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COLLABORATIVE STUDY ON IRON ABSORPTION
IN NINE CENTERS

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COLLABORATIVE STUDY ON IRON ABSORPTION IN NINE CENTERS

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COLLABORATIVE STUDY ON IRON ABSORPTION

IN NINE CENTERS

A. INTRODUCTION

Studies in recent years, sponsored by WHO (1) and by WHO and PAHO (2), have substantiated the high prevalence of iron deficiency anemia in the menstruating and pregnant female population. There is likewise ample evidence of iron deficiency in infancy (3), and recent surveys in the United States substantiate a high prevalence in childhood and adolescence as well (4). The borderline iron balance in these vulnerable populations is explained by a restricted iron intake in the face of increased requirements due to growth, menstrual blood loss, or fetal iron requirements. In a recent study in Latin America, iron deficiency was shown to be the preponderant, indeed the only statistically identifiable cause of anemia in pregnant women (2). Most nutritional anemia is of mild or moderate severity, but iron depletion renders the individual more vulnerable to severe anemia which may be life threatening. While symptoms may not be recognized in the sedentary individual, maximal work performance is impaired in proportion to the decrease in hemoglobin concentration (5, 6). In view of the widespread prevalence of iron deficiency and its anemia, estimated to involve hundreds of millions of the world's population, this deficiency state can be assumed to impose a considerable health and economic disability. Furthermore, it affects most commonly those people of limited economic means who can least afford the disadvantage.

In explaining the prevalence of iron deficiency, attention has been focused on the extremely low iron intake in man and on the low availability of food iron. Iron intake has decreased in many countries over the past 50 years due to a more sedentary life with its decreased caloric (and iron) intake, to the discontinuance of iron cooking utensils, and to the greater cleanliness in food preparation. It is also recognized that iron in vegetable food is poorly absorbed, and that chelates such as EDTA employed as food preservatives interfere with iron absorption. The correlation between low plasma protein (albumin) concentration and iron deficiency (2, 7) which is found in low economic groups is probably explained by a limited intake of animal protein, which enhances food iron absorption. In several countries in Central and South America, in spite of ample iron intake, iron deficiency anemia is prevalent, this may perhaps be attributed to low availability of iron ingested.

Extensive fortification of food with iron is being carried out in various countries throughout the world, e.g., flour in the United States and several Latin American countries, England and Sweden, and rice in Taiwan and the Philippines. Yet the effectiveness of these supplements has not been demonstrated. There is good evidence that some of the iron salts used are of limited effectiveness. For example, large particle ferrum reductum appears unavailable (8), although small particle ferrum reductum is as effective as ferrous sulphate (9). Orthophosphate has been found to be 0.3 times as effective, and pyrophosphate 0.05 times as effective as ferrous sulphate (9). Thus there appears to be a real need to evaluate iron fortification procedures.

Recent evidence has been presented to indicate that an extrinsic iron salt tag, added as a tracer, will indicate the amount of nonheme iron being absorbed from a total meal, regardless of the original form of the food iron (10). A salt such as ferrous sulphate, which is effective in fortification may also be evaluated by this extrinsic tag (11). These studies indicate that all nonheme iron including that contributed by fortification iron of high availability enters a common pool whose availability is determined by the composite food articles of the meal. While heme iron is not measured by this single tag, it represents a lesser fraction of food iron, especially in the low income vulnerable population. Furthermore, in contrast to nonheme iron whose absorption is profoundly affected by diet composition, heme iron absorption bears a fairly constant relationship to the absorption of iron ascorbate and therefore is predictable if the individual's absorption is calibrated by a reference dose of iron ascorbate. A method therefore exists by which it is possible to evaluate the effectiveness of food fortification prior to field trial.

There are several critical links which must be evaluated if food fortification with iron is to be successful. 1) An iron salt must be employed which is available for absorption. 2) A food vehicle must be identified which will permit dispersal of the fortified food to a large segment of the population in need, in a form which will be acceptable from the standpoint of appearance, taste, stability, and cost (12). 3) Pilot studies must be carried out to determine the availability of this fortification iron and the type of meal in which it will be ingested. 4) Finally, field studies must be conducted to demonstrate its practicality and efficacy. All of these elements will be considered in the present study.

