures.

RESULTS TO DATE

Approximately 2,000 sera have been tested for leptospiral antibody using twofold serum dilutions against 15 leptospiral serotypes as antigen in the microscopic-agglutination test method. Fifty-nine percent of the sera were positive to one or more serotypes at a 1:100 or higher serum dilution. The majority of the reactors were to antigens which are within the hebdomadis serogroup, i.e., hardjo (45.7 percent), wolffi (41.5 percent), sejroe (34.3 percent) and hebdomadis (30.9 percent).

Six Leptospira isolations have been made from 161 animals and cross-agglutination patterns indicate that all of them are serotypes within the hebdomadis serogroup. Cross-agglutination-absorption tests show antigenic identity between all six isolates, and absorption tests are now in progress for definitive classification.

SIGNIFICANCE

Additional information will be obtained on the epidemiology, prevalence, and significance of hebdomadis antibody titers in bovine leptospirosis. The six Leptospira cultures isolated from kidney material belonging within the hebdomadis serogroup represent the first bovine isolations of hebdomadis serotypes in Argentina.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Mr. Donald M. Myers, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

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

Timetable: 1974.

CONTAMINATION OF HORSE MEAT WITH SALMONELLAE: AN ECOLOGIC STUDY

PROBLEM

Control laboratories in countries importing Argentine horse meat report finding a relatively high percentage of Salmonellae in the meat. Several papers and communications confirming this observation have also been published. To adequately choose the points of the processing line where the application of preventive measures would be more effective, it is necessary to determine the relationships between the sources, the carriers, and the mechanisms of transmission and growth of Salmonella. It is also necessary to identify the most frequent serotypes to learn their distribution, behavior, and the way in which they reach the final product. Those are the aims of the present study, which is being carried out in close collaboration with a horse slaughterhouse and a horse meat export plant.

METHOD

Using the techniques for the detection of Salmonella routinely employed at our laboratory, the presence of the organism has been investigated in fecal specimens from meat handlers, horses, and birds captured in the horse yards, in samples of fresh and frozen horse meat, and in samples taken from surfaces, tools, and processing water.

RESULTS TO DATE

Feces from more than 100 horses, 206 sparrows, and 80 meat handlers have been studied. In addition, 400 samples of frozen meat, 100 of fresh meat, 100 samples taken from surfaces and tools, and some samples of water have been also examined. Salmonella serotypes found to date (Good, Montevideo, Vaertan, Derby, Anatum, Hato, Meleagridis, Haelsingborg, Bredeney, Oranienburg, Riggil, Typhimurium, Otmarshen, and Raus) and their frequent isolation have led to interesting conclusions.

SIGNIFICANCE

Points in the processing line requiring greater attention were pinpointed and advice has been given to veterinary inspectors and slaughterhouse managers, thus resulting in a decrease in the incidence of contamination of Argentine meat.

PUBLICATIONS

1. Quevedo, F. et al. Contaminación de carne equina con Salmonella; un estudio ecológico. I. Equinos portadores. Gaceta Veterinaria 35:119-23, 1973.
2. -----. Contaminación de carne equina con Salmonella; un estudio ecológico. II. Salmonellas en gorriones capturados en corrales de caballos de abasto. Gaceta Veterinaria 35:474-78, 1973.
3. -----. Contaminación de carne equina con Salmonella; un estudio ecológico. III. Importancia del manipulador humano. Gaceta Veterinaria, in press.
4. -----. Contaminación de carne equina con Salmonella; un estudio ecológico. IV. Salmonella en las superficies del frigorífico. Gaceta Veterinaria, in press.
5. -----. Contaminación de carne equina con Salmonella; un estudio ecológico. V. Salmonella en carne equina, to be published in Gaceta Veterinaria.
6. -----. Isolation of Salmonella from sparrows captured in horse yards. Am J Trop Med Hyg 22:672-74, 1973.
7. -----. Sources of Salmonellae in horse meat, in preparation.

OTHER DATA

Grantee: Dr. Fernando Quevedo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World
Health Organization, United Nations
Development Program, and the Governments
of the Americas.

Timetable: 1969-1972.

EXAMINATION OF MILK SAMPLES FOR MYCOBACTERIA

PROBLEM

There is no national program for the eradication of bovine tuberculosis in Argentina. The isolation and typing of mycobacteria from milk samples can provide valuable information on the importance of milk as a source of infection and as the possible origin of non-specific sensitization. Isolation of mycobacteria from milk can also aid in the determination of hygienic measures needed to protect the health of the consumers.

The present investigation undertakes to examine samples of sediments of industrial centrifuges of raw milk, carried out previously to pasteurization and samples of pasteurized milk obtained from the same establishment.

METHOD

Sediment samples from 100,000 liters of raw milk have been studied. This milk was obtained from farms where tuberculin tests are made under an agreement between the farmers and the milk processing plant.

In the laboratory, samples are decontaminated by a combined method using sodium lauryl sulfate (Tison and Tacquet method) and 5 percent oxalic acid, and inoculated into five tubes of Lowenstein-Jensen and five tubes of Stonebrink media.

Samples without and with previous treatment with chlortetracycline are inoculated to guinea pigs.

RESULTS TO DATE

A hundred twenty nine strains have been isolated from raw milk sediment samples and are being typed. One strain of M. avium and two strains of M. bovis have been identified. The other strains belong to different groups of "atypical" mycobacteria and the typing is not yet completed.

SIGNIFICANCE

In a country where no official program to control bovine tuberculosis exists, it is important to know the actual conditions in regard to mycobacterial contamination of milk. The question arises as to whether private agreements between dairymen and the milk processing establishment can prevent contamination of the raw milk with tubercle bacilli and secondarily whether or not the pasteurization techniques insure that viable mycobacteria will not be present in the final product.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Dr. J. D. Roswurm, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization. United Nations Development Program, and the Governments of the Americas.
Timetable:	1973-1974.

MICROBIAL CONTAMINATION OF PORK PRODUCTS. PRELIMINARY STUDIES FOR THE PREPARATION OF MICROBIOLOGIC CRITERIA

PROBLEM

Pork products are frequently considered responsible for outbreaks of food poisoning. In our previous studies we showed that 68 percent of pigs excreted Salmonellae and 3 percent of them enteropathogenic E. coli. This leads to the conclusion that if hygienic measures during the processing of these products are not thoroughly enforced, pork products will reach the consumer with a high degree of contamination, and will constitute a public health danger.

It is necessary to assess the microbiologic composition of the different pork products to be able to set microbiologic criteria for control activities.

METHOD

Pathogenic microorganisms and a number of bacteria are being studied as contamination indicators in various pork products. Results are related to different factors depending on the composition of the products.

RESULTS TO DATE

We investigated two types of fresh sausages: sausages with a content of less than 3 percent spices, and sausages with a higher content (8 percent) of spices. A marked difference in the degree of contamination was found according to the spice content. Fresh sausages with 3 percent or less spices were much more contaminated.

Salmonellae were found in more than 70 percent of the samples studied so far.

SIGNIFICANCE

Results obtained so far confirm that pork products are potentially dangerous. In the particular case of fresh sausages, a greater proportion of spices would apparently result in products with a better bacteriologic quality.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee:	Dr. Fernando Quevedo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.
Timetable:	1973-1976.

QUANTITATIVE EVALUATION OF MICROBIAL FLORA IN VACUUM PACKED REFRIGERATED MEAT (COMPARISON WITH CHEMICAL AND ORGANOLEPTIC CHARACTERISTICS)

PROBLEM

The volume of exportable refrigerated vacuum packed meat continues to increase. Some lots, however, do not reach the quality standards required by the purchasers. One of the possible causes of this deficiency is the material used for packing. We hope to replace it with a material of superior quality.

METHOD

With the aid of the manufacturers, experimental batches of refrigerated vacuum packed meat are being prepared, using different packing materials.

Microbiologic composition of meat and certain organoleptic and chemical characteristics are studied from the packing stage and for about 4 weeks.

Microorganisms capable of producing meat alterations and those that may be harmful to the consumer's health are being studied.

RESULTS TO DATE

So far three experimental batches only have been studied. The microbiologic modifications correlated with the organoleptic and chemical changes.

SIGNIFICANCE

One of the objectives of the study is to determine and predict possible microbiologic, chemical, and organoleptic alterations that could affect vacuum packed meat. A comparison is being made between three different packing materials. Another objective is to learn

about the microbial interrelationships that may be established under some processing conditions. In addition, we hope to relate the changes in the microbial flora to the chemical and organoleptic modifications that may occur in varying time periods. Finally, a detailed study on the metabolism of the isolated organisms will be carried out.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Fernando Quevedo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1973-1975.

DETECTION OF ENTEROPATHOGENIC ESCHERICHIA COLI IN SAMPLES OF HUMAN AND ANIMAL ORIGIN

PROBLEM

It seems that enteropathogenic Escherichia coli (EEC), which causes gastroenteritis in children, often with serious consequences, can be spread by foods of animal origin that may be contaminated by human carriers, water, and also probably by animal feces.

Serologic studies are being carried out on E. coli strains isolated from different sources to estimate their incidence in the mentioned habitats, and to contribute to knowledge of the epidemiology of gastroenteritis in Argentine children.

METHOD

E. coli strains isolated from human and animal feces and from foods of animal origin are sowed on MacConkey agar medium. The strains are then characterized biochemically and serologically.

RESULTS TO DATE

A total of 779 strains from different sources of E. coli were studied. Nine of them were found to be EEC. The serotypes identified to date are: 055:B5; 0125:B15; 0126:B16; 0111:B4; 0112:B11; and 0128:B12.

SIGNIFICANCE

The presence of EEC in food handlers and in animals destined for human consumption justifies the increasingly important study of these microorganisms in the hygienic control of foods.

This project also contributes to the epidemiologic study of diarrhea in children.

PUBLICATIONS

1. Imas, B. et al. E. coli enteropatógeno infantil en manipuladores de alimentos y en animales destinados al abasto. In preparation.

OTHER DATA

Grantee: Dr. Fernando Quevedo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1971-1974.

CONSERVATION OF AVIRULENT ANTHRAX VACCINE AT DIFFERENT TEMPERATURES

PROBLEM

Sterne vaccine is considered the best immunizing agent against animal anthrax. This vaccine, which should be kept at low temperature, may be stored for 2 years at 5°C without loss of viability.

In some tropical countries, the maintenance of the vaccine at 5°C could be very difficult under field conditions. The present study examines the viability and immunogenicity of ready-for-use Sterne vaccine, that is kept at 22°C and 37°C for 6 months and at 4°C for 3, 4, and 8 years; and a concentrated suspension of this vaccine maintained for 11 years at 4°C, and diluted 3 days before testing for potency and viability.

A standard vaccine prepared 1 month before the test and kept at 4°C was used as a control for all subsequent tests.

METHOD

a) Sterne-vaccine production in Gladstone and Fildes media, using strain CN 3472 (M. Sterne) Bacillus anthracis. Concentration: 2×10^7 spore/ml. Diluent: 50 percent saline solution + 50 percent glycerol + 0.05 percent saponin.

b) Quality control of the vaccine: 1) viability test in Gladstone and Fildes media; 2) potency test in guinea pigs (American short haired males). The challenge was made 25 days after vaccination using 0-10⁶ lethal doses for guinea pigs; 3) a safety test was carried out in cattle and sheep (using 5 and 10 doses of vaccine in each species).

c) Storage of the vaccines: 4°C; 22°C (room temperature); 37°C (incubator).

d) Viability and potency tests of vaccines: 8, 16, 24, 60, and 180 days after storage as well as of concentrated vaccine kept for 3, 4, 8, and 11 years.

