ATTACHMENT VI

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FORTIFICATION OF SUGAR WITH IRON

FIELD STUDY PRIOR TO IMPLEMENTATION AT A NATIONAL LEVEL

Principal Investigator: F. E. Viteri, M.D., D.Sc.
INCAP, Guatemala

Co-principal Investigator: M. Layrisse, M.D.
IVIC, Venezuela

43p.

1. Introduction

Epidemiological evidence collected in the five countries from Central America and Panama during the INCAP-OIR Nutrition Surveys in 1965-1967 demonstrated the existence of a severe and widespread problem of iron deficiency anemia in the population.[1,2] This situation is representative of most tropical developing countries.[3] Further studies have corroborated that this deficiency responds dramatically to the oral administration of 60 mgs. of iron as ferrous sulfate daily for two months, without any other public health measure[4].

INCAP studies have also revealed that iron deficiency anemia significantly hampers the capacity to perform heavy physical exercise in agricultural workers[5,6]. This effect is manifest even when the hemoglobin concentration is only 2 grams below the norms for the Central American population[7]. Iron deficiency also decreases the immune response[8] and the bacterial killing rate of leukocytes[9,10].

In view of this, INCAP has carried out research aimed at finding a suitable iron compound for fortifying a common food vehicle with iron[11-13]. Its efforts in this area can be summarized as follows:

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a) Two common vehicles have been considered adequate for iron fortification in Central America: salt and sugar. Because of econo reasons and ease of control of adequate levels of fortification, as well as for certain characteristics of iron absorption in the prese of reducing carbohydrates, sugar has been chosen as the most suitab vehicle. Sugar in soft-carbonated drinks is also being considered as a vehicle for iron fortification in Venezuela[14,15]. Sugar as a vehicle has the added advantage that refreshments sweetened with iron-fortified sugar are commonly consumed between meals, thus avoiding possible inhibition of absorption because of food factors.

b. The compound used for fortification must be very carefully chosen primarily for its effectiveness. Indeed, fortification of foods has failed because of the poor absorption of iron from compounds which otherwise fulfill many "food technological" criteria (4,16). On the other hand, many biologically available iron compounds have major disadvantages from the standpoint of food industry (soluble ferrous compounds are highly reactive, discolor the food a give a metalic taste to it) except when mixed with special vehicles such as milk[18], which is not available for the great majority of the population in developing areas. Another major disadvantage to the use of many of these soluble and highly ionizable iron compounds in food fortification programs in countries where most of the diet is of vegetable origin (cereals, pulses, leaves) is that the iron in the compound used for fortification becomes a part of the common food iron pool and is ineffectively absorbed. Layrisse et al.[19] have recently demonstrated that, for example, ferrous sulfate is poorly absorbed when added to a typical diet such as that consumed in Central America, and that as the amount of ferrous sulfate is

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increased in the diet to reach high levels of iron fortification its relative absorption markedly decreases becoming more and more ineffective as the iron dose increases. It appears, then, that the addition of highly ionizable, soluble iron compounds which very rapidly become part of the common food iron pool will not be an optimal form of iron fortification. Exceptions to this general rule are: i) the simultaneous administration of relatively high levels of vitamin C, which in most instances would have to be added as a supplement itself, [20-22] or ii) the inclusion of meat or other sources of heme iron, or of larger amounts of high quality proteins, mainly of animal origin[23-25]. This we also know, is not feasible in the near future for the Central American populations nor for most other developing nations. In contrast to the above type of iron compounds, heme iron (that contained in myoglobin and in hemoglobin) behaves in a different fashion, mostly because it forms an independent intestinal iron pool from that of non-heme ("inorganic") iron[23,26]. Heme iron mixed with cereals and pulses has many ideal characteristics such as a more effective absorption, a clear increase of its absorption in iron deficiency states in the presence of any type of food, and a relatively higher proportion of its absorption independent of dose[24,28]. The amount of non-heme iron in the intestine does not affect its absorption either[19,25]. Ideally, the fortification of the Central American diet with iron should be based on the administration of heme iron, but again one falls into the unfortunate situation of the rural poor who cannot afford to consume meat. A logical substitute for heme iron would be a commercially available compound which would behave somewhat like heme iron; INCAP's research has

been oriented towar finding such a compound. Our results indicate that the double iron-sodium salt of ethylene diaminotetraacetate (NaFeEDTA) behaves differently to "non heme iron" under certain conditions by enhancing the bioavailability of iron in the presence of inhibitors in relation to that of inorganic iron. In this respect it has some of the characteristics of heme iron as described in a summary form in Annex 1. . Essentially, its absorption increases in iron deficiency in the presence of foods which interfere with an increased absorption of iron from ferrous sulfate[11,13]; the iron in NaFeEDTA appears to be at least partially protected from food compounds which inhibit non-heme iron absorption, [12,13] and the dynamics of intestinal absorption of iron from NaFeEDTA, evaluated by changes in serum iron, resemble that of hemoglobin and contrast with that of ferrous sulfate[12]. We have evidence also that the iron absorbed from NaFeEDTA is highly available for hemoglobin synthesis[29,30]. From the physiological point of view, then, NaFeEDTA behaves somewhat like heme iron and as such may approach the ideal iron compound for fortification in areas where meat consumption is very small. Iron absorption from it is at least as good as from ferrous sulfate, being superior under certain conditions The EDTA portion of the molecule is essentially unabsorbed[31].