In the past two years a collaborative group of investigators under the sponsorship of WHO and the International Atomic Energy Commission have been studying techniques of evaluating food iron absorption and ways of fortifying food. These investigators include Dr. Layrisse in Venezuela, Drs. Cook, Walker, and Finch in Seattle, Dr. Hallberg in Göteborg, Sweden, and Drs. Baker, Sood, and Rao in India. While these individuals will obtain financial support for their studies from other sponsors, their work will provide a theoretical basis for the more applied studies proposed in this program. It is assumed that most of the work concerning iron salts appropriate for fortification will be carried out in these laboratories.

At the present time it is considered that ferrous sulphate is the salt of choice for fortification. The addition of ferrous sulphate to flour, bread and milk has been shown to be practical and these vehicles seem suitable in some areas. Other vehicles which may have advantages in distribution are salt and sugar. The addition of ascorbic acid to salt or some other vehicle is an important consideration in those areas where dietary iron appears ample, since such a reducing substance increases availability for absorption of iron already present. Losses of vitamin C during cooking will need to be considered in this approach.

The fact that the availability of nonheme iron of food depends on the composite effect of the whole meal makes it important to examine regional diets on a meal basis. Such a re-analysis of prior nutritional reports from Latin American countries has been carried out by Dr. Valassi (Appendix 1). It will be necessary to evaluate, employing the double isotope method, the

effectiveness of fortification iron in the type of meal in which it will be employed. On the basis of the amount of iron and its availability, calculations can be then made of the effectiveness of the proposed fortification. Only those supplements shown to be effective will be studied by field testing.

B. SPECIFIC AIMS

To investigate means of fortifying food with iron in a manner which is both practical to accomplish and which is effective in improving iron balance of the infant and adult menstruating female.

The collaborative study will be oriented to examine the following items:

1. Iron absorption from regional diets from the main meal of the day. The diets from the following eight countries in Latin America will be investigated: Argentina, Brazil, Chile, Colombia, Guatemala, Mexico, Peru, and Venezuela.
2. Absorption by infants and children of fortification iron from low fat powdered milk and high protein infant foods commonly used in Latin America.
3. Protocols will be prepared to carry out field studies to evaluate the effectiveness of fortification of foods with iron.

C. METHODS AND PROCEDURES

The present study consists of regional investigators and a reference center. The participating investigators include Dr. Kremenchuzky in Buenos Aires, and Dr. Gutnisky in Corrientes, Argentina, Dr. Simmons and Dr. Alvaro

Vieira de Milo in Recife, Brazil, Dr. Stekel in Santiago, Chile, Dr. Lema in Medellín, Colombia, Dr. Viteri of Guatemala City, Guatemala, Dr. Loria in Mexico City, Mexico, Dr. Reynafarje in Lima, Peru, and Dr. Layrisse of Caracas, Venezuela. Drs. Stekel and Simmons are concerned with infant and child studies, while the other investigators are concerned primarily with studies in adults.

A reference center for studies on iron fortification of food in Latin America will be located in Caracas, Venezuela, at the IVIC Laboratory. The reference laboratory for previous collaborative studies on nutritional anemia were also carried out at this laboratory which is well equipped for the proposed studies. The hematology laboratory at the University of Washington under Dr. Cook is prepared to provide back-up assistance to the reference laboratory at Caracas. The IVIC laboratory will assume responsibility for shipment of radioisotopes to the collaborating units, for providing containers for the shipment of samples, for determination of heme and nonheme iron content of meals, for analysis of ^{55}Fe and ^{59}Fe in blood samples drawn to evaluate iron absorption, and for calibration of other standard laboratory procedures to be employed in these studies. Insofar as participating laboratories demonstrate competency to carry out analyses, they will do so. Appropriate standardization will be carried out by mailing samples between peripheral and reference laboratories. Data will be analyzed in the Caracas laboratory and the Seattle laboratory, and results will be circulated to the participants. The laboratory in Seattle is capable of providing standardization service and is now acting as a WHO reference center for plasma iron and iron-binding capacity measurement.

