PD-AB6-783
84413

ICCNA PROGRESS REPORT

IRON DEFICIENCY SUPPORT PROGRAM

AID COOPERATIVE AGREEMENT DAN-5115-A-00-7098-00

1992 - 1993

TABLE OF CONTENTS

Page

Progress Report 1

Appendix A: Publications (7)

Appendix B: Project Reports/ Study Protocols

1. Wheat Fortification (Grenada)
2. Gastric Delivery System Trial (Tanzania)
3. Gastric Delivery System Trial (Indonesia)
   a. Final Project Report
   b. Trial Proposal
   c. Carol Flowers' report
4. Joint Micronutrient Mission (Philippines)
   a. JMM report
   b. Daily log

ICCNA PROGRESS REPORT

IRON DEFICIENCY SUPPORT PROGRAM

AID COOPERATIVE AGREEMENT DAN-5115-A-00-7098-00

September 1992 - August 1993

The following report summarizes activities performed under a one year extension of the AID Cooperative Agreement which covers the period from September 1, 1992 to August 31, 1993. The objective of this project is to facilitate efforts in developing countries to assess the prevalence of iron deficiency in different segments of the population and to develop novel approaches to reduce the prevalence of nutritional iron deficiency anemia. The program at the International Center for the Control of Nutritional Anemia (ICCNA) is divided into intramural activities at the Kansas University Medical Center and extramural activities in various developing countries. The major focus of the intramural program is to develop and refine methods to assess the prevalence of iron deficiency anemia and to develop more effective means of adding iron to the diet of populations at increased risk of nutritional anemia. Because of the reduced level of funding during this no cost extension of the Iron Deficiency Program, activities have been sharply curtailed during the past 12 months.

I. PUBLICATIONS

1. **Cook JD, Skikne BS, Baynes RD. Serum transferrin receptor. Annu Rev Med 1993;44:63-74.**

A variety of key studies on the measurement and application of serum transferrin receptor measurements have been published under support of this program. The present publication is an extended summary of the serum transferrin receptor covering all biochemical, chemical, and nutritional aspects of this new iron measurement. This article describes the precise biochemical composition of the circulating transferrin receptor as a soluble truncated form of intact receptor. The latter is measured by an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies developed against human placental receptor. The review describes the early clinical experience with this measurement which indicates that it is a measure of the total body mass of tissue receptor. Elevations in the serum transferrin receptor level occur whenever there is an expanded erythroid activity. However, for the epidemiologic assessment of iron nutriture, the key finding is that the serum transferrin receptor is elevated with normal erythroid activity because of the increased synthesis of the transferrin receptor on each red cell precursor in patients with iron deficiency. This review outlines the evidence that the serum transferrin receptor is the first iron measurement which permits a distinction between true iron deficiency anemia and the anemia associated with various chronic disease states. The evidence is based on clinical studies showing that in 17 patients with

iron deficiency anemia, the serum receptor was elevated in all but 2 whereas in 15 patients with acute bacterial infection and 5 with acute viral hepatitis, the serum receptor was invariably within the normal range. More importantly, in 41 patients with the anemia of chronic disease, only 4 had a slight elevation in serum receptor level. Complete separation between patients with iron deficiency anemia and those with the anemia of chronic disease was possible when the reduction in circulating hemoglobin concentration was greater than 25 g/L.

This review also outlines the use of the serum transferrin receptor in the assessment of iron deficiency during gestation. Our studies have demonstrated that an elevation in serum transferrin receptor during gestation invariably reflects compromised iron status. Consequently, an elevation in serum receptor was observed only in women with depleted iron stores as reflected by a serum ferritin concentration below 16 µg/L. In 11 out of 13 women with iron deficiency anemia, as defined by a variety of laboratory measurements, the serum transferrin receptor was elevated in all but 2 instances.

This same monograph outlines the important future application of serum transferrin receptor in monitoring iron status of a population. The serum ferritin determination reflects iron status in individuals with remaining iron stores while the serum transferrin provides a quantitative measure of the degree of tissue iron deficiency. Consequently, our preliminary studies indicate that the ratio of serum ferritin/serum receptor can be used to provide a quantitative estimate of body iron in each sampled individual.