c) The iron compound to be used in fortifying sugar must be stable and must not produce undesirable changes in the appearance of sugar or in its culinary properties. The addition of one part per 1,000 of NaFeEDTA (which provides 13 mg. of iron per 00 g. of sugar) produces only a hardly detectable yellowish discoloration to sugar and does not interfere in its culinary properties such as the product

of jams or jellies, cookies, cakes, sweet gruels or INCAPARINA etc.). Furthermore, the iron compound should not add a metalic ta to beverages sweetened with iron containing sugar. NaFeEDTA, in contrast to ferrous sulfate or ferrous fumarate, is imperceptible when added to lemonade, coffee or tea. This last drink provides al an interesting characteristic: when sugar containing ferrous sulfat is added it turns black immediately. This color is almost irrevers by the addition of lemon. In contrast, sugar containing NaFeEDTA produces a progressive darkening of the tea which reverts to its natural color with the addition of only a few drops of lemon (11-14 Stability trials are under way in climatic chambers and in two area in Guatemala: the tropical coast and highlands. So far the characteristics of the sugar have not changed significantly in eith location except for a slight darkening in both areas when the forti sugar has been only minimally refined (please see annex 2). [Refine white sugar is now being tested under similar conditions and after three months no change in appearance nor in organoleptic characteri have occurred in the sugar maintained in the climatic chamber simul tropical coast environments. Bread and home made jams have been te by different p nnels with excellent results.]

d) The mechanisms of the addition of iron compound to sugar mu be easily accomplished, and the cost of supplementation must be low. NaFeEDTA can be easily added to sugar in its latest stages of production. The very fine yellow powder sticks to the sugar crystal which still has about 1% moisture, remains with the crystal in the process of drying and does not segregate from the sugar in the bag. Samples obtained throughout a period of sugar production when

fortification with NaFeEDTA was aimed at 15 mg of iron/100 g of sugar resulted in a stable level of iron fortification which was 15.6 mg/100 g. of sugar with a range between 13 and 18 mg/100 g. There has been no segregation of sugar kept in bags for twelve month

We estimate that the cost, in terms of available iron, is only

two to three tir the cost of $FeSO_4$. This relatively greater co is, in our opini..., outweighed by the stability characteristics c NaFeEDTA and by its culinary superiority. The daily administrati of 6.5 mg of iron per person (contained in 50g. of fortified suga represents a yearly investment of US$0.063 (6.3 cents) per person The increase in cost of sugar would be less than US$0.0016 (0.16 cents) per pound, that is less than 2%. The costs could probably be reduced further.

e) The compound chosen for the fortification of iron must no! be a hazard to the health of the population. In the case of NaF we have consulted the FDAat the United States who have indicated that Na_2EDTA is an accepted food additive for over 30 uses, that $Na_2CaEDTA$ has over 50 accepted uses and that the level to be add in the fortification of sugar is well within the acceptable inta levels in the United States[32,33]. Calculations of the possibl losses of nutrients from the intestine if total exchange of iron for calcium would occur indicates that less than 15 mg of calciu per day would be lost in the feces. In Central America calcium intake is very high and we do not foresee that this would cause problem even in the case that all of the EDTA would exchange wit calcium in the gut. Less than 5% of EDTA is absorbed from the i tine by the human being. In terms of other cations and trace minerals it appears that the presence of NaFeEDTA in the gut actually facilitates the absorption of other metals such as zinc If this were so, this would be an added advantage to the administration of this salt, since zinc d e f i c i e n c i e s

are also highly prevalent in most developing countries where iron deficiency is also a public health problem[35,36].

In any case, among the objectives of this proposal is the evaluation of trace mineral nutrition in populations receiving NaFe EDTA fortified sugar.

f) Iron fortification of sugar with NaFeEDTA must not interfere with Vitamin A fortification of sugar.

At present, two countries in Central America are fortifying sugar with retinyl-palmitate to provide 1,500 ug of retinol/100 g of sugar[37]. It is expected that all the other countries in Centr America and many countries in South America and the Caribbean will also fortify sugar with vitamin A. The technique developed for thi process involves the preparation of a vitamin A-rich sugar premix which contains 1.5 x 10^6 ug of vitamin A/100 g. and an antioxidant. This is then mixed with sugar in a proportion of 1g per kilo at the same stage of production of sugar when NaFeEDTA would be added also in a proportion of 1g per kilo of sugar. From the technological point of view these additions do not interfere with each other. Stability trials are underway with sugar enriched with both vitamin A and iron. These involve the use of a climatic chamber at INCAP t duplicate extreme tropical conditions and animal trials to evaluate the availability of vitamin A. In theory, however, NaFeEDTA should not decrease at all the stability of vitamin A if all the iron in it is chelated (as is the case). Furthermore, EDTA and an antioxidant are actually used to increase the stability of vitamin A preparatio

In brief, INCAP believes that it has developed the process for fortification of sugar with iron which promises to be a very effecti measure to prevent iron deficiency and even, in the long run, treat individuals who at the start of fortification were iron deficient.

The dangers of iron toxicity in Central America and Panama with the proposed levels of fortification are essentially nil; on the one hand iron absorption from NaFeEDTA in iron repleted individuals is clearly lower in relation to that in iron deficient subjects[12,13]. The amount of iron absorbed from NaFeEDTA in iron repleted individuals is in the same proportion as other food iron. Furthermore, hemochromatosis in Central America is essentially unknown; in contrast, iron deficiency occurs in over 50% of the population in tropical areas.

The most important aspect of this project is the careful periodical evaluation of the effectiveness of iron fortification of sugar with NaFeEDTA at a population level. This evaluation must involve two aspects: a) Those related to the delivery of iron to the population, and b) the quantification of the changes that are taking place in the iron nutrition of those receiving iron-fortified sugar. Recent advances in the evaluation of iron nutrition by the use of serum ferritin determinations[38-40] make this a very valuable tool, since it allows the estimation of iron reserves: a change of 10 ng of ferritin/ml reflects a change of approximately 100 mg of iron reserves[39]. Serial evaluations of serum ferritin, hematological variables, free erythrocyte protoporphyrines (FEP) and serum iron and iron binding capacity permit a quantitative estimation of the actual changes which have occurred in the body iron pool as well as in some of the most important functional aspects of iron nutrition.