I. Studies on Infants and Children

A. Fortification salt and vehicle

The major focus of studies in infancy is on the absorption of fortification iron in milk or in other feeding mixtures ingested without other food items present to affect absorption. The feasibility of adding ferrous sulphate to milk has already been demonstrated, and isotope studies have shown a 7% availability of 10 mg. of iron added to 100 gm. of powdered low fat milk (13). The availability of other iron salts should be investigated only when there is an impelling reason why such a salt would be advantageous over ferrous sulphate. In general, such comparative studies of iron salts are better carried out in adults for technical reasons. The effect of adding ascorbic acid to milk and improving availability of fortification iron will also be evaluated. This will be particularly important when milk is to be used with cereal. A number of low-cost high protein infant feeding preparations are being consumed by a substantial population of infants in different regions of the world (Appendix 2). It is considered important to examine availability of fortification iron added to these. Initially, high protein infant foods in current use in Central and South America will be tested.

B. Dietary considerations

After initial studies have been carried out with milk alone, food articles ingested along with milk in infancy and early childhood will be identified and absorption studies carried out on those which are extensively eaten (Appendix 1).

C. Preliminary absorption studies

Questions to be answered concerning fortification salt, vehicle, or effects of food mixtures will be tested by the double-isotope absorption method. In this procedure it is necessary to evaluate the level of absorption in the individual subject by the administration of a single dose of reduced iron (0.05 μC of ^{59}Fe /kg in a total dose of 3 mg of iron) (14). The child will be fasted overnight before the administration of the material, and further oral intake will be withheld for 2 hours. This will be followed the next day by a test substance or meal given with an extrinsic tag of 0.1 μC /kg of ^{55}Fe in 0.1 mg of iron as FeCl_3 . A blood sample will be obtained at 2 weeks at which time absorption will be calculated with an assumed blood volume of 70 ml/kg. Infants so tested will have a health history with particular reference to any evidence of acute inflammation at the time of study, past dietary habits and food intake as well as a physical examination. Other measurements include height, weight, plasma iron, total iron-binding capacity and red cell protoporphyrin, hemoglobin and hematocrit, total protein and albumin by electrophoresis, and blood smear. To establish the availability of any test substance, approximately 20 infants will be tested. It is anticipated that 100 to 200 subjects will be studied in this fashion during the first and second year in Chile. From these preliminary isotope evaluations, it will be possible to determine whether sufficient additional iron is provided through fortification to normalize the iron balance of all but the severely anemic children over the period of a year. Initial studies at the Institute of Nutrition - Recife, will involve the measurement of iron absorption employing radioiron as an extrinsic tag

given to 20 infants who will ingest iron fortified milk powder reconstituted with water. A second group of infants will be given the same material with added cassava and sugar.

D. Field trials

Initial field trial studies on infants and children will be carried out in Chile. Here health care is provided to around 80% of the population by the National Health Service. Proposed studies will be performed in infants from low and middle-low socio-economic conditions who have by past studies been shown to have a high incidence of iron deficiency. Recent studies in the 12 to 18 month age group indicate a prevalence of anemia of about 25% in the middle-low group and 40% in the very low socio-economic group (15). The feeding of infants follows a standard pattern in Chile. Breast feeding is usually less than 3 months, and babies are weaned to a low fat powdered milk formula. Corn starch or wheat flour is usually added. Powdered low fat milk is distributed by the National Health Service through clinics in Santiago and is often the only regular source of animal protein available.

The proposed study is an extension of an ongoing program in which low fat powdered milk enriched with 10 to 15 mg of ferrous sulphate per 100 gm powder will be used. This has been shown by isotope studies to supply about 0.5 mg a day of additional iron to the infant. The field trial will consist of a study of 300 infants to be fed iron fortified milk and 300 infants receiving the same milk without iron. The study will be conducted in two outpatient clinics in the city of Santiago. The infants will be observed after birth under a system of continued medical care and will enter this

study at 3 months. The study will be terminated at 15 months. Milk will be given to each mother at regular intervals at the clinic and taken home. D-Xylose will be used as a means of monitoring consumption of milk given as a food supplement. In addition to the usual program (weight and height record, immunizations, etc.), regular visits will be paid to the home by nurses to check on whether instructions are being followed. An estimate will be made of the amount of milk consumed by the child. At 3, 9, and 15 months of age, laboratory studies will be conducted including hemoglobin and hematocrit, blood smear, plasma protein with paper strip electrophoresis, serum iron, total iron-binding capacity, and red cell protophyrin.