RESULTS TO DATE

The effectiveness of attenuated anthrax vaccine stored at 4°C for a number of years was investigated by performing potency tests and counting viable spores. Of 11 batches examined, one showed a 79 percent reduction in viable spores over a 10-year period. The maximum reduction observed in the other 10 batches over 10 years was only 27 percent.

Guinea pigs immunized with vaccine stored in concentrated suspension for 10 years, or in a diluted suspension for 5 years, resisted the challenge of 10⁶ lethal doses of B. anthracis, type Pasteur IV.

Storage of the vaccine at 25°C and 37°C for 6 months did not affect the number of viable cells or its immunogenic effect on guinea pigs.

SIGNIFICANCE

Determination of the limits of storage and temperature which affect the potency of the vaccine would help to solve vaccination problems in tropical countries, in which B. anthracis infection is prevalent.

PUBLICATIONS

1. García-Carrillo, C. Conservación a diferentes temperaturas de la vacuna virulenta contra el carbunco. Bol Of Sanit Panam 74:405-10, 1973.

OTHER DATA

Grantee:	Dr. Casimiro García-Carrillo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.
Timetable:	1972-1973.

RESISTANCE TRANSFER FACTOR (RTF) IN ENTEROBACTERIACEAE
ISOLATED FROM PIGS RECEIVING PROPHYLACTIC
DOSES OF ANTIBIOTICS

PROBLEM

The importance of antibiotic-resistant Enterobacteriaceae and their ability to transfer this resistance, increased by the non-therapeutic use of antibiotics, has been pointed out in our research with horses and cattle. The aim of this study is to compare the incidence rates of resistant and RTF-positive organisms in two groups of animals, one of them having received prophylactic doses of antibiotics.

METHOD

Using the methods currently employed in our laboratory, Escherichia coli and Salmonella were isolated from feces of two groups of 50 pigs; animals in one of the groups had received prophylactic doses of antibiotics for the previous 5 days.

Sensitivity to antibiotics was determined by the Bauer and Kirby method. The transference of resistance was tested with the technique described by Schroeder et al.

RESULTS TO DATE

Salmonellae were isolated from 21 pigs pertaining to the group treated with antibiotics and from 47 pigs from the other group. The serotypes were identified as Newport, Give, Montevideo, Derby, Tennessee, Bredeney, Infantis, Cubana, Anatum, Corvallis, Hartford, Fresno, California, Cerro, Panama, and Emsbuettel.

The strains from serotype Panama proved to be resistant to streptomycin, chloramphenicol, tetracycline, and sulfamides. The presence of RTF was demonstrated in these strains. A total of 272 strains of E. coli were isolated, 109 of them from the group treated with antibiotics, which showed to be resistant to one or more drugs. From the nontreated group of pigs, 20 strains proved to be resistant. The presence of RTF could be demonstrated in a great number of E. coli strains.

SIGNIFICANCE

This project contributed to demonstrate that small doses of antibiotics exert a pressure on the selection of E. coli strains resistant to drugs.

PUBLICATIONS

1. Quevedo, F. et al. Los cerdos como excretores de salmonelas, Rev Agr Vet 2:4-8, 1973.
2. Michanie, S. et al. Resistance transfer factors in Enterobacteriaceae isolated from pigs, in preparation.

OTHER DATA

Grantee: Dr. Fernando Quevedo, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1971-1972.

INVESTIGATIONS ON THE ECOLOGY OF THE TRANSMISSION OF JUNIN VIRUS

PROBLEM

The main purpose of these studies is to determine the mode of transmission of the Junin virus, the agent of Argentine hemorrhagic fever, from rodents to man.

METHOD

Invasion of homes and buildings in urban and rural situations will be studied through a combination of techniques utilizing traps, tracking boards, and examination of the fecal material of cats.

The role of mosquitoes will be determined through attempts to isolate virus from them during the peak of the annual outbreak of the disease.

Other avenues of possible transmission will also be investigated.

RESULTS TO DATE

None thus far.

SIGNIFICANCE

To date the mode of transmission of the Argentine hemorrhagic fever remains unknown, but because of its close relation to the Bolivian hemorrhagic fever, the disease is suspected to pass between rodents by the fecal-urine-oral route. Presumably this is one possible route for infection in man, but there remain cases which are difficult to explain by this route. The role of mosquitoes has been inadequately investigated for both of these diseases. Finding the mode or modes of transmission may offer suggestions for control of the disease or protection of man from infection.

PUBLICATIONS

1. Lord, R. D. et al. Frequency of rodents in habitats near Pergamino, Argentina, as related to Junin virus. Am J Trop Med Hyg 20:338-42, 1971.
2. ----- et al. The tracking board: a relative census technique for studying rodents. J Mammal 51:828-29, 1970.

OTHER DATA

Grantee: Dr. R. D. Lord, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1974-1978.

HISTOLOGIC AND HEMATOLOGIC STUDIES IN APPARENTLY NORMAL LABORATORY AND WILD ANIMAL SPECIES

PROBLEM

The use of new animal species as experimental models requires previous knowledge of their normal histology and hematology. These values will serve as reference for alterations that might occur in the course of an experiment.

This project involves the study of the above constants in gerbils, "mulitas," (Dasypus hybridus) and vampire bats (Desmodus rotundus).

METHOD

Standard hematologic and histologic techniques will be used: fixation in neutral formalin, imbedding in paraffin, hematoxylin-eosin and Giemsa staining. Electron microscopy techniques will be used for "mulitas" and gerbils: fixation in glutaraldehyde and osmium tetroxide, imbedding in epon 812, sections in ultramicrotome Porter Blum MTI and observation with a Siemens 101 electron microscope.

Studies will be made of the leukocyte ultrastructure of peripheral blood in "mulitas" and of the epididymus and testicle ultrastructure in gerbils.

RESULTS TO DATE

1) The study of some hematologic constants in the "mulita" has been completed.

A total of 26 animals, 10 males and 16 females, were used, from which blood was obtained by cardiac puncture at time of capture. The following hematologic determinations were performed by standard methods: number of erythrocytes and leukocytes per mm^3 , erythro sedimentation; hematocrit; percentage and absolute count of leukocytes; mean globular volume; mean globular hemoglobin and concentration of globular hemoglobin; study of the ultrastructure of neutrophile leukocytes and lymphocytes; and determination of rectal temperature and of the sexual chromatin of leukocytes.

2) The study of some hematologic constants in the gerbil has been completed. One hundred gerbils of both sexes were used and the following determinations were performed by standard hematologic techniques: erythrocyte and leukocyte count, erythro sedimentation, hematocrit, and percentage and absolute count of leukocytes.

3) The histologic study of gerbils, "mulitas," and vampire bats is under way.

SIGNIFICANCE

The determination of hematologic values and the knowledge of the normal histology in apparently normal animals is basic, as a term of reference for future studies, in which the animal species will be used as experimental models.

PUBLICATIONS

1. González, M. I. and R. Battolla. Determinación de algunos valores hematológicos normales del merión (Meriones unguiculatus). Gaceta Veterinaria, in press.
2. Cuba-Caparó, A. et al. Determinación de algunos valores hematológicos en mulitas (Dasypus hybridus) aparentemente normales, submitted for publication.

OTHER DATA

Grantee: Dr. Alberto Cuba-Caparó, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.

Funded by: Pan American Health Organization/World Health Organization, United Nations Development Program, and the Governments of the Americas.

Timetable: 1972-1974.

PATHOLOGY OF LABORATORY ANIMALS

PROBLEM

The main purpose of this study is the health surveillance of laboratory animals that are used by the different units of the Pan American Zoonoses Center.

To achieve the aforementioned objective, two classes of animals were studied: 1) apparently healthy animals and 2) sick animals.

METHOD

1) Apparently healthy animals: the health of these animals is controlled through necropsies on breeders discarded because of their advanced age.

At necropsy, material is collected for microbiologic and histopathologic examinations of organs showing macroscopic lesions.

2) Sick animals: If an illness occurs in an animal colony, necropsy is performed and samples are taken for the microbiologic and histopathologic examination of organs involved.

RESULTS TO DATE

1) Apparently healthy animals: At necropsy of guinea pigs discarded because of their advanced age, liver necrosis and a parasite of peripheral blood leukocytes were found. According to the information available, this Hepatozoon type of parasite has never been described in guinea pigs. The infection it causes is widely spread in the colony and affects mostly older animals, although it has also been observed in younger and even suckling animals.

The infection progresses asymptotically, without clinical signs, in spite of the intensity of the parasitism and the severity of the hepatic lesions observed in some cases.

2) Sick animals: An illness, reported in guinea pigs maintained under overcrowding conditions, and characterized by uncertain,

nonspecific symptomatology (anorexia, adynamia, and dyspnea), has been studied. The general condition of the animals was rapidly impaired and they died in a few days. At necropsy no specific macroscopic or microscopic alterations were found. Bacteriologic examination of the liver, spleen, lung, and intestine contents was carried out. Cultures of microorganisms isolated from these materials were inoculated into healthy animals under normal housing conditions and also under overcrowding conditions similar to those experienced by the guinea pigs under study.

It is concluded that the process studied is not specific but is caused by overcrowding and poor hygiene, and by temperature and feeding deficiencies.

SIGNIFICANCE

It is hardly necessary to emphasize the importance of monitoring the health of laboratory animals to be used in scientific experiments. The early diagnosis of diseases that may affect a laboratory animal colony is of great importance to prevent its further spread.

PUBLICATIONS

1. Battolla, R. E. and J. E. Roncaroli. Necrosis hepática focal en cobayos causada por un protozoario del tipo de hepatozoon sp. Gaceta Veterinaria, in press.
2. Cuba-Caparó, A. Necrosis hepática focal en cobayos (Cavia porcellus) causada por un esporozoario del tipo de hepatozoon sp, submitted for publication.

OTHER DATA

Grantee:	Dr. Alberto Cuba-Caparó, Pan American Zoonoses Center, Ramos Mejía, Buenos Aires Province, Argentina.
Funded by:	Pan American Health Organization/World Health Organization, United Nations Development Program, and the Government of the Americas.
Timetable:	1973 - continuing.

INTER-AMERICAN INVESTIGATION OF MORTALITY IN CHILDHOOD

PROBLEM

The overall aim of the Inter-American Investigation of Mortality in Childhood was to develop death rates as accurate and comparable as possible in selected communities, taking into account the nutritional, sociological, and environmental factors responsible for excessive mortality. The data provided by the study were used to compare underlying and associated causes of death, investigate relationships with infectious diseases, nutritional deficiency states, and sociological conditions, and to recommend actions indicated by the findings.

METHOD

The deaths of 35,095 infants and children under 5 years of age in 15 widely separated areas of the Americas were 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. To permit a full evaluation of the factors resulting in the death of these infants and young children, tabulations on the pregnancy history, the status of the infant at birth, breast feeding, growth and development, and social and environmental conditions have been included.

In order to compare the data on the children who died with similar data for the general population, approximately 20,000 living children under 5 years of age have been studied through household surveys in which probability sampling techniques have been used.

RESULTS TO DATE

A comprehensive report on the investigation was published in English and Spanish in 1973 under the titles of Patterns of Mortality in Childhood and Características de la Mortalidad en la Niñez, PAHO Scientific Publication No. 262. This geographic study, and the previous one on urban mortality¹ in several areas of the Continent, point to distinctly different and intriguing patterns. They show the great value of comparing mortality in widely separated areas, thereby leading to a better understanding of health problems.