INCAP is convinced that the only effective measure in the prevention of iron deficiency in the Central American Istmus is food fortification with iron. Most probably this concept is extensive to the rest of Latin America and the Caribbean. It also believes that

it is at the verge of being able to advise the general use of sugar enrichment with NaFeEDTA to the Central American countries but before it does so, a pilot study in two different ecological settings in Central America must be conducted and carefully evaluated. Fortification of fish sauce with NaFeEDTA has recently proven effective in a small field trial conducted in Thailand by Garby and Areekul[41]. This proposal has as its objective precisely the implementation and careful evaluation of such pilot study.

2. Objectives

The purpose of this project is to evaluate the biological effectiveness and the general feasibility -including costs- of sugar fortification with NaFeEDTA in large populations where iron deficiency is highly prevalent.

3. Scope of Work

The sample will be formed by primary school children and their families. They will be representative of two of the major ecological areas in Central America, that is the tropical lowlands and the high lands. All the families will be rural. The iron losses in the high lands resemble those in non-tropical areas while those in the lowlands are representative of most tropical-hookworm-infected areas. Four communities, two in the lowlands and two in the highlands of Guatemala each having approximately 400 children in the public schools will be subjects of study. The total sample in each community will be around 1800 individuals. One of each pair of communities will be experimental and the other will serve as control; each pair will differ in that the experimental community will consume iron-fortified sugar while the control community will consume plain sugar. Both exp

America and Panamá according to the attached table) and most probably also a den program which, besides providing dental care, will include the distribution of fluoride tablets. If considered appropriate, other health related activities no interfering with the iron fortification program could be implemented; their natu will be decided based on the characteristics of the communities. Blood and spot urine samples will be taken from all the school children and their families. Di intake of the whole family will be measured. A simple clinical form will be filled for each member of the study.

Trace mineral and folate nutrition will be monitored in a subsample. The study will last 3 years. Samples will be obtained for 2 1/2 years. The remaining 6 months will be devoted to data analysis and writing of final results.

Another possible design could be the study of two or four communities in a sequential fashion that is, taking first basal data prior to fortification and then proceed with the fortification in all the communities. This sequential design has several disadvantages compared to the parallel study of an experiment and a control community, which I pass to enumerate:

1) The duration of the investigation would be lengthen by at least eighteen months since basal data collection would take that long to obtain, in order to have as a minimum three points in terms of hematological and biochemical paramet initial and two more, eight months apart. Statistically, having only two basal points is too risky and we would be more at ease if three points were obtained prior to fortification.

2) The risk in terms of validity of data in a sequential design is that the control is out of time phase, and the changes which are occuring throughout time in these communities and which are out of our control, could clearly influence the results and we would not be able to evaluate them. In this sense, the parallel collection of data in an experimental and control situation

HEMATOLOGICAL VALUES BELOW WHICH TREATMENT FOR ANEMIA WILL BE INSTITUTED

Age (years)	SEX	Altitude 0-749 m		Altitude 750-1499 m		Altitude 1500-2800 m	
		Hb(g/dl)	Hct (%)	Hb(g/dl)	Hct (%)	(Hb (g/dl)	Hct (%)
1-4	MF	11.3	32.6	11.4	34.0	11.6	34.3
5-8	MF	10.9	33.0	12.4	35.6	13.2	38.9
9-12	MF	11.8	35.3	12.5	37.6	13.4	40.2
13-16	M	12.4	36.9	13.2	39.6	14.6	43.8
13-16	F	12.7	38.0	13.2	39.3	13.6	41.3
17-20	M	13.4	40.8	14.4	45.2	15.0	49.2
17-20	F	11.8	35.2	12.4	38.8	13.6	41.2
21-49	M	13.3	40.2	14.3	43.6	15.3	46.2
21-49	F	11.8	35.2	12.4	39.5	13.1	42.4
50+	M	12.2	35.0	13.1	39.0	14.4	42.9
50+	F	12.1	36.5	12.7	38.5	13.4	40.6

3) The costs would be higher in the sequential design: even if only two communities are studied (one in the highlands and one in the lowlands) the total costs would not be reduced as compared to the study of a control and experimental communities in a parallel fashich; on the contrary, the costs would increase because of the need to lengthen the period of study. It is obviou that with four communities under a sequential study the costs would almost double

Finally, a careful review on ethical considerations of both designs has been done and neither is considered to pose any ethical question since the study does not present any hazard nor violates any of the human rights of the groups involved. In the parallel design (control and experimental community at each altitude above sea level) the control community will receive benefits identical t those in the experimental community except for a delay of maybe two to three yea in receiving the benefit of sugar fortification, which we expect this study to show. In the sequential design all the study communities will not receive the benefits of sugar fortification with iron for at least eighteen months.

In conclusion, we strongly suggest following the parallel design of two communities at each altitude of sea level, one control and the other experimental

4. Methods

a) Intake of sugar and iron

The diet of the whole family will be studied in the families of the members a stratified subsample described in table 1. Food intake will be estimated by t 24 hour recall[42] method before the fortification program starts and every 8 months from then on. Particular attention will be paid to intake of iron, divid into animal sources and vegetable sources, the intake of manufactured products rich in iron, such as fortified food and formulae, vitamin and mineral pills containing iron, medicinal iron, etc. Interpretation of the data will be based on INCAP food composition tables[43]. Besides total iron will be determined in composite food samples of diets of one of each twenty families[44]. This will b

The program of Vi[illegible]n A fortification of Sugar which is already in progress, is obtaini[illegible] [illegible]a on sugar consumption in various places in Guatemala and El Salvador. This information is being already of great value, but still the actual consumption of iron fortified sugar in the communities under study must be carefully documented in order to be able to calculate total iron intake and the proportion of it coming from sodium iron EDTA. The reason for the four dietary surveys throughout the study is precisely to be able to calculate actual nutrient intakes in the communities with emphasis on dietary sources of iron, as well as to be able to describe the diets in terms of fiber, phytates and other mineral intakes which are necessary in order to evaluate trace mineral nutrition also with and without NaFeEDTA intake.