The dietary intake of the infants involved in the study will be evaluated prior to the initiation of the field trial and at its termination by accepted dietary survey techniques.

Preliminary studies are also being carried out in North East Brazil by Dr. Simmons, to evaluate the feasibility of field trials in this setting. The infant feeding practices in this region follow a certain pattern. Infants from three to six months of age are weaned from breast milk to a gruel made from cassava and sugar. The effectiveness of fortified (iron) skim milk powder administered to infants and children, essentially on a cassava diet, will be evaluated.

II. Adult Studies

A. Availability of iron in typical diets

Studies will be first carried out on typical diets as identified by dietary surveys to determine the availability of the food iron naturally present. The heme and nonheme iron content of the diet will be determined

not only from food composition tables but also by chemical analysis of the homogenized "typical" meal in the reference center. The meal employed would be typical for a significant segment of the population in general. A study of this type will be carried out by each of the investigators from the following countries: Argentina (two), Colombia, Guatemala, Mexico, Peru, and Venezuela.

Subjects studied will be characterized by hemoglobin and hematocrit, smear, plasma iron, and iron-binding capacity, and red cell protoporphyrin. In respect to the isotope studied, the following experimental design has certain advantages. To obtain the most accurate total pattern of absorption, four studies in a single group of 20 subjects will be carried out in each geographic area. The individual absorption measurements would be:

1. the measurement of absorption of an extrinsic tag from a single test meal considered typical of the local diet of a designated field test population,
2. absorption of a reference dose to characterize the subjects studied, and
3. and 4. the simultaneous absorption of tagged fortification iron and of an extrinsic tag added to the meal.

To obtain the total pattern of absorption, the extrinsic tag will be administered with designated main meal of the day on alternate days, times five. On the other days, breakfast will be omitted and the reference dose of radioiron ascorbate will be administered. From the radioactivity in the circulating blood on the 14th day, a determination of the percent absorption of each isotope will be determined and thereby the total amount of dietary nonheme iron absorbed and its ratio to the reference dose.

Any change in percent absorption brought about by changes in total iron content will be assessed by the proposed studies. Under consideration is the amount of iron absorbed, that is, the amount of nonheme iron in the diet, times the percent absorption. The total nonheme iron content of an aliquot of the diet will be determined at the reference center and absorption will be calculated as an absolute amount. Actually, percent absorption will not vary greatly in the proposed studies of absorption with varying levels of iron since the supplement will probably not double the total dietary iron. However the increase in amount of iron absorbed will be the critical determination.

In nonhookworm areas, an increase in iron absorption of 0.5 mg/day due to the supplement is considered satisfactory; in hookworm areas, an increment of 1 mg would be desirable. In the adult, the dose of radioiron employed in each tag meal or reference dose will be less than 5 μ C of ^{59}Fe . Further details concerning these studies of availability will require definition of the iron salt (or enhancing agent) and the diet to be evaluated. However, preliminary studies on availability can probably be started in most areas within 6 to 12 months.

B. Field Trial

The field trial will consist of 1) dietary characterization as discussed in the following paragraph (C), 2) hematologic determinations as listed previously on all subjects at the beginning and every 6 months thereafter, until the study is discontinued, and 3) evaluation of the efficacy of food fortification.

The population of subjects to be studied will be females between the ages of 15 and 40, since this is an iron-deficient population which would

be most likely to show the effects of improved iron balance. It is recognized that over a period of one year there may be up to 40% loss of women from the study due to pregnancy. It is therefore considered desirable to start with 400 subjects in the control group and 400 in the fortification group. While it may be necessary to work with smaller numbers, the anticipated efficacy of the fortification as disclosed by preliminary isotope studies may permit a more accurate prediction of the number of subjects required.

A control group is necessary since there may be unforeseen variables in these studies which would change the baseline of the population. A minimum of 12 months is considered necessary; this will obviate seasonal variations in diet, iron balance and hematologic values. It may be that a longer period (18 to 24 months) will be desirable in some instances; this will be assessed by the ongoing results of the study.

C. Dietary Considerations

Background dietary information is required from representative samples of each population in whom food iron fortification studies are to be conducted. The communities selected for these studies should preferably meet the following criteria: Low migration rates and food patterns representative of a region as a whole. Sample size of 400 females ranging in age from 15 - 40 years will be considered in conformity with the hematological studies.