Besides these two books, the pamphlets, Excerpts and Datos Seleccionados were also published in 1973 for use in teaching and at meetings.

Nutritional deficiency was the most serious health problem uncovered in the investigation. When found in conjunction with low-birth weight, it endangered the survival of infants, young children, and probably of future mothers and hampered their growth and development. The distribution of neonatal deaths by infant weight at birth suggested excessive frequencies of low-birth-weight infants in Latin American projects. Further study of hospital births showed that the proportion of live births, with birth weights of 2,500g or less, is much higher in Latin America than in the United States and Europe. In addition, the number of babies weighing at birth 2,501-3,000g was relatively high, and of those 3,501g and over, low. Infants with birth weights of 3,501-4,000g have the lowest mortality in New York City. Because of the synergistic relationships of immaturity, nutritional deficiency, and infectious diseases, these babies with deficient weight are at great risk of death and if they survive, their growth and development may be hampered. The results of the investigation clearly point to the need for programs directed to health of women in the reproductive period, especially during pregnancy. Infants of healthy mothers have better chances to be born with normal weights and defensive mechanisms than do babies of unhealthy mother.

Of the 35,095 deaths studied in the investigation, 2,107 were not registered and were discovered through searches by the staff of the projects. In the four cities, Santiago, San Salvador, La Paz, and Cartagena, over 30 percent of neonatal deaths in hospitals were not registered. During the investigation, Legarreta et al.⁵⁷ found that 56.1 percent of the neonatal deaths in six large maternities were not included in official statistics records. Discovery of such unregistered deaths, many of which were of low-birth-weight babies, resulted in uncovering the serious problem of immaturity. Based on findings of the investigation, recommendations for the improvement of registration of births and deaths were made in a paper prepared for use at the United Nations Expert Committee meeting for the Improvement of Demographic Statistics. Hospitals should have the responsibility for preparing and registering certificates of births and deaths occurring in their institutions.

New analyses have revealed the three important determinants of infant mortality: birth weight, birth order, and maternal age group. A monograph in which mortality considering these important factors are analyzed is being prepared for publication. Because of the value of data on these three determinants for health planning, procedures have been established in large maternities in Recife for recording outcome of pregnancies on new summary forms.

The Investigation uncovered excessive frequencies of deaths from congenital nervous system anomalies in three projects. Further exploration in the El Salvador and Sherbrooke projects has revealed relatively high frequencies of anencephalus in fetal deaths. Thus research into causation of congenital anomalies of the nervous system in two Latin American countries, El Salvador and Mexico, and in the Quebec Province of Canada is indicated.

Study of biologic and socioeconomic factors revealed that lack of breast feeding was involved in excessive infant mortality from diarrheal disease and nutritional deficiency. The mother's level of education was shown to be a valuable indicator of socioeconomic status. A negative correlation was established between postneonatal death rates and the percentages of homes with piped water among families of infants dying in the postneonatal period. Coordinated, community centered research was clearly demonstrated to be a sound method of uncovering health problems and providing data needed for the establishment of effective programs.

SIGNIFICANCE

The importance of this research program has been recognized widely in the Continent for orientation of actions in maternal and child health. In December 1971, the Ministers of Health of the Andean Region stated the need for strengthening community centered research in their countries in order to measure and uncover the real magnitude of basic health problems and also to find the best methods for the efficient use of resources for solution of the problems disclosed. They pointed out the value of joining their efforts for coordinated research such as has been done in the Inter-American Investigation of Mortality in Childhood.

The success of the Investigation requires application of the recommendations for reduction of mortality and improvement of the health of future generations. To stimulate action in each project the computerized tabulations have been reproduced and are being discussed with the local staff in all projects. This material serves for analyses prepared locally and for teaching. A new project is being developed for full utilization of the results in maternal and child health programs and for teaching students of public health, medicine, and midwifery. This regional project should be influential throughout Latin America.

The World Health Organization is conducting studies of perinatal, infant, and childhood mortality in areas of Afghanistan, Algeria, Sierra Leone, Sudan, and Trinidad and Tobago, using procedures adapted from the ones followed in the Investigation. Thus the continental study is being utilized to reveal problems in other areas of the world. As stated by the director of one of the divisions of WHO, the Investigation

will be a basic reference for studies of child mortality for many years to come.

PUBLICATIONS

1. Aceves-Sainos, D. Investigación interamericana de mortalidad en la niñez. Planificación de los trabajos en el área de Monterrey, N.L., Mexico, Salud Pública de México 11:471, 1969.
2. Kessner, D. M. et al. Infant death: an analysis by maternal risk and health care, National Academy of Sciences, 1973.
3. Laurenti, R. A investigação interamericana de mortalidade na infância em São Paulo, Brasil. Rev Saúde Púb, São Paulo, 3:225-29, 1969.
4. ----- et al. Sub-registro de nascimento de crianças falecidas com menos de um ano de idade. Rev Saúde Púb, São Paulo 5:237-42, 1971.
5. Legarreta, A. et al. Omisión del registro de defunciones de niños ocurridas en maternidades, Santiago, Chile, Bol Of Sanit Panam, Vol. 75, 1973.
6. Pan American Health Organization. Mortality in Childhood. In: Gazette No. 19, 1973.
7. Puffer, R. R. and G. W. Griffith. Patterns of Urban Mortality, PAHO Sci. Pub. No. 151, 1967.
8. ----- . Initial phases of the Inter-American Investigation of Mortality in Childhood. Bol Of Sanit Panam 65:114-26, 1968; English edition, 65:35-46, 1968; and 66:341-43, 1969.
9. ----- and C. V. Serrano. Inter-American Investigation of Childhood Mortality. Washington, D.C. Technical Front Section of War on Hunger. U.S. Agency for International Development, Vol. 3, No. 11, November-December 1969.
10. ----- et al. The Inter-American Investigation of Mortality in Childhood. Assignment Children. United Nations Children's Fund, No. 15, July-September 1971.

11. ----- and C. V. Serrano. The role of nutritional deficiency in mortality. Bull of Sanit Panam (English edition) 7:1-25, 1973.
12. ----- and ----- . La deficiencia nutricional y la mortalidad en la niñez. Bol Of Sanit Panam 75:1-30, 1973.
13. ----- and ----- . Patterns of Mortality in Childhood, PAHO Sci. Pub. No. 262, 1973.
14. ----- and ----- . Características de la Mortalidad en la Niñez, PAHO Pub. Cient. No. 262, 1973.

OTHER DATA

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

Funded by: U.S. Agency for International Development (in
part, 1968-31 March, 1973).

Timetable: 1968 - continuing.

SURVEY OF SMOKING PATTERNS IN LATIN AMERICAN CITIES

PROBLEM

Background Information

According to a PAHO investigation in the early sixties, 20 percent of all deaths occurring in men 35 to 64 years of age in 10 Latin American cities were due to smoking-related diseases. Considerable variation was noted among the cities in the mortality from specific forms of cancer, and one of the most striking findings was the very high death rate from malignant neoplasms of the lung in La Plata, Argentina. Even though there is some knowledge about tobacco consumption on a national scale in a few Latin American countries, there is still no detailed information available on the smoking habits of the Region's urban populations in general.

Research Objectives

1. To estimate the extent of smoking in the populations of eight large urban centers of Latin America (Bogotá, Caracas, Guatemala City, Lima, La Plata, Mexico City, Santiago, and São Paulo), and to investigate some of the more significant social, cultural, and demographic characteristics of current and former smokers and lifelong nonsmokers.
2. To determine the beliefs and attitude in these communities about smoking and its effects on health.
3. To correlate smoking patterns with certain types of morbidity, such as respiratory symptoms, disability, and days lost from work or school, and with mortality rates from smoking-related diseases.

METHOD

A probabilistic 1,600-person sample of the population 15-74 years old in each of the cities were interviewed about their smoking behavior, their attitudes toward smoking, the reasons that motivated their acceptance or rejection of the habit, and a number of socio-cultural variables. The frequency of smoking was examined according

to sex, age, race, education, socioeconomic level, and other significant variables. The intensity of the habit was correlated with the local patterns of mortality, respiratory symptoms, disability, and absenteeism from work or school.

To facilitate comparison of Latin American and United States data, use was made of the questions in the survey instrument employed by the USPHS National Clearinghouse for Smoking and Health in studies carried out in the fall of 1964, spring of 1966, and fall of 1970.

RESULTS TO DATE

Preliminary analysis of the data shows the following:

1. The proportion of current smokers ranges between 33.8 percent and 55.1 percent for men and from 5.7 percent to 26.1 percent for women. Cities with the highest number of smokers also had the largest percentages of heavy smokers (20 cigarettes and over daily).
2. 19.5 percent to 44.0 percent of current male smokers and 15.1 percent to 36.3 percent of female smokers took up the habit before the age of 16.
3. Although about 90 percent of current smokers in each city thought the habit was hazardous to health, less than one-third of them worried, little or much, about possible harmful effects on their own health.
4. In most cities, slightly over one-half of current smokers had any knowledge of ongoing antismoking campaigns.
5. Current male smokers were more frequently confined to bed, absent from work, or restricted in their activities during the two weeks preceding the interview than were men who had never smoked.
6. The frequency of past and present cardiorespiratory symptoms was also markedly higher among current smokers than among nonsmokers.

SIGNIFICANCE

It is expected that the investigation will:

1. Establish baselines for the evaluation of smoking control programs.

2. Provide information to member countries that will help them formulate policies regarding tobacco smoking, and plan and conduct antismoking programs.

3. Generate interest and promote active support for the fight against cigarette smoking within the health agencies and voluntary organizations of the Region's countries.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Dr. Daniel J. Joly, Regional Adviser in Cancer Control, Pan American Health Organization, Washington, D.C.

Funded by: Pan American Health Organization, United Nations Development Program, American Cancer Society, Inc., Sociedad Anticancerosa de Venezuela, Fundación Peruana del Cáncer, and Associação Paulista de Combate ao Câncer.

Timetable:	Planning and design:	March 1971
	Field work:	February 1973
	Data processing and analysis:	March 1974
	Report preparation and publication:	August 1974

WATER DISTRIBUTION

PROBLEM

Water demand data and their seasonal, daily and hourly variations are not regularly registered in Latin America's water supply systems. Per capita rates are generally adopted by textbooks or arrived at on the basis of personal judgment. Once these rates are adopted for a given water supply project, they have a decisive influence on the cost of the entire system. The School of Engineering at the University of Chihuahua, in cooperation with the Central and Municipal Boards for Water and Sanitation, has undertaken to conduct this research to determine the water demand in Chihuahua.

METHOD

Water users in Chihuahua have been divided into categories according to housing standards. Sample households have been selected in each category and their water consumption registered. Consumption also has been measured in public buildings (hospitals, schools, office buildings, etc.) and industrial establishments. The data collected will be processed by computer.

RESULTS TO DATE

Although the computerization of data by the Secretariat of Hydraulic Resources in Mexico City has not yet been completed, daily variations showing a range of 20-50 percent with respect to yearly rate of consumption have been found to exist. Per capita consumption oscillates between 95 and 315 liters per capita per day. The real average rate of consumption will be known when the computerization of data is completed.

SIGNIFICANCE

Considerable savings in water costs can be obtained by adopting more realistic per capita consumption rates at the time installations are designed.