2. **Simmons WK, Cook JD, Bingham KC, Thomas M, Jackson J, Jackson M, Ahluwalia N, Kahn SG, Patterson AW. Evaluation of a gastric delivery system for iron supplementation in pregnancy. Am J Clin Nutr (In press).**

The extensive field trial to examine the hematological efficacy of a gastric delivery system (GDS) in Kingston, Jamaica, that has been described in prior reports, has now reached the final publication stage. During the past year, several difficulties were encountered and resolved before this paper was accepted for publication. The reviewers requested that the statistical analysis be revised to include a primary analysis using repeated measures analysis of variance (ANOVA) and repeated measures analysis of covariance (ANCOVA). This new approach to the statistical analysis did not substantially alter the basic findings in this study but it did provide a means of eliminating concern about the baseline differences in hemoglobin concentration in the three study groups.

This study was performed in 376 pregnant women aged 16 to 35 years and between 14 and 22 weeks of gestation. Only those pregnant women with a hemoglobin concentration between 80 and 110 g/L were selected for the trial. These women were randomized to one of three study groups given either no iron, two ferrous sulfate tablets daily (100 mg), or one GDS capsule (50 mg) daily. There was a highly significant improvement in hematological and iron status in women given iron whereas progressive iron deficiency developed in women who were given folic acid alone.

Whereas the journal requested and, in fact, insisted on data relating to compliance in this study, we preferred to reserve this information for a separate publication. However, in the end, arguments by the editor prevailed and a brief description of the compliance data was included in this report. It was mentioned in the methods that the adherence to the supplementation program was greater than 90% in all three study groups during the 12 week trial based on tablet counts and questionnaires. Thus, there was no significant difference in compliance among the three groups. While it is still planned to publish this information separately, the study was not optimally designed to evaluate adherence. By having these women report at regular intervals throughout the study for repeated questioning, and because the study was not fully blinded, the compliance data is of questionable value.

3. **Singhal A, Cook JD, Skikne BS, Thomas P, Serjeant B, Serjeant G. The clinical significance of serum transferrin receptor levels in Sickle Cell disease. Br J Haematol 1993;84:301-304.**

As we approach wider application of the serum transferrin measurements in epidemiological studies, the extent to which this new measurement is influenced by hematological disorders which occur frequently enough to be detected in epidemiological surveys requires further study. One such genetic disorder which occurs with significant frequency in African populations is sickle cell (SS) disease. The present study was performed as part of a collaborative effort between ICCNA and the Medical

Research Council Laboratories at the University of the West Indies in Kingston, Jamaica headed by Dr. Graham Serjeant. Serum transferrin receptor levels were measured in 182 children with homozygous SS, 47 with sickle cell hemoglobin-C (SC) disease, and 41 normal controls. A novel aspect of this study is that all measurements were obtained when these children reached their eighth birthday. As anticipated, highly significant elevations in serum transferrin receptor were seen in SS as compared to normal controls. A less dramatic increase was seen in children with SC but the elevation was significant as compared to controls. Interestingly, the serum transferrin receptor level in male children with SS and SC was higher than in females in contrast to studies in normal populations in which male teenagers have been shown to have higher levels than females. There was a significant correlation with serum transferrin receptor and the reticulocyte count providing evidence that the elevation in serum transferrin receptor levels in SS relates to the expanded erythroid activity. This study therefore defines one mechanism for an elevated serum transferrin receptor level that may be seen in epidemiologic surveys and that is not the result of iron deficiency. Another important potential cause of an elevated serum transferrin receptor level in epidemiologic studies is the anticipated increase in patients with malaria because of the accelerated red cell destruction and parallel increase in red cell production. Studies are needed to study the effect of active and/or latent malarial infection on serum transferrin receptor levels.