Besides the central dietary survey outlined above, two collateral surveys will be performed on smaller stratified samples covering 25% of the subsample aimed at the two most vulnerable groups in terms of iron nutrition: 1) sugar int[illegible] in the young child, and 2) sugar intake in the pregnant and lactating woman. In both of these groups actual weight of sugar consumption will be obtained.

b) Biochemical determination of iron and folate nutritional status and hematological condition in the population.

Blood samples will be collected in the same families, and at the same time as the dietary survey is carried out. Hemoglobin concentration, hematocrit, red blood cell counts, red cell indices, serum iron and total iron binding capacity (TIBC) will be determine by routine methods of hematological techniques[7,45]. Free erythrocyte protoporphyrines (FEP) will be determined by the method of Heller et al.(46). Serum ferritin will be measured by radio inmuno assay at IVIC, Venezuela at Dr. M. Layrisse's Laborato by the method of Miles et al.[47] Total serum proteins will be estimated by refractometry[48] and whole blood folates in half of the stratified sample by Herbert's method using L. casei[49].

All the members in the study will be monitored by hematocrit determinations to asses their hematological condition.

Complete hematology, serum iron, TIBC, serum ferritin and whol blood folates will be determined in a stratified subsample totallin 1,480 individuals distributed as shown in table 1. The venous bloo needed for these determinations will be obtained by two physicians in the project and by the trained auxiliary nurses. Only periphera veins will be punctured; blood from children will always be drawn by the physicians.

TABLE 1

STRATIFIED SUBSAMPLE FOR COMPLETE HEMATOLOGICAL AND BIOCHEMICAL STUDIES

AGE SEX GROUPS		COMMUNITIES			
		CONTROL		EXPERIMENTAL	
AGE (years)	SEX	HIGHLAND	LOWLAND	HIGHLAND	LOWLAND
1-4	M-F	30	30	30	30
5-8	M-F	30	30	30	30
9-12	M-F	30	30	30	30
13-16	M-F	30	30	30	30
17-49	M	100	100	100	100
17-49	F	100	100	100	100
50 +	M	25	25	25	25
50 +	F	25	25	25	25
	TOTAL	370	370	370	370

GRAND TOTAL 1,480

c) General morbidity surveillance and registration and surveillance of cases of anemia in out patient clinics.

A careful record will be kept of cases diagnosed as iron deficiency anemia either at the public health out patient clinics servin the study villages or in any of the surveys. The record will cover a 3 month period prior to the initiation of fortification and will last throughout the period of intervention. All of these cases will receive therapeutic iron using the routine system employed by the public health department: $FeSO_4$ by mouth. Their hematocrit will be checked after 2 m. of therapy. A simple clinical form will be filled for each number of the families composing the sample. The population will also be monitored throughout the study with regards to hookworm and T. Trichura parasite loads. For this purpose a fecal sample from each subject will be formalin-fixed and Stoll counts [50] performed at least once at the beginning and near the end of the study. This means performing 14,400 egg counts. [All the procedures and the data to be obtained concerning parasite loads have been consulted with Dr. Layrisse who will also continue to assist us directly on this matter.] A record of general morbidit will be kept at the clinic, covering all the population in the study villages. Adequate treatment of all diseases will be instituted by the clinic staff in the project.

Fortnightly, morbidity surveys will be performed in each of the four

communities, using survey instruments which have been developed by the Medical Division in close cooperation with Environmental Biology and Human Development. This information will effectively complement that obtained in the clinics.

d) Evaluation of trace mineral nutrition in subjects receiving iron fortification with NaFeEDTA.

Plasma and urine samples for determination of trace minerals, particularly zinc[51] and Copper[52] in plasma and zinc and magnesium[53] in urine, will be collected from all the subjects in the subsample indicated in table 1. The urinary outputs of zinc and magnesium will be evaluated in single urine samples, relating the urinary content of these elements to that of creatinine.

Even though the probability of producing iron overload is essentially nil, careful clinical evaluation will be performed on any case which could be suspected of developing an iron overload syndrome. The subjects will be hospitalized at INCAP's clinical center for careful iron metabolism studies, including urinary output after desferrioxamide.

e) The data to be collected will cover the following areas:

--Dietary: 24 hour family intake by recall method 4 times in each community, that is, prior to intervention and every 8 months for the two years of intervention. The number of families under study will be approximately 150 in each community, the total being 600 families.

-- Measured iron intake: by analysis of food composits on two occasions in each community. For this purpose one food composit will be prepared for each 20 families. The total number of composite samples will be 140, representing 7 in each community, each time.

-- Iron nutritional status in the population: These data involve the following information:

i) Hematological: Hematocrit in all the subjects, who will total about 1800 in each community. Hemoglobin, hematocrit, red blood cell counts and red cell indices (MCV, MCH and MCHC) in the stratified subsample of 1480 individuals covering all ages and both sexes.

ii) Iron status: serum iron, total iron binding capacity, free erythrocyte protoporphyrines and serum ferritin will be determined in the same stratified sub-sample. Therefore, detailed iron status will be measured longitudinally in 1480 subjects in the 4 communities (total 5,920 samples in 28 months). Besides, each hematological survey will aim to cover 7,200 individua consequently the total number of samples for hematocri determination throughout the study will be 28,800.

-- Folate nutritional status: This will involve whole blood folate determinations in half of the subsample having detailed hematological and iron studies. The total numbe of analyses will be 2,960 in 28 months.

-- Morbidity studies: The study villages will have an out-patient clinic staffed with trained personnel where a careful record of cases of anemia and general morbidity will be kept throughout the study. Medical and dental attention will be identical in experimental and control communities, and will be strengthened by our personnel.