PUBLICATIONS

1. Universidad de Chihuahua, Escuela de Ingeniería.
Informe sobre el estado de la investigación de
consumos de agua potable en la ciudad de Chihuahua.
Mexico, 1972.

OTHER DATA

Grantee: Eng. José Lozano Franco, School of
Engineering, University of Chihuahua,
Chihuahua, Mexico.

Funded by: Pan American Health Organization.

Timetable: 1972-1973.

RURAL WATER CONSUMPTION DEMAND

PROBLEM

There is an almost absolute lack of information on the per-capita water consumption rates in the rural communities. There is also a similar lack of information on the daily and hourly variations of the water demands. This project aims at obtaining factual data through registration of actual water consumption in six rural communities of Panamá.

METHOD

The time period of the project is estimated at a minimum of 2 years to get a continuous flow of data covering yearly and seasonal variations. Water meters with automatic registration mechanism will be installed. Engineering students and local people will collaborate in the research. Surveys on the population living in the selected communities will be conducted.

RESULTS TO DATE

After some delay water meters have been installed and some data have been already collected.

Six forms have been prepared to correlate the metered water data to climatic, economic, social, and other factors. The forms contain information on (a) general matters; (b) public services; (c) housing; (d) facilities within the house; (e) water consumption categories; (f) hourly rate of flow.

SIGNIFICANCE

Per-capita water consumption and total population are the two factors that determine the rate of flow to be provided and consequently the size characteristics and the cost of the water supply system.

PUBLICATIONS

1. Montemayor, H. M. Informe No. 1. Proyecto de investigación sobre consumo domestico de agua in comunidades rurales. Panamá, 1972.
2. -----. Informe No. 2. Proyecto de investigación sobre consumo domestico de agua in comunidades rurales. Panamá, March 1973.

OTHER DATA

Grantee: Mr. Hector M. Montemayor, Faculty of Engineering,
Universidad de Panamá, Panamá.

Funded by: Pan American Health Organization.

Timetable: 1971-1973.

MODEL FOUR-FILTER WATER TREATMENT PLANT FOR THE CITY OF EL IMPERIAL

PROBLEM

One important problem in water treatment plants, which adversely affects the developing countries, is caused by the number of valves, appurtenances, control panels, etc., that are used in the conventional plants. This factor has an adverse impact on the cost of a plant's construction, operation, and maintenance.

The purpose of this project is to explore the possibilities of reducing to a minimum the use of mechanical equipment by relying almost entirely on hydraulic conditions, in order to obtain considerable savings in the cost of building and operation of water treatment plants.

METHOD

The project consists of the building of a pilot treatment plant with a battery of four filters. Variables will be studied and conclusions will be applied to the design of the plant for the city of El Imperial, of 8,000 people, in the Department of Lima. The pilot plant is to be built at the Laboratory of Hydraulics of the National University of Engineering.

RESULTS TO DATE

The model plant was built. Comparisons were made under different water conditions. Turbidities up to 10,000 ppm were used in specially conditioned water. Different rates of flow were passed through the filters. Design parameters obtained from the test run in the pilot plant have been used in the design of the life-size plant for El Imperial. The project is now terminated.

SIGNIFICANCE

With simplified design, savings in the cost of building and operating a water treatment plant may be substantial.

PUBLICATIONS

A final report is in preparation.

OTHER DATA

Grantee: Eng. Harry D. Dawson, Department of
Environmental Sanitation, National
University of Engineering, Lima, Peru,
in cooperation with CEPIS.

Funded by: Pan American Health Organization.

Timetable: 1971-1973.

EVALUATION OF THE INFLUENCE OF FILTRATION RATE ON WATER QUALITY AT LAS VIZCACHAS WATER TREATMENT PLANT IN SANTIAGO

PROBLEM

Water treatment plants are designed to meet water demands for "design periods" of usually 10, 20, or 30 years. Inability to expand their capacity after the design period ends, usually because of financial difficulties, is a common problem. To improve the rate of flow through the plant without impairing the quality of the water might be a good temporary solution. This project was undertaken to study the work "elasticity" of the rapid filters at Las Vizcachas water treatment plant in Santiago, Chile, and to determine whether they could absorb increasing filtration rates.

METHOD

A new experimental filter of 0.806 m^2 has been built.

Different dosages of coagulants were compared with varying rates of flow through the filters. Correlation was established between these parameters and the uniformity coefficient of the sand media used for the filtration bed.

RESULTS TO DATE

The collection of data has been completed.

Changes in the staff of the Section of Sanitary Engineering have caused a temporary delay in the preparation of the final report. The report will be available in 1974.

SIGNIFICANCE

The "load" capacity of Las Vizcachas water treatment plant would be greatly increased, thereby bringing considerable savings on plant expansion costs.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Eng. Isaac Faiguenbaum, School of Physical
and Mathematical Sciences, University of
Chile, Santiago, Chile.

Funded by: Pan American Health Organization.

Timetable: 1969-1973.

BEHAVIORAL PATTERNS OF DYNAMIC AND UP-FLOW SLOW FILTERS

PROBLEM

The purpose of this project is to trace the behavioral patterns of the dynamic filters as applied to water treatment. The project also aims at: determining how dynamic filters now in operation in Rosario behave; opening fields of usefulness for this type of filters; simplifying the filters' design; testing the retention capacity of the filter's beds; simplifying filter washing and cleaning operations; and at establishing certain design standards.

METHOD

To achieve a more simplified filter design, the following steps continue to be taken:

a) Field research:

1. Selection of three filters
2. Studies of filtering media
3. Hydraulic studies
4. Efficiency of turbidity removal
5. Observation of hydraulic head-loss

b) Laboratory research:

1. Design and construction of pilot plants
(dynamic and up-flow slow filters)
2. Selection of filtering media
3. Observations of performance of pilot plants

c) Processing and interpretation of data gathered

d) Conclusions.

RESULTS TO DATE

In order to run comparable tests, one of the filters was made to work under a hydraulic head of 1.0 cm and a surface run-off speed of 13 cm/s. Another filter was made to run under a hydraulic head of 5.0 cm and a surface run-off speed of 2 cm/s. During a continuous run of 24 days it was observed that in the filter with 2 cm/s surface speed a rapid accumulation of particles occurred resulting in the formation of a mud layer. The first filter showed a much slower deposit of particles due to its greater kinetic energy resulting from a considerable higher surface speed.

A greenish color was observed in the filters' supporting layers, especially between the gravel's interstices, suggesting the growing of gas-producing algae.

The penetration of mud into the filtering layers was of 4 cm in both filters.

Losses of head at different speeds were checked and registered.

SIGNIFICANCE

Dynamic and up-flow slow filters are in a state of development. Parameters of design leading to substantial construction savings and to more efficient running and maintenance operations will be drawn.

PUBLICATIONS

1. Carcedo, M. Informe sobre el proyecto de investigación de comportamiento y aplicabilidad de filtros dinámicos y filtros lentos ascendentes (correspondiente al periodo entre el 30/X/72 y el 30/IX/73). Rosario, October 1973.

OTHER DATA

Grantee: Prof. Mario Carcedo, Sanitary Engineering
Center, National University of Rosario, Argentina.

Funded by: Pan American Health Organization and the
National University of Rosario, Argentina.

Timetable: 1972-1973.

SEDIMENTATION TANKS

PROBLEM

No definite conclusions have been reached concerning the advantages and disadvantages of vertical-flow settling tanks as opposed to horizontal-flow installations. A number of factors affect the performance and efficiency of each type.

At the Vizcachas water treatment plant in Santiago, Chile, there are circular settling tanks, originally of horizontal-flow design, which have been modified to function through combined vertical and horizontal flow. The purpose of the present research is to gain a better understanding of this particular type of tank and to compare the performance of horizontal- and vertical-flow installations.

METHOD

A compilation of existing literature on the subject was prepared.

Two different types of pilot settling tanks were designed and built, and the efficiency of both units was measured. They were made to work under different conditions, but under the same hydraulic head and with water of the same quality.

RESULTS TO DATE

Pilot plants and scale-model tanks were designed and built. Conditioning of the settling tanks at the Vizcachas plant was completed. Tests have been run on speeds varying from 15 cm/min to 60 cm/min on horizontal- and vertical-flow basis. No significant results have yet been achieved regarding the total amount of settled material. Retention period of 2 to 4 hours have been considered.

SIGNIFICANCE

If conclusive results are derived from the research on combined vertical- and horizontal-flow installations, they can be incorporated in the design of future circular settling tanks.

PUBLICATIONS

1. Schifini, J. P. Memorial acerca de investigación sobre sedimentadores. Santiago, 1970.
2. Universidad de Chile, Facultad de Ciencias Físicas y Matemáticas, Departamento de Obras Civiles, Sección de Ingeniería Sanitaria. Estudios sobre sedimentadores. Informe de avance. Santiago, December 1970.

OTHER DATA

Grantee: Eng. Juan Pablo Schifini, Faculty of Physical and Mathematical Sciences, University of Chile, Santiago, Chile.

Funded by: Pan American Health Organization.

Timetable: 1970-1973.

STABILIZATION PONDS IN ARGENTINA

PROBLEM

Stabilization ponds seem to offer an adequate solution for the treatment of sewage from middle-sized and small communities. This process is efficient, simple to operate and maintain, and relatively cheap. It could be successfully used to treat wastes from slaughter houses, a problem of great importance in Argentina.

Nevertheless, the effect of specific geographic conditions (seasonal variations of temperature, hours of sunshine, altitude, etc). on the efficiency of the method needs to be investigated. The object of this project is to find out how these geographic parameters affect the efficiency of a stabilization pond located in the proximity of Buenos Aires.

METHOD

Two stabilization ponds were built, one in 1968 and one in 1969, and put into operation. In the first stage of the study, the efficiency of the lagoons was examined, the flow rate control was established, and a laboratory analysis was performed. Rainfall, temperature, evaporation, and sunlight hours were recorded.

In the second stage of the project, the effluent of the facultative lagoon has been studied and influence of algae on the biochemical oxygen demand (BOD) has been determined. Performance of the two lagoons under different loading conditions has been recorded. Studies now center on the efficiency of the lagoons and on the reduction of BOD.

RESULTS TO DATE

Results show that the biochemical oxygen demand (BOD/5 days/20°C) varied as follows:

-Influent: 76 - 177 ppm.

-Effluent of anaerobic lagoon: 42 - 105 ppm.

-Effluent of facultative lagoon: 32 - 127 ppm.

-Unfiltered (algae) samples: 41 - 127 ppm.

-Filtered (algae) samples: 23 - 48 ppm.

There are also variations in the dissolved oxygen (DO) (1.2 to 16.0 ppm) in relation with temperature of liquid in lagoon (10.5 to 26.5°C), temperature of the environment (14.5 to 30°C) and sky (from cloudy to sunshine). There are also variations of DO in relation to depth of sampling (0.10 m, 0.40 m, 0.70 m).

The results up to now show a classic pattern of variation in the BOD and DO.

SIGNIFICANCE

It is expected that this research will provide information on the efficiency of natural treatment of sewage through the stabilization pond process in the area of Buenos Aires.

PUBLICATIONS

1. Universidad de Buenos Aires, Facultad de Ingenieria, Instituto de Ingenieria Sanitaria. Estudios e Investigaciones Tecnológicas. Publicación No. 1:18-19, Buenos Aires, 1969.
2. Universidad de Buenos Aires, Facultad de Ingenieria, Instituto de Ingenieria Sanitaria. Lagunas de Estabilización. Publicación No. 9:121-40, Buenos Aires, 1971.