4. **Cook JD, Skikne BS, Baynes RD. Iron deficiency: The global perspective. In: Hershko C, ed. Progress in Iron Research New York:Plenum Press, Co., 1993 (In press).**

This review outlines the global problem of iron deficiency anemia and includes sections on the liabilities of iron deficiency (mental and motor development, work capacity, and premature birth), the prevalence of iron deficiency with emphasis on newer methods to assess prevalence, and finally, intervention methods covering the topics of iron supplementation and iron fortification. The novel feature of this review is that it will be the first article on iron deficiency that will be included in a monograph that has previously dealt only with problems of iron overload, namely idiopathic or hereditary hemochromatosis. The present article outlines the high prevalence of iron deficiency anemia in the world and emphasizes the important contribution that the serum transferrin receptor assay will make in future epidemiological studies. To reconcile the efforts of investigators concerned with iron overload to remove all forms of fortification iron from the diet, this report discusses the concept of targeted fortification in which food vehicles fortified with iron are directed only to segments of the population at greatest need of iron such as infants, school children, and pregnant women. The review also describes the use of NaFeEDTA as an iron fortificant.

5. **Baynes RD, Shih YJ, Cook JD. Mechanism of production of the serum transferrin receptor. In: Hershko C, ed. Progress in Iron Research New York:Plenum Press, Co., 1993 (In press).**

This monograph summarizes a series of studies in this laboratory over the past two years that have been performed in an attempt to develop simplified and less expensive methods for obtaining immunologic reagents for the measurement of serum transferrin receptor. Currently, our assays use monoclonal antibodies which involve technology that is not feasible for the majority of laboratories in developing countries. An alternate approach for the production of immunologic reagents is the use of domain specific anti-peptide antibodies. In this report, the use of polyclonal antibodies against peptide sequences in the endodomain and ectodomain of the intact transferrin receptor is reported. The use of these two sequence locations, in tandem provides a means of distinguishing the soluble truncated form of serum transferrin receptor in serum from circulating intact receptor that may appear in certain hemolytic states such as SS disease or patients with malarial infection. Although much of this report is technical and at a stage that is not immediately applicable to epidemiologic work, it does open the door for an avenue of investigation which could greatly reduce the cost and complexity of producing immunologic reagents for iron-related proteins such as ferritin and serum transferrin receptor.

6. **Trowbridge FL, Harris SS, Cook J, Dunn JT, Florentino RF, Kodyat BA, Mannar MGV, Reddy V, Tontisirin K, Underwood BA, Yip R. Coordinated strategies for controlling micronutrient malnutrition: A technical workshop. J Nutr 1993;123:775-787.**

This monograph summarizes the outcome of a workshop held in November, 1991 to describe the potential for coordinating strategies for controlling malnutrition due to iodine, iron, and vitamin A either by coordinating epidemiologic surveys or developing multiple fortification systems for specific food vehicles such as salt, rice, or sugar. This monograph includes recommendations for the formation of national coordinating bodies for micronutrient deficiency control and an expanded international network that would provide efficient and rapid exchange of information related to micronutrient deficiency.

7. **Cook JD. The effect of endurance training on iron metabolism. Sem Hematol (In press).**

The influence of iron deficiency on work capacity has been the topic of extended discussion during the past decade and has provided much of the momentum for attempting to reduce the global prevalence of iron deficiency anemia. In the present monograph, the effect of more intensive work activity, namely that involved with endurance training, is described. This is a topic that is a matter of current interest in industrialized countries and has been the subject of over 100 publications during the past decade. This article discusses evidence that intense aerobic training is associated

with only a modest decrease in body iron reserves due, in part, to the development of so-called sports anemia. The latter refers to the decline in hemoglobin concentration which occurs during the early stages of an intense conditioning program. Review of existing data indicates that the development of iron deficient erythropoiesis or frank iron deficiency anemia is uncommon except, perhaps, in adolescent girls who are otherwise highly susceptible to the development of iron deficiency. Most of the modest influence of endurance training on iron status can be explained by the mobilization of storage iron required to accommodate an expansion in circulating red cell and muscle mass. The explanation for more overt iron deficiency anemia in a few highly conditioned athletes is probably due to accelerated blood loss from the gastrointestinal tract. Importantly, few, if any, studies of the effect of oral iron on athletic performance have shown a measurable benefit. It is recommended that the widespread use of high doses of iron supplement in those engaged in aerobic training be curtailed and that the modest increase in iron requirements by these groups be offset by the intake of an adequate quantity of dietary heme iron.