Dietary information for three meals per day by the recipe method (food preparation and consumption) (16), will be obtained on approximately 10 percent of the women included in the sample. Within the selected population each day of the week will be equally represented. Aliquots of each

of the three meals per day will be procured for chemical analysis. A duplicate sample of all the meals will be frozen or lyophilized so that analysis can be performed in the future.

Iron analysis will be carried out at the reference laboratory in Caracas and contractual arrangements will be made with a laboratory such as the Wisconsin Alumni Research Foundation to perform analysis on factors believed to be important in the availability of food iron: protein, ascorbate, phosphate, phytate, Vit. C, and calcium. Drinking water will be analyzed for its iron content and its ability to destroy ascorbate.

In addition, information will be obtained on the mode of food production, processing, distribution, storage, purchasing and food preparation practices. Observations will also be made with regard to other practices related to ingested items, i.e., pica, water supply, and sanitary practices. Seasonal food changes will be noted in those areas where this occurs. In addition to the preliminary studies needed to identify the field trial diet, surveys would be conducted at the end of each field trial in order to assure that no change has occurred during the period of study. Monthly visits would be made by field assistants to check on the use of the fortified food by the women under study. Samples of the fortified food would be collected from selected homes of cohort women for analysis of iron content. This continuous sampling will serve as a form of quality control of the operation. In addition, quality control will be conducted in every lot of the fortified food before it is introduced in the field trial area. Formats for dietary data collection, tabulation, and coding will be standardized. Furthermore, a set of standard

computer programs will be used to handle data produced by the above mentioned forms. Use of standardized forms and programs should produce the required data much sooner and less expensively. Another advantage is the fact that results from different surveys are more easily comparable when use is made of a standard system.

Evaluation of Diets: From the data obtained, the quantity of each food item consumed per meal per day will be derived. The intake of nutrients per meal per day will be quantified using food composition tables (17). The calculated dietary data will be compared with international standards for evaluating nutrient adequacy as to age and sex (18-19).

Calculations will be made of the amount of iron and protein contributed per meal by various food groups such as vegetable products (grains, roots and tubers, legumes, vegetables, and fruits) and animal products (meat and fish, liver, milk, and milk products). Eggs, in view of their adverse effects on iron absorption will be separately listed. Ascorbic acid values will be corrected for food preparation losses. Phytates, phosphates, calcium, and amino acid content will also be calculated.

D. Fortification Salt and Vehicle

Consideration of iron compounds to be employed in fortification and their vehicles depends on the availability of the iron salt for absorption and on the feasibility of adding iron salt to raw materials used in home preparation of traditional foods with apparent efficiency, facility, and economy, and the quantity of the vehicle consumed by the target and other segments of the population. In addition to considering the iron compound itself, ascorbic acid will also need to be evaluated as a means

of enhancing iron absorption. These considerations must be on a regional basis and in most instances, additional preliminary work will be required before specific protocols are established.

At the present time, dietary information on proposed field trial populations in different areas varies greatly. Information is available in Mexico, Chile, Colombia, and Venezuela; less is known in others. In all instances, there is a need for further definition before preliminary isotope studies of availability are performed, since these studies must be carried out on a diet characteristic of that consumed by the field trial population.

After a diet has been defined for field trials, a group of approximately 20 subjects will be studied in whom the designated diet along with iron and/or ascorbic acid will be administered and its availability determined. The absorption of this fortification iron will be expressed in relation to the absorption of a reference dose of iron ascorbate as previously described.

These studies are required prior to the field trial to ensure the adequacy of the supplement proposed. Subject variations are in general corrected by a reference dose of iron salt, but it is better to start with high absorbers. Iron-depleted subjects or adult women are the most desirable, since their basal absorption will be high and thus representative of the iron-depleted or deficient target population. It is probably best to use subjects selected from the field test population in evaluating availability of the diet to be employed. However, it is not necessary as long as the diet of the field trial population is the one employed.

The vehicles that are being considered for fortification with iron salt are the following: Argentina and Peru - wheat flour; Venezuela - maize; Mexico and Colombia - salt; and, Guatemala - sugar. The type of iron salt employed must be defined to ensure the compatibility of iron compounds with various food systems.