OTHER DATA

Grantee: Eng. Carlos Carrique, Sanitary Engineering Institute, University of Buenos Aires, Buenos Aires, Argentina.

Funded by: Pan American Health Organization.

Timetable: 1972-1974.

STABILIZATION PONDS IN CHILE

PROBLEM

Stabilization ponds, because of their relatively low cost, are being increasingly recognized as one of the most suitable approaches to sewage treatment in the developing countries. There has been no experience in Chile with this process. The purposes of the present research are to study the efficiency of stabilization ponds under the climatic conditions prevailing in the city of Melipilla, Chile, to compare performances with conventional sewage treatment systems, and to determine the load capacity of the lagoons.

METHOD

A pilot stabilization pond was built by the Ministry of Public Works in the city of Melipilla. At the same time, the existing literature on stabilization ponds was compiled and reviewed. Meteorologic data were collected. Different loads and rates of flow were applied to the lagoon, and the biochemical oxygen demand (BOD), the dissolved oxygen (DO), and total solids were measured to test its efficiency.

RESULTS TO DATE

Marked reductions in the BOD of the influent resulted when sewage passed through the facultative stabilization ponds (from 32 percent to 68 percent overall BOD reduction). Algae has developed very well. Records are now being taken of sunshine hours and of temperature of water and of air. These data have not yet been correlated to BOD reduction and DO content. A conventional sewage treatment plant (biological trickling filter process) has been completed. This will permit efficiency comparisons with the lagoon.

SIGNIFICANCE

The results will provide realistic information about the feasibility of this process in Chile.

PUBLICATIONS

1. Universidad de Chile, Facultad de Ciencias Físicas y Matemáticas, Departamento de Obras Civiles, Sección de Ingeniería Sanitaria. Memorial del proyecto de investigación sobre lagunas de estabilización. Santiago, 1972.

OTHER DATA

Grantee: Eng. Juan Pablo Schifini, Sanitary Engineering Section, University of Chile, Santiago, Chile.

Funded by: Pan American Health Organization.

Timetable: 1970-1973.

PERFORMANCE OF FACULTATIVE STABILIZATION PONDS IN TREATING MILK INDUSTRY WASTES

PROBLEM

Water pollution due to milk industry wastes is spreading in the Province of Santa Fé, Argentina. The project aims at studying the performance of the stabilization pond water treatment process when applied to milk industry wastes.

METHOD

Research consists of several activities: (1) study of the technical literature on stabilization ponds; (2) determination of physical, chemical, and biologic characteristics of the waste effluents from selected dairy plants; (3) analysis of the performance of full-sized stabilization ponds; (4) analysis of the performance of laboratory-scale stabilization ponds; (5) study and interpretation of the data gathered; and (6) formulation of conclusions.

RESULTS TO DATE

It has not been conclusively proved that facultative stabilization ponds have definite advantages over other milk waste treatment processes. Biochemical oxygen demand (BOD) and dissolved oxygen (DO) vary following the same patterns as other treatment processes. No attempt was made to experiment with an anaerobic process for this type of wastes.

SIGNIFICANCE

It was hoped that design parameters could be determined to appropriately use facultative stabilization ponds in the treatment of wastes coming from the dairy industry. This has not been confirmed by the research project.

PUBLICATIONS

1. Carcedo, M. Estudio sobre el Comportamiento de Lagunas de Estabilización Facultativas en el Tratamiento de los Desagues Provenientes de la Industria Lactea. Rosario, 1973.

OTHER DATA

Grantee: Eng. Mario Carcedo, Center of Sanitary Engineering of Rosario, Argentina.

Funded by: Pan American Health Organization and the University of Rosario, Argentina.

Timetable: 1971-1973.

ORTHOKINETIC FLOCCULATION

PROBLEM

Orthokinetic flocculation is a hydraulic phenomenon that has not been thoroughly studied in its application to water treatment processes. Only scant information is available about this technique. A systematic study of the subject is the objective of this research project.

METHOD

Water with artificially controlled turbidity is being circulated through a hydraulic flocculator. Rates of flow, velocities, amount of turbidity, coagulant dosage, and other factors, will be systematically varied. A number of combinations will be studied.

RESULTS TO DATE

A hydraulic flocculator was designed according to specifications that made the building of the structure a special and complex operation. The flocculator is on the premises of the School of Engineering's hydraulic laboratory. Runs and small adjustments were made to test the hydraulic performance of the unit. Several tests were performed with water having turbidities of 10 and 20 ppm. The project had to be postponed for a period of time because the principal investigator was on a field mission.

SIGNIFICANCE

The results of this project will improve the efficiency of hydraulic flocculators of helicoidal flow through new design approaches. These units could be used to serve populations with limited economic resources who cannot afford costly equipment.

PUBLICATIONS

None thus far.

OTHER DATA

Grantee: Eng. Pedro Martínez Pereda, School of Engineering, National Autonomous University of Mexico, Mexico City, Mexico.

Funded by: Pan American Health Organization.

Timetable: 1970-1973.

THE MATHEMATICAL STRUCTURE OF LIFE EXPECTANCY AND MODELS FOR ITS CONTROL

PROBLEM

The goal of this study is to develop a cost-benefit method to evaluate the impact of medical programs that change the mortality structure of a population, where "benefit" is related to increased years of life as a function of a person's age.

METHOD

A differential equation for "life expectancy" is used to examine a control problem formulation of an optimal approach in altering the life expectancy of a less favorable population toward that of a more favorable population. The "costate variable" of life expectancy is "probability of survival." The differential equations related to probabilities of dying as a function of age, due to various causes, are developed, and the discrete analogue is a Markov Chain Process which yields the "life table" of actuarial statistics.

RESULTS TO DATE

A numerical example is given in Reference 1 to illustrate the use of the formulations. Mortality for females in the countries of Costa Rica, 1963 (See Table 1 of the cited report) and Puerto Rico, 1963 (See Table 2 of the cited report) for age intervals of 5 years is used.

SIGNIFICANCE

Life expectancy, needless to say, is one of the important measures of human well-being. One of the fundamental goals set forth in the Charter of Punta del Este is to increase life expectancy at birth throughout Latin America by a minimum of 5 years within the first decade.

The methodology and computer programs developed in this study will expedite investigation of the potential impact of medical programs on life expectancy.

PUBLICATIONS

1. Parker, R. and J. Ortiz. The mathematical structure of life expectancy and the life table. PAHO Document: RD 1974.

OTHER DATA

Grantee: Dr. Rodger D. Parker, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, in collaboration with Mr. Jorge Ortiz, Department of Research Development and Coordination, Pan American Health Organization, Washington, D.C.

Funded by: Pan American Health Organization.

Timetable: 1972-1973.

AN EVALUATION METHOD TO MEASURE THE STEADY IMPACT OF BIRTH CONTROL ON HEALTH SERVICES

PROBLEM

A mathematical model that forecasts the steady state vital statistics and longitudinal population characteristics resulting from various input options is desired to form the basis for cost-effectiveness studies of PAHO activities.

METHOD

Using a Markovian approach with absorbing barriers, the explicit steady state formulas have been determined to calculate the impact of altering age-specific fertilities on the age distribution of the population, its growth and birth rates, family size and death rates per disease category. The computer programs for these calculations are fully checked out and available for other users.

RESULTS TO DATE

Data from Costa Rica have been used to illustrate the application of the model. Tables 3 and 4 of the resulting report^{2/} show the marked impact on 5-year birth rates per 100,000 caused by changing fertility rates alone. For example, in going from the baseline fertility rate ($r=1$) to those necessary for zero population growth ($r=.308135$) the death rate caused by tumors would increase from 355 to 1,520, whereas, the death rate caused by diseases of early infancy would drop from 91.5 to 13.2.

SIGNIFICANCE

The model has been developed to aid in the investigation of time-projected impacts of PAHO programs. Program changes are reflected in alterations of age-specific fertilities and the impact on medical services and concomitant required resources is ultimately determined from the alteration in population and mortality structures predicted by the model.

PUBLICATIONS

1. Nakamura, M. Some programming problems in population growth. Operations Research 21:1048-63, 1973.
2. Parker, R. and J. Ortiz. The steady state impact of birth control on health services systems. PAHO Document: RD/13/1, 1974.

OTHER DATA

Grantee: Dr. Rodger D. Parker, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, in collaboration with Mr. Jorge Ortiz, Department of Research Development and Coordination, Pan American Health Organization, Washington, D.C.

Funded by: Pan American Health Organization.

Timetable: 1972-1973.

A SYSTEMS APPROACH TO THE PREDICTION OF CANCER MORTALITY

PROBLEM

The Birth-Life-Death model developed by Ortiz and Parker, which forecasts age and disease related to mortality and population structure, will be modified to predict the expected future pattern of cancer mortality in Latin America.

METHOD

The computer program developed for the Birth-Life-Death model has been altered to yield the appropriate results.

RESULTS TO DATE

In order to develop an example, data from Costa Rica have been stratified into seven malignant tumor categories and have been analyzed. A report is now in process.

PUBLICATIONS

1. Ortiz, J. and R. Parker. A Birth-Life-Death model for planning and evaluation of health services programs. Health Serv Res 6:120-43, 1971.

OTHER DATA

Grantee: Mr. Jorge Ortiz, Department of Research Development and Coordination, Pan American Health Organization, Washington, D.C., in collaboration with Dr. Rodger D. Parker, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland.

Funded by: Pan American Health Organization.

Timetable: 1972-1973.

A SYSTEMS ENGINEERING STUDY AT THE CHILDREN'S HOSPITAL IN SAN JOSE, COSTA RICA

PROBLEM

The main objective of this study is to analyze current operating conditions in a number of areas of the hospital for the purpose of designing more efficient systems.

Students from the school of industrial engineering will be used in carrying out the project. Particular areas of investigation will be: food processing, outpatient services, and the emergency room.

In the food processing area the goals are: to simplify the information system, to modify equipment layout, and to improve work methods and environmental condition.

In the outpatient and emergency areas the particular objectives are to simplify the information system, to reduce the total waiting time of patients, to construct plans for expanding physically, the emergency area, and to improve the scheduling process.

METHOD

1) Food processing area

For the programming of diets the following techniques have been applied: (a) diagnosis of the present information system; (b) design of new forms and procedures; (c) a plant layout study for a more effective storage of food.

2) Outpatient and emergency areas

The following techniques have been applied. Work sampling study of the different behavior patterns of patients, doctors, auxiliaries; waiting lines studies in the surgical and emergency wards; statistical analysis of the effective utilization of both wards; and design of a physical expansion of the surgical emergency ward.

RESULTS TO DATE

Preliminary reports for each project have been prepared, describing the studies carried out and the results aimed at. Hospital authorities have already initiated the necessary measures for putting the recommendations into effect.

In the subarea programming of diets, improvements have consisted in elimination of 60 percent of transcriptions; elimination of the unproductive use of 40 supervisor-hours per month; introduction of telephone communications to increase efficiency in correct assignment of diets to patients who change during the day; and in 250 percent extension of the usable food storage area.

In the subarea preparation of formulas, the improvements have consisted in elimination of the excessive ambient heat; establishment of the precooling zone, with consequent improvement in the thermic output of the autoclave and particularly in utilization of the refrigerators; elimination of 50 percent of the loading and unloading in intermediate transport; replacement of an average of 40,000 transcriptions, of 4 items each, per month, performed manually, by a stamp whose use requires approximately 50 percent less man-hours and which gives 80 percent more reliability in the transcription; and in increased efficiency resulting from the new information procedures.