General morbidity surveys will be carried out every two weeks in all

-- Evaluation of trace mineral nutrition in subjects receiving iron fortification with NaFeEDTA: The number of analyses in serum and urine for each of the trace minerals (Zinc, copper and magnesium) will be 1/2 of the stratified subsample. If results indicate the need for larger numbers, the other half of the collected samples will be analyzed.

-- Cost of fortification and control of the program: This activity will involve periodic cost evaluation of the process of fortification and of the delivery system in its totality. Cost of treatment for iron deficiency anemia in the communities under study will also be obtained throughout.

-- Sample number: Finally, a word on sample numbers. When the presence of anemia is estimated on probability basis for each age-sex group,[(7)*] a near 7% prevalence of subjects with hemoglobin concentrations or hematocrit values carrying a high probability of belonging to an anemic population (and therefore classified as anemic) is expected even in normal populations. The sample size needed for valid estimations of changes in prevalence of anemia when it is not very much higher than 15% must be large, ideally between 1000 and 1200 in each community. That is why we will attempt to hav hematocrit determinations in all the sample: 1800 in each community. The stratified subsample where complete hematological, iron and folate studies will be performed, will consist of 1480 individuals (740 controls and 740 experimental). This large number of subjects will insure complete longitudinal studies in near 1000 individuals.

4. Collection, storage and analysis of data, and possible publications

INCAP is implementing a very strong data processing center which includes computer facilities of greater capacity than the ones existing now (a new Hewlett Packard 3,000 is being installed in May) and all the organization necessary for data handling and statistical advice. INCAP already has ample experience in longitudinal field programs and specifically at the Biomedical Division we have now the experience of at least two field programs, one on subclinical malabsorption and the other on energy supplementation to agricultural workers, which have required the stablishment of very complete files with clearly designed variables, that allow us to perform essentially all the analysis and statistical tests which are required to demonstrate the effect (or lack of it) of nutrition intervention programs. In the case of the energy supplementation program, various theoretical models are also being tested, based on longitudinally collected data. In other words, with our present experience and the facilities which are now a reality at INCAP, I do not foresee any limitation in terms of data processing, stablishment of files and analysis of data.

It is hard to indicate a priori how many articles will come out of any investigation. My personal philosophy is that even if I can publish many articles to fill my curriculum (but which have not enough to say), I rather publish less articles each of which will carry a significant contribution to the problem at hand. I can foresee, as a result of this investigation, articles on each of the areas in which effort is going to be invested and which include dietary data, iron and folate interactions both with hematological variables and among themselves effect of iron fortification on general morbidity, trace mineral nutrition (including content in foods and general nutritional status with regards to zinc, copper and magnesium, without and with addition of iron EDTA), cost considerations (including cost effectiveness and cost benefit analysis) and a monograph on iron fortification and on the possible solutions that will come from this investigation.

EVALUATION OF THE EFFECTIVENESS OF IRON FORTIFICATION

This program aims at supplying a small additional amount of biologically available iron to population groups who have a high prevalence of iron deficiency because of: a) inadequate iron intake: too little and poorly absorbed, and b) elevated requirements due to pregnancy and childbirth and/or chronic blood loss.

The populations at large will receive iron fortified sugar which will provide 13 mg of iron/100 g of sugar. This level appears to be enough as a fortification measure based on the following considerations which are available since having written the application:

1. Twenty two subjects in the lowlands consumed fortified sugar containing 13 mg of iron laveled with a tracer dose of ^{59}Fe as they would normally consume sugar throughout the day. The mean ± S.D. iron absorption was 5.6 ± 5.0. There were 2 clearly iron deficient subjects (% sat of TIBC 9.9 and 11.1%) and 2 possibly deficient subjects (% sat of TIBC 13.5 and 18.6%). Their respective absorptio were 16.8, 14.0, 2.8 and 16.2%. Based on these results, iron fortif sugar at 13 mg Fe/100 g sugar would increase the mean absorbed iron in adults consuming 50 g of sugar by 0.4 mg/day. In iron deficient subjects, absorbed iron may be as high as 1.1 mg/day. The amount of iron absorbed from a totally vegetable diet based on the staples consumed in Central America varies between 0.7 and 1.0 mg/day*. Fortified sugar would increase the total absorbed iron to between 1.[illegible] and 2.1 mg/day, depending on iron nutritional status. These levels

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* Calculated from data in Annex 1 and the dietary intake data fr the Central American survey, and from Layrisse et al.,Blood, 4[illegible] 689, 1975.

would be adequate to meet iron requirements even when about 0.6 mg of iron would be lost each day in males because of hookworm infect (hookworm load = 3,000 eggs/g of feces) or in the course of pregna In Central America only 1.4% of the population surpass the indicate level of hookworm load (Viteri et al., Arch. Latinoam. Nutr., 23:3 1973).

2. A small pilot study being conducted at present in a lowlan area in the coast of Guatemala, where sugar fortified to 15 mg of I per 100 g of sugar has been consumed by the population for 10 mont after having received supplemental iron (100 mg/day for 4 months), showing that the fortified sugar is maintaining iron nutrition and hematological normality in a large proportion of the population in of variable degrees of hookworm infection. These results contrast with: a) Members in the same population who, after receiving supple mental iron, did not receive fortified sugar. A larger proportion previously iron deficient subjects are again iron deficient and an among this group, compared to those receiving fortified sugar; and Previous studies conducted with the same experimental design in ot populations but with sugar fortified with $FePO_4$ (which is actually unabsorbable with the Guatemalan diet) resulted in a rapid deterior tion in iron nutrition, similar to what is occurring with a).