E. Regional Field Studies

Six regional programs have been suggested which include studies on both rural and urban populations of low income. Rural populations are available for study in Mexico, Guatemala, Colombia, and Venezuela. In Mexico there are two small communities (less than 10,000 people) near Mexico City with similar eating habits, one of which will be given fortified food and the other will serve as a control. These people have in the past cooperated in nutrition studies. In Guatemala, rural communities are already participating in studies carried out by INCAP on nutritional anemia. In Colombia, there are also two rural communities of about 2,000 each with a low migration rate which are close to the laboratory facilities of the nutritional division. Both the food processors and the people are willing to cooperate in fortification studies. In Venezuela there are two small villages with a high prevalence of iron deficiency and anemia which can be used. Urban populations can be studied in Lima, Buenos Aires, and Corrientes. In Lima, a population of girls living and eating at a high school could be used. The feasibility of this will depend on exploring their availability for the study and the appropriateness of the school diet. In Buenos Aires female students of the School of Nutrition and Nursing and women working and eating in factories will be used. Again, this will require exploration. In Corrientes, an urban factory population would be studied.

It is recognized that hookworm infestation is an important consideration. The populations of subjects in Medellín and Corrientes have heavy infestations. On the other hand, Mexican subjects are free of infestation and in Lima, Caracas, and Buenos Aires, the load is very slight. It seems desirable to have these extremes represented if the value of iron supplementation for different populations is to be evaluated. Quantitative egg counts will be performed to establish the size of the load and the amount of blood loss. If it appears desirable, ⁵¹Cr blood loss studies will be carried out.

The purpose of fortification is not to treat anemia, but to prevent it. Since iron-depleted individuals will have the highest absorption and show the greatest improvement, a female population drawn from the low income group with a high prevalence of iron deficiency should preferably be selected for field study. This would reduce the number of subjects required to the minimum. A number of parameters will be analyzed to demonstrate a positive iron balance. Included are the mean hemoglobin and hematocrit concentrations, plasma iron and iron-binding capacity and red cell protoporphyrin. Calculations will be made from the sequential changes in hemoglobin and hematocrit in each individual as to the amount of iron available from the supplement per day. This will be compared to the isotope studies to evaluate their predictability of field trial results. The plan for data collection and statistical analysis of each study will be worked out by the program director and statistical consultants (Dr. Cook and PAHO) before the study begins.

D. Significance

There is a great concern in the developed and developing countries regarding the problem of nutritional anemia and how it is affecting the life at all levels, economic, social, and political. Recognizing the difficulty of changing dietary practices, it seems reasonable to develop methods for fortifying foods already included in the regular diet, as this type of a program could be more easily implemented in the community.

Fortification with iron or iron and ascorbic acid appears to be a justifiable approach. Fortification of cereals has already been demonstrated to be feasible under experimental conditions. The major impact of iron fortification, however, should be in the community where the anemia problem is primordial. There are very few studies in human populations aimed at evaluating the efficacy of food fortification with iron. This type of approach can be undertaken in developing countries because of the following factors:

- a. The feasibility of studying the effectiveness of this procedure in areas with a high prevalence of iron deficiency anemia.
- b. The relative immobility of village population group in developing areas will permit an evaluation of consequences of the proposed nutrition interventions.
- c. The minor changes in average nutrient consumptions over period of time.

A successful fortification program should lead to a reduction in the prevalence of iron deficiency anemia and thereby contribute significantly to the health and well being of the population at large.

In addition, an answer to the feasibility of fortification of different types of diet with iron will assist USA, as well as other countries in planning fortification programs.

By approaching this problem as a collaborative group, it should be possible to provide various services required to answer both theoretical and practical problems of food fortification with iron under different environmental conditions.

E. Facilities

The Instituto Nacional de la Nutrición in Mexico City is an official branch of the Ministry of Health of the Mexican government. Extensive research laboratories are located in the institute; Dr. Loria and his associates have had extensive experience with general nutritional studies and more specifically with evaluation of the status of iron metabolism in rural areas in Mexico. Laboratory procedures to be used in studies of iron absorption would be performed in the hematology department of the institute which has had extensive experience with this type of study.