In the subarea emergencies, the following has been recommended: doubling of the capacity of the surgical emergency service by constructing a new ward, in view of the fact that there are infectious and non-infectious emergencies and that in 60 percent of cases the patient has to wait to be attended to; extension of the observation ward to increase the number of beds from 13 to 17 and provide the correct amount of space per bed, and enlargement of the waiting room for parents.

SIGNIFICANCE

This system's engineering study is the first of its kind undertaken by a hospital institution and the School of Engineering of the University of Costa Rica. It has created an awareness on the part of the industrial engineering students about the human elements involved in designing and improving hospital systems. On the part of hospital management, it has developed an awareness of the available quantitative methodologies.

Finally as one specific result, the program is expected to produce an improvement in the efficiency of a number of operational systems in the Children's Hospital.

PUBLICATIONS

1. Castillo, V. R. Un estudio realizado en la sala de formulas del Hospital Nacional de Niños, San José, Costa Rica, 1973.
2. Quesada S., C. M. Sistema de información de la Sección de Dietética, San José, Costa Rica, 1973.
3. Rey M., P. J. Servicio de Emergencias, San José, Costa Rica, 1973.

OTHER DATA

Grantee: Dr. Pedro Jesús Rey Merino, School of Industrial Engineering, University of Costa Rica, Ciudad Universitaria "Rodrigo Facio", Costa Rica.

Funded by: School of Engineering, University of Costa Rica and Pan American Health Organization.

Timetable: 1973.

A METHODOLOGY TO REDIRECT SOCIAL SECURITY SYSTEM PATIENTS TO CLINICS

PROBLEM

This study was carried out at the Social Security System of Monterrey, Mexico. Its objectives were:

- 1) To reassign each System's subscriber to a clinic where he and his family may obtain medical attention. As the number of subscribers increases new clinics will be in operation.
- 2) To decide which (if any) of the old clinics should be closed.
- 3) To devise a method to reassign patients from some clinics that are working at overload capacity.

METHOD

Information was obtained about the location of each existing clinic, the number of people assigned to it, and the region it serves. The location of new clinics and their capacity was then determined, and the routes of the local transportation system (LTS) identified. Each clinic was assigned a circular zone of approximately 18 blocks of radius, delineated by landmarks, avenues, parks, and other geographical boundaries. Taking into account the number of people living in each zone, the LTS routes and the main access arteries, each clinic was assigned a new area, making sure that the number of patients it will serve will not be greater than the clinic's capacity. Once geographic zones were allotted to each clinic, adjustments were made to balance outpatient populations.

RESULTS TO DATE

Reassignment of a number of subscribers was suggested for 1973 so that patients do not have to walk more than five blocks to either a clinic or to a route of the local transportation system. New assignments are proposed for 1974, 1975, and 1976. It was estimated that for 1975 most of the clinics would be saturated, unless the old clinics.

were kept open. Through population increase projections, three zones in the city were identified as requiring the construction of new clinics by 1975.

Since not all medical specialties are represented in a clinic, a patient may have to seek care for a specific condition in another clinic. A new assignment method was suggested by which a clinic would give (or receive) service to (or from) no more than two other clinics--as opposed to as many as five clinics before this system.

SIGNIFICANCE

This study, which was part of a project to encourage the use of industrial engineering techniques in hospital and medical care systems in Latin America, was instrumental in the effective reassignment of Social Security Systems patients to appropriate clinics. It also recommended zones for the development of new clinics and the exchange of medical specialty services among the clinics in the system.

PUBLICATIONS

1. Pan American Health Organization. Estudios de Ingenieria Industrial en Sistemas de Salud. Document RD 13/2, April 1974.

OTHER DATA

Grantee: Dr. Gonzalo Mitre-Salazar, Department of Industrial Engineering, Technological Institute of Monterrey, Monterrey, Mexico.

Funded by: Pan American Health Organization.

Timetable: 1972.

NATIONAL SYSTEM FOR MAINTENANCE AND ENGINEERING OF HEALTH-CARE FACILITIES

PROBLEM

Immediate Objectives

Reduce the backlog of essential maintenance and the accumulated deficit of current investment in maintenance and repairs of health-care facilities. Increase the normal useful life of facilities and equipment. Establish a national system for hospital and health-care facilities maintenance and engineering.

Long-Range Objectives

Development of human resources for maintenance and engineering. Assignment of economic resources and financing. Development of a national maintenance system, emphasizing regional programs. Management and administrative improvement in the utilization of the existing and future maintenance resources.

METHOD

Development and implementation of national maintenance and engineering services for health-care facilities in Venezuela, at four levels of operation--central, regional, subregional, and local--by clearly identifying the functional relationship of the various levels of the system. Organizational structures will be developed and introduced at each level within the framework of the administrative reform. The management component will be developed and introduced at all levels along with education and training, special studies, and technical assistance.

RESULTS TO DATE

The objective of the plan and activities for the Center for Hospital Maintenance and Engineering are being carried out effectively. All necessary technical manuals have been written or are in the stage of development. Programs have been prepared and used for the training

of personnel. Interministerial technical committees have been established, through which technical recommendations and modifications can be incorporated during the course of design and construction. A comprehensive program for the operation, maintenance, and inspection of equipment through technical training courses in electrical systems, hospital maintenance and engineering, refrigeration and air conditioning, boiler operation and maintenance, emergency power plants, environmental sanitation, sterilization equipment, and laundry supervision is being instituted.

SIGNIFICANCE

The special studies carried out as a result of the total program indicate that effective cost savings can be made and the delivery of medical care improved. Special studies through operations research are required to maintain the health-care facility at the optimum.

PUBLICATIONS

Listed below are technical manuals published by the Center for Hospital Maintenance and Engineering, Caracas, Venezuela:

1. Operación y Mantenimiento de Equipos de Esterilización, 1968.
2. Métodos y Procedimientos de Limpieza, 1969.
3. Equipos de Rayos X y su Mantenimiento, 1969.
4. Mantenimiento de plantas y red de distribución de vapor, 1969.
5. Conservación de acabados en edificios, instalaciones y equipos, 1969.
6. Operación y mantenimiento de incineradores, 1969.
7. Equipos de prevención de incendios y su mantenimiento, 1969.

8. Impermeabilizaciones y su mantenimiento, 1969.
9. Operación y mantenimiento de equipos de refrigeración y aire acondicionado. 1969.
10. Operación y mantenimiento de equipos de cocina, 1969.
11. Mantenimiento de Areas Exteriores, 1969.
12. Tratamiento de aguas para equipos e instalaciones de hospitales, 1969.
13. Acondicionamiento ambiental de hospitales, 1969.
14. Estudio y proyecto de organización para el mantenimiento de plantas termoeléctricas de emergencia, 1969.
15. Operación y mantenimiento de equipos de lavandería, 1970.
16. Incineración de desperdicios en hospitales, 1970.
17. Preparación de una caldera para el reconocimiento y prueba hidrostática, 1970.
18. Recomendaciones para la solución de trampas de vapor, 1971.
19. Programa de adiestramiento para personal técnico de mantenimiento de hospitales, 1971.
20. Adiestramiento en operación y mantenimiento sobre plantas de generación de vapor, 1971.
21. Normas para Recepción de Centros Médico-Asistenciales, 1971.
22. Curso de Electrónica Aplicada para Técnicos Electro-Mecánicos, 1972.
23. Programa del Curso: Técnico de Mantenimiento para Plantas de Generación de Vapor, 1972.
24. Guía del Instructor para Curso Técnicos de Mantenimiento para Plantas de Generación de Vapor, 1972.

25. Curso para Técnicos Regionales de Plantas de Generación de Vapor, 1972.
26. Curso para Operadores de Plantas de Generación de Vapor, 1972.
27. Partes y funcionamiento. Simpson 260 (Multímetro), 1972.
28. Manual del Curso para Operadores de Equipos de Esterilización, 1972.
29. Mantenimiento de quemadores de gas y gasoil - Ing. Victor Salazar Hamana, 1973.
30. Programa de mantenimiento preventivo para equipos de refrigeración y aire acondicionado. Vittorio Della Sala, 1973.
31. Algunas recomendaciones de operación sobre los defibriladores de corriente continua para cardioversión. Vittorio Della Sala, 1973.
32. Procedimientos del Departamento de Mantenimiento Hospitalario. 1973.
33. Curso de producción y supervisión de lavandería, 1973.
34. Seguridad y Protección contra incendios en hospitales. (Manual para organización y funcionamiento). Shelley Rule, 1973.
35. Manual de organización para seguridad y prevención de incendios. Universidad de los Andes de Mérida. Shelley Rule, 1973.
36. Sistema Total de Mantenimiento para Hospitales, 1974.

OTHER DATA

Grantee: Center for Hospital Maintenance and Engineering,
Ministry of Health and Social Assistance,
Caracas, Venezuela.

Funded by: Pan American Health Organization and
United Nations Development Program.

Timetable: 1972-1975.

ASSESSMENT OF THE STATE OF THE HEALTH PLANNING PROCESS

PROBLEM

Latin American countries have been using new approaches to health planning during the last 10 to 12 years. Planning methods and techniques have been devised; a sizable number of personnel has been trained and planning has been incorporated as a normal activity of many health organizations. Planning processes have evolved in many different ways and partial efforts have been made to study their state of development. These efforts have mostly approached the problem from a formal organizational viewpoint. This project aims at determining the actual state of development of the processes, ascertaining the benefits achieved with them, and detecting both the areas that are hampering its progress and the strategic ones that might promote its further development.

METHOD

Development of a conceptual framework and of a theoretical model of a planning process. Design and field trial of a questionnaire and data collection forms. Field work: collection of data in each country, processing, analysis, and publication of results.

RESULTS TO DATE

The conceptual framework and the theoretical model were drafted during 1973 and are now being revised following a field trial.

SIGNIFICANCE

The state of development of a planning process conditions the planning methods to be used, as well as the general orientation that must be adopted. The provision of specific approaches for each particular case will thus be expedited.

PUBLICATIONS

1. Pan American Health Planning Center. The conceptual framework of health planning processes. In preparation.

OTHER DATA

Grantee: Pan American Health Planning Center,
Santiago, Chile.

Funded by: Pan American Health Organization and
United Nations Development Program.

Timetable: 1973-1974.

EVALUATION OF THE HEALTH PLANNING PROCESS OF ANTIOQUIA, COLOMBIA (PLANSAN)

PROBLEM

Health planning was introduced in Colombia during the midsixties. The impact it has had on the health services administration is a matter of particular concern for the government of the country and for PLANSAN. This project is intended to evaluate the health planning process of the Antioquia Department of Health, through a method which could subsequently be applied to other areas and to the health sector of Colombia as a whole, and which would allow for the adoption of measures to improve and further develop the process.

The project has been divided into four subprojects, as follows:

I. Intersectoral analysis, which intends to study the impact of health planning on the development of the health sector and on the health sector's relation to other social and economic areas.

II. Sectoral diagnosis and health institutional analysis, which is geared to study the impact of health planning on the macrostructure of the health sector and on each one of its main institutions.

III. Intrainstitutional analysis, which intends to ascertain the degree to which the planning process has been incorporated by each health institution and the characteristics of the process.

IV. Macroproblem analysis, which is addressed to the formulation of plans oriented to correct the main problems of health planning.