It appears that 15 mg Fe/100 g sugar is able to maintain iron nutrition in a population which received first a large iron supplem We propose to add 13 mg of Fe/100 g sugar instead of 15 mg Fe/100 g sugar because the iron content of FeNaEDTA is near to 13%. The lev of addition to sugar would be simply 1 part per 1000 parts. We believe that both levels of iron will be equally effective. In con to the pilot study being conducted now, the proposed study will consist of sugar fortification to populations who did not receive s

vious iron supplementation and where only anemic subjects will receive iron therapy. Consequently, we expect to evaluate the program on the following grounds:

1. Serial changes in iron nutrition in non anemic subjects but who are iron deficient at the start, as detected by low serum ferr low serum irons and % saturation of TIBC, and elevated free erythr protoporphyrins (FEP). Assuming the additional amount of iron to l absorbed by iron deficient subjects will bring iron balance to a retention of + 0.5 mg/day, the iron reserves will have increased about 350 mg in 2 years. Consequently, the following changes are expected:

a) higher serum iron and normal (> 20%) saturation of TIBC (maybe in 8-16 m.)

b) lower FEP (maybe also in 8 to 16 m.)

c) a progressive increase in serum ferritin concentration, reachin on the average 35 ng/ml in two years.

2. Lower rates of recurrence and of new cases of anemia in th experimental communities compared to the controls. Since for ethi reasons all cases of anemia discovered in the course of the progra will receive treatment, the incidence of anemia will constitute an evaluation tool.

4. Significance of Work

The desired significance of the data collected is to substantiate the effects of sugar fortification with iron in two different echological areas in Central America: (a) lowlands (tropical) and (b) highlands. In both areas there is iron deficiency, mostly due to inadequate food iron availability. In both areas there is also iron-deficiency anemia in vulnerable groups (children, adolescents, women of childbearing age, pregnant women and older citizens). The situation in the highlands is similar to that of most other parts of the world, including the U.S.A. In the lowlands, the added problem of chronic blood loss due to N. Americanus and T. trichura infections, induces a more severe iron deficit and, consequently, a greater prevalence of anemia in the general population. In the lowlands, anemia is more frequent among mature men in prime productive age.

The trial in both ecological situations is designed to evaluate the effectiveness of iron fortification under conditions of inadequate iron utilization alone, and that with added chronic blood loss. If effective in both conditions (as is expected from absorption data in annex 1 and from prior experience in small trials in progress), the fortification with this form of iron will have great significance in that it could be applied almost universal This investigation will also indicate the problems involved in large scale sugar fortification programs.

The sources of the data are self explanatory. It is important to stress that in the experimental populations the prevalence of iron deficiency and anemia is expected to decrease slowly during the two years of fortification and essentially no new cases of iron deficiency or anemia should occur, in contrast to the control

populations. Moreover, with the specific data which serum ferritin determinations provide, an estimate of progressive changes in iron deposits can now be obtained, which reflect cummulative iron absorption.

5. Dissemination and Utilization

The results of the research will be disseminated by the following media:

(a) Publication of scientific articles in world - read journals

(b) Formal presentations at scientific group meetings including physicians, public health personnel, etc.

(c) Through teaching at INCAP's undergraduate and graduate courses and at the Central American and Venezuelan universities, where the principal and co-principal investigators are professors.

(d) Through the system of advisory services which INCAP has throughout the Americas in its role of Technical project of the Pan American Health Organization.

(e) The principal and co-principal investigators have been members of WHO advisory groups on Nutritional Anemias and their findings will be presented there.

(f) Whether the investigation indicates that iron fortificatio should or should not become a public health measure yet in Central America and other countries, the results will be presented, discussed and evaluated by a committee integrat by appointees of various health and development oriented organizations and by members of the sugar industry government and the universities. This committee will produce documents which will weight heavily in government judgement of the results obtained and, we believe, will

9. Development Implications

This project will have a very significant impact in improving the health of the population in developing areas, particularly Central America and Panama; by extension, this could easily apply to many other parts of the world where the U.S. and other national and multinational organizations are engaged in accelerating social and economic development. We firmly believe that proper health and adequate nutrition are prerequisites for any development action to take place[55]. Iron deficiency and anemia are associated with decreased host-defense mechanisms against bacterial infection[9,10] and to decreased physical working capacity[5,6,56,57]. Populations in developing areas have a high rate of bacterial infection and depend economically on physical work. The importance of improving iron nutrition as a basic need for development is, therefore, clear evident.

The principal investigator together with other members of INCA including psychologist and biochemists are beginning to explore the effect of iron deficiency and anemia on learning and behavior of preschool and schoolchildren. In this regard, significant differenc have already been found in a pilot study involving lowland first grade children attending public schools.- Iron deficient children performed poorly in tests requiring prolonged and demanding attention[58]. This may be related to alterations in catecholamine metabo

to reach them and of carefully programmed actions in cooperation with Governments and with Regional Economic and Health Development and Planning Agencies are common activities at INCAP now. The presence of a Ph.D. in Economics within the Biomedical Division primarily concerned with doing feasibility studies, cost effectiveness and cost benefit analysis of nutrition interventions and implementation of delivery systems is also a major advantage in terms of future implementation of iron fortification in Central America.

Another very important resource in Central America is INCAE (The Central American Institute of Administration), with whom INCAP and specifically the Biomedical Division has maintained close working relations for several years. INCAE has very close ties with the graduate school of business in Harvard and we plan to consult them regarding studies of feasibility for implementing iron fortification in sugar. A cooperative program in terms of fortification of corn aimed at improving protein nutrition is in progress with Dr. James Austin of the Graduate School of Business in Harvard. His consultantship will be considered for this program when the time comes.

10. Other Organizations to be Involved in Project

(a) INCAP: Nutrition and Productivity, Physiology and Biochemistry of Nutrition, Food Science and Technology, Health intervention, Food and Nutrition Policies.

(b) Instituto Venezolano de Investigaciones Cientificas (IVIC): Laboratory of Patho-Physiology.

(c) Epidemiology divisions of the Public Health Department and Social Security of Guatemala (where the research will take place). They will help gather and analyzed information on anemia prevalence and incidence and on the expenses incurred at the national level in treating anemic atients.

(d) Associations of sugar producers will help INCAP with their expertise in sugar technology in order to solve all the details involved in the technical process of iron fortification.