In Guatemala City, INCAP is well known as a center for nutritional research. Dr. Fernando Viteri and his associates have previously carried out extensive studies in rural areas on nutritional anemia. In addition, a metabolic ward is available for studies on hospitalized infants, and adult hospital facilities are also available.

The University of Antioquia through the departments of biochemistry and nutrition and the department of medicine in Medellín, Colombia, have had an ongoing program of study of nutritional anemia. A metabolic unit

has been available for both adults and children, and a modern clinical laboratory with standard laboratory equipment and liquid scintillation spectrometer is available. The school of dietetics and nutrition has also been involved in dietary surveys and would be available for participation. This center, under the direction of Dr. Lema, is engaged in nutritional studies of the general type proposed.

In Lima, the hematologic and nutritional departments of the Institute of Andean Biology of the University of San Marcos would be prepared to participate in this program. These laboratories are located at the Loayza Hospital, where the studies listed under (A) and (B) would be carried out.

In Chile, Dr. Abraham Stekel is working at the Pediatric Research Laboratory of the University of Chile. This laboratory has extensive facilities and personnel devoted to the study of basic and practical aspects of infantile nutrition. Extensive experience has also accumulated in relation to dietary surveys, food analyses, and field trial of new products. The National Health Service clinics of the city of Santiago provide a place for carrying out field trial studies. Already Dr. Stekel is working at this location in studies concerning iron fortification.

In Buenos Aires, Dr. Silvio Kremenichuzky is working at the National Commission of Atomic Energy and the Center of Nuclear Medicine at the Hospital de Clínicas José de San Martín. Personnel from the department of nutrition would also collaborate in the preparation of meals, dietary controls, etc. Facilities at the Center of Nuclear Medicine are extensive in respect to isotope equipment.

In Corrientes, Argentina, Dr. Abraham Gutnisky is located at the Universidad Nacional del Nordeste and has equipment within the department of physiology for carrying out hematologic procedures required in this study.

In Recife, the Nutrition Institute which is a part of the Federal University of Pernambuco has a large nutritional unit concerned with experimental and applied nutrition. Personnel from the Department of Pediatrics of the University Hospital will also collaborate. The Institute operates and advises seven nutritional centers in northeast Brazil.

F. Collaborative Arrangements

The Pan American Health Organization (PAHO) - Regional Office of the World Health Organization, serves the governments of the Americas with a single functional program. Its field activities are administered from six zones and 28 country representative offices, each maintaining regular contact with the health authorities of the governments.

Close relationship and ready consultation with the collaborating institutions will assist greatly in carrying out the proposed multinational research project. At the headquarters in Washington, the Director and his technical staff undertake the basic planning and coordination of activities. This office also is responsible for activities in U.S. and Canada; and through the various administrative and technical units, it will be responsible for co-ordinating activities of the co-operating institutions.

Publications

From the onset, it should be stressed that this is a cooperative study. The data emanating from this project will be published with the

consent of the appropriate authorities. Each country would be free to publish its own data as it desires. The main interest of this regional project is to obtain data for the development of effective programs to control nutritional anemias in the hemisphere. It is hoped that a report of this study will be published in a monograph form with joint authorship.

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SUMMARY

The objective of this study is to investigate means of fortifying food with iron in a manner which is both practical to accomplish and which is effective in improving iron balance of the infant and adult menstruating female.

This study will be carried out in collaboration between a number of investigators in Latin America and a reference laboratory in Caracas, Venezuela. It will interdigitate with studies on the mechanism of food iron absorption carried out by Dr. Cook (Seattle) and Dr. Layrisse (Caracas) and with similar studies of the World Health Organization and the International Atomic Energy Commission. The design of this study will be such as to provide a mechanism for developing and evaluating food fortification programs in infants and adult females. There are four essential features of this study:

1. to identify "typical" basal diets in eight locations in Latin America, and to determine the iron content and availability of food iron in these diets;
2. to study the feasibility of fortification of specific food items in the "typical" basal diets which are practical for dispersal and utilization by the population at large;
3. the determination by isotope techniques of the availability of this fortification iron when consumed in the usual diet;
4. to carry out field trials including both a supplemental and control population so as to determine the effectiveness of food fortification in improving iron balance and decreasing the prevalence of iron deficiency anemia.