METHOD

Subproject I: Selection and study of the evolution of indicators for each one of the sectors. A panel of experts is interviewed, information is collected, described, and interpreted. Relational hypothesis are tested.

Subproject II: Techniques for the study of the public sector, adapted and improved for the study of the health sector, are employed.

Subproject III: A model has been studied and adopted. Interviews with the agents of the planning process were held. Official documents and reports of institutions are analyzed.

Subproject IV: Each macroproblem is analyzed within the conceptual framework of health planning; political analysis is used to formulate plans of action.

RESULTS TO DATE

Subproject I: All information was collected and processed and a descriptive study was completed. Analysis is in its last stage.

Subproject II: All information was compiled. Description of the situation was published. Analysis is under way.

Subproject III: The model of analysis was designed. Information was obtained through questionnaires; provisional publication of results has been made on the interviews, the attitudes of agents, and on the model used. Analysis is under way.

Subproject IV: Awaiting for completion of Subprojects I, II, and III.

SIGNIFICANCE

The project will throw light into the utility of the planning process as it has been developed to date in Colombia; it will also provide criteria to adopt certain techniques and procedures both for the evaluation of health planning processes and for the introduction of new approaches to planning.

PUBLICATIONS

1. PLANSAN. Planificación de la Salud en Antioquia, Protocolo General, Publicación No. 1, 1971.
2. -----. Indicadores Sectoriales, Departamento de Antioquia Proyecto I, Etapa A, Publicación No. 3, 1973.
3. -----. Diagnóstico del Sector Salud y Análisis de sus Instituciones, Departamento de Antioquia, Proyecto II, Publicación No. 4, 1973.

OTHER DATA

Grantee: PLANSAN Research Group, Dr. Francisco Henao,
Executive Director, Antioquia, Colombia.

Funded by: Pan American Health Organization; Ministry of
Health of Colombia; Sectional Health Service
Antioquia; Secretary of Health, Education, and
Welfare of Medellín, Colombian Institute of
Social Security; and School of Public Health of
the University of Antioquia.

Timetable: First stage: 1970-1972.
Second stage: 1973-1975.

DESIGN OF A GUIDE FOR HEALTH POLICY FORMULATION

PROBLEM

A sound and well-stated health policy is of paramount importance in the development of health planning and administration. It should provide planners and administrators with a clear view of the goals to be achieved in regard to the state, configuration, and performance of the health services system, as well as with the guidelines to orient all decisions and actions towards such achievement. A quick review of the existing health policies of the countries of the Region show them to be rarely well formulated, incomplete, and lacking in internal and external consistency, feasibility, and viability. In short, they are not operative enough to serve planners and administrators as effective guides for their work.

It is believed that policy formulation could benefit from the use of a systematic approach--a set of procedures--that could constitute a guide for policy makers in the consideration and statement of adequate health policies. The purpose of this project is to devise such a guide and to design the analytical tools to be employed therein.

METHOD

Development by the Health Planning Center of a conceptual framework, followed by a theoretical model of a policy formulation procedure. Design of analytical tools for the study of consistency, feasibility, and viability. Experimental application of the method in three countries, and final adjustment of the guide.

RESULTS TO DATE

The conceptual framework and the theoretical model for policy formulation were drafted. A first approach to the field trial was partially carried out in two countries and had to be postponed because of internal reasons in the countries. The design of analytical tools is in progress. The results obtained to date have been widely used for training purposes in planning courses.

SIGNIFICANCE

This approach to policy formulation will probably expedite the process and facilitate communication and understanding between the policy makers and technical staff of the health services system. Planning and management procedures should also gain by this research.

PUBLICATIONS

1. Pan American Health Planning Center. A procedure for the health policy formulation (first draft). (Mimeographed, for use in health planning courses), 1973.

OTHER DATA

Grantee:	Pan American Health Planning Center, Santiago, Chile.
Funded by:	Pan American Health Organization and United Nations Development Program.
Timetable:	1973-1974.

REGIONAL HEALTH PLANNING

PROBLEM

Each geographic region of a country has its own approach to the planning of its overall development, which may differ somewhat from the average national development policy. On the other hand, the latter often requires the redistribution of economic activities through the strengthening of certain areas or poles of development. Whereas the economic sector has been successful in gathering knowledge on regional planning and on the utilization of certain techniques for that purpose, the social sector, health in particular, is always called to participate in regional development programs. They face these commitments, however, with a limited knowledge and not-well-organized methods.

This project seeks to throw light on this problem and to devise adequate methods for the elaboration of a general regional development planning.

METHOD

Design of a preliminary conceptual framework; direct participation in regional development projects to gather factual information; development of methods; practical application to actual projects; adjustment of framework and methods.

RESULTS TO DATE

A preliminary conceptual framework was developed and applied in two regional development projects of Venezuela, at the request of that country, in a collaborative enterprise with the Latin American Institute for Social and Economic Development (ILPES). Methods are being devised and adjusted.

SIGNIFICANCE

Real integration of development planning can be achieved in an easier way when planning is made at the regional level. This research has significance both from substantive and methodologic viewpoints.

PUBLICATIONS

Preliminary reports under preparation.

OTHER DATA

Grantee: Pan American Health Planning Center,
Santiago, Chile.

Funded by: Pan American Health Organization and United
Nations Development Program.

Timetable: 1973-1974.

PAHO REGIONAL LIBRARY OF MEDICINE AND THE HEALTH SCIENCES

PROBLEM

A survey of library resources in Latin 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

The PAHO Regional Library of Medicine and the Health Sciences (RLM) was established in 1967 to contribute to the strengthening and improvement of the teaching, research, and practice of medicine and allied professions in Latin America. It carries out regular functions designed to accomplish the following aims:

To provide workers in the health sciences with increased access to a more comprehensive collection of publications in these sciences, and, by so doing, to make Latin America self-sufficient in library resources and services in the biomedical field.

To use modern methods of data processing and communication, including the MEDLINE system, to make these materials more accessible, and to disseminate biomedical information to the health sciences community;

To build up a pilot biomedical audiovisual unit and to establish a dissemination system for audiovisual materials.

To serve as a demonstration and training center to foster and encourage the development of medical libraries in Latin America;

To encourage cooperative library programs among medical libraries in Latin America; and

To build up its own facilities through the development of journals and monograph collections, as well as selected secondary reference materials.

RESULTS TO DATE

A library building provided by the Escola Paulista de Medicina has been adapted for RLM's use, and equipment is being obtained as needed. Since 1971 the building has been expanded from 1,950 m² to 3,340 m² to satisfy RLM's growth in services and to provide space for audiovisual activities.

The systematic acquisition and maintenance of bibliographic materials, serial publications, monographs, and documents is being continued. A collection of periodicals from 1960 onward of 4,817 biomedical journal titles--1,699 of which are obtained by subscription, 265 by exchange, and 854 by grants from the editors--is well on its way to being completed.

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 including audiovisuals is also being developed.

A Scientific Advisory Committee to the Library has held six annual meetings so far.

In January 1969, interlibrary loan services to all parts of Brazil were begun, and based on experience in operations in that country RLM extended its services to other Latin American countries in 1970. Up to 1973 a total of 156,581 interlibrary loan requests from 180 libraries were processed and services were begun to Latin American countries, especially Argentina, Peru, and Venezuela. RLM was able to satisfy 97,031 (62 percent) of the requests from its own resources. An additional 37,398 (24 percent) were answered through other libraries in São Paulo participating in the program, and the remaining 22,152 (14 percent) were forwarded to the U.S. National Library of Medicine (NLM) in Bethesda, Maryland, for processing. Up to 1973, 2,190 reference requests for special bibliographies were also received: 1,833 of these were attended to by RLM itself; 147 were sent to NLM in Bethesda for bibliographic retrieval by the MEDLARS/MEDLINE computer-based system, and 210 were fulfilled by the MEDLINE system operating in São Paulo.

Ten subcenters have been developed in Brazil--Fortaleza, Belém, Recife, Salvador, Brasília, Rio de Janeiro, Niterói, Belo Horizonte, Curitiba, and Porto Alegre--and in addition, four specialized sub-systems for dentistry, nursing, pharmacy, and veterinary medicine were established. Once these libraries' resources were pooled and operations throughout Brazil fully established, RLM started its cooperation with national centers elsewhere in Latin America, to establish a Pan American network for biomedical scientific communications. Gift and exchange activities were conducted with 535 libraries in Brazil and 237 similar institutions in other Latin American countries. A total of 164,790 duplicate journal volumes or issues were distributed since 1969.

In 1970 a Selective Dissemination Information Program was begun to spread biomedical and scientific information to selected groups of workers in hospital administration, medical education, health and population dynamics, maternal and child health, nuclear medicine, preventive and social medicine, and parasitology and tropical medicine. At the present time, 115,612 articles have been delivered to 976 participants.

MEDLINE service was initiated on a pilot basis in March 1974. By arrangement with the U.S. National Library of Medicine, which provides the software, and the State of São Paulo's Atomic Energy Institute which leases computer time, MEDLINE is routinely performing 2 hours of search per week at the computer site. A three-phase MEDLINE deployment program has been initiated under grants from the United Nations Development Program, the Government of Brazil, and the State of São Paulo. MEDLINE service will start at RLM, at Brasília, Rio de Janeiro, and in northeastern Brazil, and will be extended later to other areas.

In 1974 also, RLM's audiovisual program was activated with the acquisition of hardware and software, and arrangements were made for coordination with the Latin American Center of Educational Technology for Health (CLATES).

Advanced courses have been offered to medical librarians throughout Latin America. In 1973, 46 librarians received PAHO fellowships to attend the courses. Several seminars for guidance and orientation of medical educators and librarians were sponsored and attended by RLM.

SIGNIFICANCE

By providing specialists in the health sciences with increased access to scientific literature, the PAHO Regional Library of Medicine and the Health Sciences is contributing to the acceleration and improvement of biomedical education, research, and practice in Latin America.

Satisfactory, prompt service and improved bibliographic resources are generating an increase in requests for scientific literature. Biomedical librarians throughout Brazil have responded with increasing interest by producing collective catalogs and improving cooperative interlibrary loan service among local medical libraries.

Cooperative efforts have been made to improve scientific information in most of the Latin American countries. An inter-American network of biomedical communications is being developed and studies are being made of the existing facilities and needs. The installation of the MEDLINE data-base in São Paulo is the first step toward establishing this network in Brazil and other countries of Latin America.

Special emphasis on medical education is an important aspect of RLM's audiovisual activities.

Several projects are being presented to international agencies involved in aiding research, with the idea of giving Latin American scientists access to a wider and more complete collection of medical information that is basic for creative research work, and of avoiding repetition of research.