11. Cost of fortification and control of the program

A careful evaluation of costs will be conducted at the end of the first and of the second year of fortification, based on data from the sugar mills and the cost of NaFeEDTA addition, the operation of fortification itself, etc. At the sa time an assessment will be made of the cost of the control process, routine colle of sugar samples, analysis, surveillance of the process in the mills, etc. The a cost will then be calculated, per capita, and per person assumed to have been pro These costs will be compared with the expenses in which the public health departm incurred in treating iron deficiency anemias in the control communities. These d will be projected to a National and Regional level. [Private industry has already contributed to the implementation at the sugar mills of iron fortification syste Two Chemical Engineers whose specialty is sugar production will be actively parti in the study. The program of Food Technology at INCAP is already workingin clos cooperation with us in performing all of the stability trials.]

Fortification of [illegible]r with iron has been monitored by us in initial pilot trials, by means [illegible]etermination of iron in sugar using atomic absorption spectrophotometry in twenty samples of each production batch consisting, so far, of 400 bags of one hundred pounds each. This means taking a sample from each ton of fortified sugar. This form of control will continue for study purposes . Based on the variability which will be found in the different production batches throughout the two years of fortification planned in this study, recommendations will be drawn for the minimum number of samples required for analysis once the whole sugar production of each country begins to be fortified with iron.

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9. Supervision of the studies

The technical responsibility for the implementation and careful control of the field studies described in this document rests with INCAP. The design and performance of special techniques at the field or laboratory level will be both the responsability of INCAP and of Dr. M. Layrisse at IVIC. All the details involved in the implementation of the experimental design will be discussed with Dr. M. Layrisse and submitted to an expert committee who will have a dual role: Advisory and Coordinating. In this last capacity it will try to steer all the research on food-iron-fortification throughout the world to follow the guidelines states in the WHO-AID-IAEA meeting in Geneva in 1974. This will insure that the present research and that performed to improve iron nutrition in other parts of the world will have a commonality and will, therefor be as universally useful as possible.

To accomplish this, we propose to gather at INCAP as soon as possible a committee of 8-10 members to review and build upon the present proposal one which will be followed by everybody embarking in iron-fortification or in measures aimed at improving iron nutrition. INCAP will provide all the information that will be collected to a National or Regional ad-hoc Evaluating Committee, which will be responsible to the government of the country for a final assessment of the effectiveness and the cost of sugar fortifi tion with iron. It is proposed that the committee be composed of delegates from the Ministry of Health, Pediatric Association, Hematological Association, Association of Sugar Millers, the Acade of Science (or the National Research Council) and INCAP. Finally, Dr. M. Layrisse and the principal investigator will meet at least 3 times per year to review the progress made. They will advise als

8. Cost of fortification.

Table B1 shows an estimate of the time assigned for the personn in each activity.

Table B2 presents the cost estimates of each activity divided i: personnel and other costs. Table B3 is the 3 year budget estimate.

INCAP will contribute with the principal investigator's salary and with all its present facilities which are well known to AID. W: request the purchase of a new Coulter Counter because the one curre: in use is near 15 years old and is the only one available for this type of work. The purchase of two microhematocrit centrifuges and 2 freezers is indispensable for the progress of the work as planned The U.S. $8,000 quoted in this application is only an estimate of current costs to U.S. Government agencies. Two vehicles for exclus use of the program are also needed. As is well known to AID, the whole professional staff of INCAP is available for consultation. In this regard it is of particular importance to indicate that through the current reorganization taking place at INCAP, statistic advice and data processing will improve substantially starting in the first half of the present year.

12. Related Activities

Other projects previously undertaken and/or currently in progre by the principal investigator:

1. WHO support on "sugar fortification with iron" Dates 1974-1976. Amount: $10,000 to 13,500 (U.S.)/year. Purpose: Stu: bioavailability of various iron compounds and properties of sugar enriched with such compounds. Radioactive iron absorption is studied in small samples. (Annex 4)

the Advisory and Coordinating Committee any worthwhile matter.

10. Calendar (Figure 1)

Initial explorations in several communities and recruitment of the four who will continue in the study, and training of personnel will take 4 months. Baseline studies in the chosen communities will be conducted for four months prior to the iniciation of fortificatio The study will continue for 24 months after iron fortified sugar is distributed to the population. This sugar will have the same price as the presently consumed unfortified sugar. The collection of dietary data, blood samples, clinical records, physical working capacity, and trace mineral studies will be repeated every 8 months for two years. The last 6 months will be required for final data analysis. Partial data analysis will be available throughout the p of study.

11. Budget

For ease of presentation in terms of activities, the following have been considered the main activities:

1. Direction, organization and monitoring of project, coordina tion of collaborative efforts, relations with the research communities, education and motivation of the members being studied and of the communities in general.
2. Dietary data.
3. Measured iron intakes.
4. Iron and folate nutritional status - Clinical condition
5. Morbidity studies, including fecal egg counts.
6. Trace mineral nutrition

2. Guatemalan government support on "Nutrition and work: a combined biological and economic analysis of caloric supplementation to agricultural workers." Amount: $52,500 (U.S.)/year. Dates 1975-1976.

3. W.K. Kellogg Foundation support on " Nutrition and Productivity of Agricultural Workers". Amount: $59,000 (U.S.)/year. Dates: 1976-1977.

4. U.S. Army Research and Development Command support on "Relations between nutrition and physical working capacity". Dates 1966-1968. Several publications available as a result of this work.

13. Other Support

The proposed project has not been submitted to other organizations for sponsorship.

Human Rights: (Annex 5)

The individuals participating in the study, either in the experimental or the control communities, will be informed as clearly as possible about the study and of what would be required of them as individuals, as families and as members of the communities. The possible benefits they may receive will also be explained. A clear understanding will be established that they may withdraw from the study whenever they desire. Specifically in the case of the control communities, they will know they will not receive iron-fortified sugar in contrast to the experimental communities.

A written authorization will be obtained from each family participating in the study. It will list the members who will

TABLE B2

ACTIVITIES	PERSONNEL	OTHER DIRECT COSTS	TOTAL DIRECT COSTS	APPORTIONED RESEARCH EXPENSES	TOTAL
1. Direction, organization, etc.	33,445	7,375	40,820	7,348	48,168
2. Dietary	16,607	4,425	21,032	3,786	24,818
3. Measured intakes	4,671	9,500	14,171	2,551	16,722
4. Iron and folate status	24,683	27,750	52,433	9,438	61,871
5. Morbidity	18,593	11,570	30,163	5,429	35,592
6. Trace mineral nutrition	9,866	5,155	15,021	2,704	17,725
7. Costs of fortification & [illegible] of implementation	10,005	6,425	15,430	2,956	19,386
	117,870	72,200	190,070	34,212	224,282

TABLE B3

BUDGET

1. Personnel

a) Professionals	1st year	2nd year	3rd y
1 Physician, chief of field project	38,060	33,500	36,7
1 Physician, assistant	15,900	15,900	11,9
1 Nutritionist	15,900	15,900	11,9
b) Non professionals			
1 Assistant administrator	3,620	3,785	2,9
4 surveyors	11,140	11,740	9,2
4 Auxiliary nurses	8,920	9,230	7,2
2 Laboratory assistants	7,240	7,570	7,9
1 Lab. assist. (IVIC, Venezuela)	4,000	4,400	4,8
1 Parasitology technician	3,620	3,785	1,9
2 Data clerks	7,240	7,570	7,9
2 Drivers	2,230	2,330	1,8
	US117,870	115,800	104,5

2. Travel and Transportation

a) 3 round trip/year Caracas-Guatemala + per diem	2,400	2,400	2,4
b) Mileage charge $0.20 per km, 36,000 Km/year (gasoline and maintenance)	7,200	7,200	5,4
c) Per diem professional personnel $10 x 84 days x 3	2,520	2,520	1,8
d) Per diem for non-professionals $5 x 84 days x 9	3,780	3,780	2,8
	15,900	15,900	12,5

TABLE 53 (co

	t year	2nd year	3rd y
3. Supplies and equipment			
Coulter counter, 2 microhematocrit centrifuges and 2 freezers	8,000	------	----
O_2 cells (4)	600	600	
Spare parts for equipment	1,500	1,500	
Equipment for blood collection	1,200	1,300	1,3
Equipment for urine collection	200	200	2
Stationery and miscellaneous	1,000	1,000	1,0
Two vehicles for exclusive use of Project (Field and other activities)	12,000	------	----
	24,500	4,600	2,5
OTHER DIRECT COSTS			
4. Blood and urine analyses	7,600	15,400	7,0
5. Composite analyses of food	4,800		4,8
6. Medicines for out-patient care	6,000	6,000	4,5
7. Contractual services	2,000	2,000	1,5
8. Advisory services (STC)			
a) Short term consultants 2 months at $3,000	6,000	6,000	6,0
b) Review meeting			5,0
9. Data processing and writing report	5,400	5,000	15,0
0. Publication costs			
3 articles x 15 pages x $50/page			2,2
1. Apportioned Research Expenses	34,212	30,726	29,9
	66,012	65,126	76,5
Total Budget for each year	224,282	201,426	196,0
Total Budget for 3 year period			621,7

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Personnel not covered by AID budget

a) Professionals	Time	Costs
Principal Investigator (F. Viteri)	20%	8,050.
Co-Principal Investigator (M. Layrisse)	5%	2,015.
Scientist (B. Torún)	10%	2,615.
Medical resident (P. Aycinena)	5%	735.
Statistician (M. Guzmán)	5%	2,150.
Statistician (C. Yarbrough)	5%	1,690.
Systems Analyst (S. Sibony)	10%	840.
Economist (M. Immink)	5%	765.
Research Associate (R. García)	10%	735.
Sugar Engineer (private industry)	?	?
b) Non professionals		
2 Laboratory assistants (Physiology)	11%	1100.
1 Laboratory technician (F. Saldaña)	10%	635.
1 Secretary (D. García)	15%	670.
	TOTAL:	22,000.

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TABLE B1

Time assigned for each activity (%)

Activities	Princ. Invest.	Co-Princ. Invest.	Assist. Administrator	Field Director	Assist. Field Director	Nutritionist	Surveyors	Aux. nurses (4)	Lab. Assistants (2)	Lab. Assistants IVIC	Parasitol Tech.	Contracts
1. Direction, organization, etc.	20*	10*	80	50	35	30						
2. Dietary				5		50	50					
3. Measured intakes				2		10	5		13			
4. Iron and folate [illegible]				20	25			40	65	100		Professional
5. Morbidity				8	15		35	50			100	
6. Trace mineral nutrition				5	10	10	10	10	22			Professional
7. Cost of fortification and feasibility of implementation			20	10	15							Other professional activities at INCAP

The drivers are needed for all field activities. The data clerks will be involved in processing data from all sources.

* No cost.

RAC recommendations on proposals , reviewed at M ch 22-23, 1976 meeting -- Attachment VII

2. Fortification of Sugar and Iron: Field St y Prior to Implementation at a National Level (New) - Pan American Health Organization. Durati of project, 3 years; estimated cost, $621,799. Schweigert, Carter, Linder, Ludington.

Recommendation: That the project be approved as requested subject to incorporation by AID staff into a revised project proposal the sugges made by RAC and the peer reviewers. This revised proposal is to be r by members of the RAC subcommittee. If the revised proposal is accep to the RAC subcommittee it may be immediately implemented; if major o are made by the RAC subcommittee, the project will not be implemented further review by the RAC, preferably at the May, 1976 meeting.

*RAC review subcommittee; chairman underscored