PUBLICATIONS

1. Bibliographic Reference Lists: 6 issues
2. Catalog of Recent Acquisitions: 12 issues
3. Index of Reference Works Existing in the PAHO Regional Library of Medicine and the Health Sciences
4. Information Bulletin: 5 volumes
5. The Library for Librarians
6. List of Joint Duplicates for Exchange: 16 issues
7. Minimum Requirements for the Establishment of a Dentistry School Library (Portuguese)
8. Minimum Requirements for the Establishment of a Medical School Library (also in Spanish): 4 editions
9. Minimum Requirements for the Establishment of a Nursing School Library (Portuguese)
10. Neghme, A. La función de la Biblioteca en la Enseñanza Superior, la Investigación Científica, y la Práctica Profesional. Bol Of Sanit Panam 63:242-50, 1972.
11. Recent Information on RLM: 4 issues
12. Regulations for Interlibrary Loans: 2 editions
13. Standards for Hospital Libraries (Spanish)
14. Suggestions for the Establishment of Library Services

OTHER DATA

- Grantee: Dr. M. Martins da Silva (Project Coordinator), Pan American Health Organization, Washington, D.C., and Dr. Amador Neghme, Director, PAHO Regional Library of Medicine and the Health Sciences, São Paulo, Brazil.
- Funded by: The Commonwealth Fund, the W. K. Kellogg Foundation, the United Nations Development Program, the Brazil's Ministries of Health and of Education and Culture, the Secretariat of Health of the State of São Paulo, the U.S. National Library of Medicine, the Pan American Health Organization, and the Escola Paulista de Medicina.
- Timetable: 1967 - continuing.

PAHO/WHO IMMUNOLOGY RESEARCH AND TRAINING CENTERS

PROBLEM

Despite the broadening scope of activities in immunology, the phenomenon of immunity to infectious diseases is poorly understood and vaccination remains an empirical procedure in many countries. As immunologic techniques become more complicated and interrelations with other disciplines become wider and more frequent, the need for proper training in immunology is urgent. The PAHO/WHO Research and Training Centers in Immunology are doing much to fulfill this need, functioning as catalysts in a multidisciplinary field.

METHOD

The two PAHO/WHO Immunology Research and Training Centers set up for the Americas, one at the Butantan Institute in São Paulo and the other in Mexico City, differ somewhat in the nature of their organization and the scale of their activities. They share, however, two common functions: they are developing the potential for research and teaching in immunology at a high level, and they provide opportunities for workers in related fields such as clinical medicine, microbiology, and parasitology to gain experience of modern immunologic ideas and techniques.

The São Paulo Center, now in its eighth year of operation, admits up to 15 students to its 4-month training course each year. The course comprises an intensive program of lectures (140 hours), seminars (115 hours) and laboratory work (320 hours). Special emphasis is given to developing the trainees' capacity to analyze scientific papers and to elaborate research projects. The course is given full credit both by the University of São Paulo and by the Escola Paulista de Medicina. Postgraduate students are accepted to work in the Center for the master's or doctor's degree.

The Immunology Research and Training Center in Mexico City consists of a collaboration between 10 participating laboratories and institutes. In addition to basic general courses, the Mexican Center provides opportunities for advanced training in immunology at the graduate level.

The two Centers conduct, simultaneously with their teaching activities, research programs in basic immunology and in the immunology of diseases prevalent in Brazil and Mexico.

RESULTS TO DATE

Increasing numbers of visiting professors have acted as lecturers, short-term consultants, or collaborators in joint research projects. As a result of these activities, there has been an increase in the number and quality of the personnel engaged in the teaching of immunology in schools of medicine in Latin America and an increase in laboratory facilities for both research and training. There has been an improvement in the utilization of available resources, and a raising of standards in the diagnosis and treatment of clinical conditions related to immunology.

Broadly, the Mexican Center's research projects have focused on diarrhea, leprosy, tuberculosis, and rhinoscleroma. The Center has also played an active role in subjects of national interest such as immunization programs and transplantation legislation.

The Brazilian Center's research programs have included the study of T and B lymphocytes in circulating blood in the different forms of leprosy; production of IgE antibody in B mice; stimulation of the immune system by single and double stranded RNA; adjuvant effect of lipopolysaccharides in IgE antibody production in Guinea pigs; purification and biologic properties of lymphocytosis-stimulating factor; identification of protective antigens from Bothrops jararaca venom; and others.

SIGNIFICANCE

The teaching activities of the two Immunology Research and Training Centers have led to an increase in interest in immunology among research workers in related disciplines, such as biochemistry, bacteriology, virology, parasitology, and transplantation.

PUBLICATIONS

São Paulo

1. Albuquerque, L. R. et al. Failure of insoluble antigens to elicit anaphylactic reactions. Int Arch Allergy Appl Immunol 41:797, 1971.

2. Beutner, E. H. et al. Immunofluorescent studies of anti antibodies to intercellular areas of epithelia in Brazilian pemphigus foliaceus. Proc Soc Exp Biol Med 127:81, 1968.
3. ----- et al. Mem Inst Butantan 35:79-94, 1971.
4. Gennari, M. Estudo da ação do veneno de Bothrops jararaca sobre o complemento de cobaia. Master's thesis (oriented by Otto G. Bier), University of São Paulo, 1972.
5. Guercio, P. et al. Opsonic, cytophilic, and agglutinating activity of guinea pig γ -2 and γ -M anti Salmonella antibodies. Immunology 16:361-71, 1969.
6. Mota, I. and A. Perini. A heat-labile mercaptoethanol susceptible homocytotropic antibody in the guinea pig. Life Sci 9:923, 1970.
7. Passos, Helcio C. et al. Immunology. Submitted for publication.
8. Perini, A. Estudo sobre o efeito adjuvante de endotoxinas e de ácidos nucleicos na produção de anticorpos da classe IgE em cobaias. Master's thesis (oriented by I. Mota), University of São Paulo, 1972.
9. ----- and I. Mota. Heterogeneity of guinea pig homocytotropic antibodies. Immunology 22:915, 1972.
10. ----- and ----- . The production of IgE and IgG1 antibodies in guinea pigs immunized with antigen and bacterial lipopolysaccharides. Immunology, 1973. Submitted for publication.
11. Uriel, J. et al. Immuno-electrophoretic characterization of enzymes in the venom of Bothrops jararaca. Bull Soc Chim Biol 50:938-40, 1968.

Mexico City

1. Alarcon-Segovia, D. et al. Antinuclear antibodies in patients on anticonvulsant therapy. Clin Exp Immunol 12:39-47, 1972.

2. ----- et al. Radioisotopic evaluation of salivary gland dysfunction in Sjögren's syndrome. Am J Roentgenol 112:373-82, 1971.
3. ----- et al. Isoniazid acetylation rate and development of antinuclear antibodies upon isoniazid treatment. Arthritis Rheum 14:748, 1971.
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5. Biro, C. E. and C. Arroyave. On the existence of a live immunologically unresponsive lymphoid cell. Immunology 18:387-91, 1970.
6. Estrada Parra, Sergio and E. Garcia Ortigoza. Specificity of antibodies in sera of rabbits immunized with dinucleotide-egg albumin conjugates. Immunochemistry 9:839-42, 1972.
7. ----- and ----- . Immunochemical determination of the molecular conformation of nucleotides. Ibid 9:799-807, 1972.
8. Gitlin, David et al. The turnover of amniotic fluid protein in the human conceptus. Am J Obstet Gynecol 113:632-45, 1972.
9. Jaroslow, Bernard N. and L. Ortiz-Ortiz. Hydroxyurea and cell-cycle kinetics of cultured antibody-forming cells. Cell Immunol 2:164-70, 1971.
10. ----- and ----- . Influence of poly A-poly U on early events in the immune response in vitro. Cell Immunol 3:123-32, 1972.
11. Kretschmer, R. R. et al. Reversible neutrophil defect in ataxia telangiectasia. Pediatrics 50:147-50, 1972.
12. Kumate, Jesus et al. Neutrophil-mediated immunity in severe malnutrition. In: Proceedings III Western Hemisphere Nutrition Congress, Miami Beach, Florida, August-September 1971, pp. 346-50, Mt. Kisco, N.Y., 1972.

13. ----- et al. Content of common antigen of Escherichia coli diarrhea of newborns and infants in a Mexican preindustrial community. Ann N Y Acad Sci 176:350, 1971.
14. Mejia Laguna, J. E. et al. Prolonged survival of kidney xenografts in leucopenic rabbits. Immunology 21:879-82, 1971.
15. Montford, Irmgard and R. P. Tamayo. Two antigenically different types of macrophages. Proc Soc Exp Biol Med 138:204-07,
16. Ortiz Ortiz, L. and L. F. Bojalil. Delayed skin reactions to cytoplasmic extracts of Nocardia organisms as a means of diagnosis and epidemiological study of Nocardia infection. Clin Exp Immunol 12:225-29, 1972.
17. ----- et al. Cytoplasmic antigens from Nocardia eliciting a specific delayed hypersensitivity. Infect Immun 5:879-82, 1972.
18. ----- et al. Delayed hypersensitivity to polysaccharides from Nocardia. J Immunol 108:1409, 1972.
19. ----- et al. The assay of delayed hypersensitivity to ribosomal proteins from Nocardia. Sabouraudia 10:147-51, 1972.
20. ----- et al. Delayed hypersensitivity to ribosomal protein from BCG. J Immunol 107:1022-26, 1971.
21. Rivera, Felix Cordoba et al. La especificidad inmunológica de los anticuerpos contra piridoxal y contra fosfato-5' -de piridozal. Immunochemistry 7:543-58, 1970.
22. Rojas Espinosa, O. et al. Presence of Clq-reactive immune complexes in patients with leprosy. Clin Exp Immunol 12:215-23, 1972.

OTHER DATA

Grantee: Dr. Ivan Mota, PAHO/WHO Immunology Research and Training Center, Instituto Butantan, São Paulo, Brazil, and Dr. Jesús A. Kumate (Project Coordinator in Mexico), Hospital Infantil de México, México, D.F., Mexico.

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

Timetable: São Paulo: 1966 - continuing.
Mexico City: 1969 - continuing.

PAHO RESEARCH GRANTS PROGRAM

PROBLEM

In most countries of the Region, there is a need for strengthening of health services, cooperation among scientific and professional groups, good standards of teaching of health and related fields, and for promotion of basic and applied research.

To meet these needs, the PAHO Research Grants Program (1) provides research grants to individual investigators to enable them to carry on specific research projects; and (2) encourages the exchange of research experiences and the improvement of communication among scientists through investigators' visits to different countries.

METHOD

Preference is given to research projects in areas related to PAHO program priorities, and to applicants who are nationals of member or participating governments of PAHO.

Applications are appraised for scientific merit by referees appointed on a rotating basis, who represent the following fields: human and animal health; veterinary medical education; food hygiene, communicable diseases; cancer; cardiovascular diseases; noncommunicable diseases; sanitary engineering; education methodology and technology; mental health; alcoholism and drugs; nutrition and food sciences; malaria; vector biology and control; health education; population dynamics; pathology; mammalogy and zoonotic diseases; protozoology and epidemiology; and radiation health.

The grantee is expected to submit periodic reports on the progress of his research and a final report summarizing the whole project and the positive and negative aspects of the experience acquired.

RESULTS TO DATE

Thirty eight applications from Argentina, Brazil, Chile, Colombia, Costa Rica, Guyana, Mexico, Panama, Trinidad and Tobago, Venezuela, and the United States have been received. Twenty research grants have been

awarded to date. Four were rejected because they were outside the scope of the program and 14 others are being processed.

SIGNIFICANCE

By supporting investigators in the study of health problems prevalent in their own countries, the PAHO research program contributes to moderating the emigration of scientific personnel from Latin America and the Caribbean area.

PUBLICATIONS

1. PAHO Research Grants to Individual Investigators. Information Booklet, 1974.
2. PAHO Research Grants Program. PAHO/ACMR 13/3, 1974.

OTHER DATA

Grantee:	Dr. Humberto Torloni (Project Coordinator), Department of Research Development and Coordination, Pan American Health Organization, Washington, D.C.
Funded by:	The Wellcome Trust and the Pan American Health Organization.
Timetable:	1973 - continuing.