II. PRESENTATIONS

The following presentations and/or meetings were believed to have some impact on awareness of the problem of nutritional iron deficiency anemia and served as a format to convey the findings of recent studies at ICCNA on methods to assess iron status and approaches for reducing the prevalence of iron deficiency anemia.

1. Dr. Manju Reddy traveled to Hyderabad, India to attend an International Atomic Energy Agency (IAEA) Regional Seminar on Isotopic Tracers in Human Nutrition Research for the Asia and Pacific region. This activity was supported by an IAEA coordinated research program (CRP) contract with ICCNA to promote studies of bioavailability of iron from human diets. The technical sessions were held from November 16-20, 1992 at which time Dr. Reddy summarized the current efforts at ICCNA to standardize radioisotopic bioavailability measurements in human subjects. After presenting preliminary studies of international standardization by the provision of a farina-based standardized meal, plans were developed by which ICCNA would provide samples of ^{55}Fe and ^{59}Fe containing known quantities of isotopes. In addition, supplies of farina will be circulated to participating laboratories to permit the use of a universal standardized meal. The ability to obtain closer uniformity of human measurements of iron bioavailability is perceived to be an important step in expanding the use of these technically difficult measures of iron bioavailability to the developing world.

2. Dr. Cook attended a workshop at Nestlé in Vevey, Switzerland from January 8-12, 1993 to review progress of the collaborative studies between ICCNA and Nestlé. This collaboration is designed to use radioisotopic studies in human subjects (ICCNA) to evaluate and develop new methods for fortifying food products. The emphasis in recent studies has been on techniques to process infant foods so as to reduce their inhibiting effect on iron absorption. At this session, a review of iron absorption studies performed

in the Fall of 1992 was undertaken and an extended discussion to define studies to be performed in the Spring of 1993 was held. The emphasis in the latter period will be on the mechanism of the facilitating effect of animal tissue on nonheme iron bioavailability.

3. Dr. Cook traveled to Arusha, Tanzania to participate in the XV Annual Vitamin A meeting (IVAC). This meeting was held from March 8-12, 1993 and was attended by more than 200 participants. The inclusion of a session devoted to iodine and iron deficiency represented the first step towards an evolving micronutrient strategy and, in particular, efforts to associate or link vitamin A deficiency to other micronutrient deficiencies. This presentation covered information on the global prevalence of iron deficiency, emphasized current methods for the assessment of nutritional iron deficiency, and outlined methods for its control. This meeting provided a very effective format for interchanging information about iron deficiency with those whose primary interest lies with vitamin A deficiency.

4. Dr. Cook was invited to attend the Experimental Biology meeting held from March 28-April 1, 1993 in New Orleans, to participate in a seminar on micronutrient deficiency. The purpose was to present a paper on contemporary scientific issues facing programs to combat iron deficiency. This symposium entitled "Clinical Nutrition in Developing Countries: Towards the Application of Contemporary Concepts and Technology" included representatives from the fields of vitamin A, iodine, and iron deficiency.

Chaired by Noel Solomons and Michael Latham, topics included clinical nutrition: practice in the Third World, dietary management of diarrheal disease, hypovitaminosis A, and iron deficiency. In addition, Dr. Cook co-chaired a nutritional symposium on iron for the 1993 AIN/ASCN meeting that included a wide range of topics relating to both iron deficiency and iron excess.

5. Dr. Manju Reddy attended the Experimental Biology meeting in New Orleans and presented a paper entitled "Absorption of Nonheme Iron in Ascorbic Acid Deficient Rats". This paper represented efforts over the last two years to develop a more suitable animal model for assessing the bioavailability of iron in human subjects. Because iron absorption in the rat in higher and less responsive to factors known to influence the absorption of dietary iron in humans, it was believed that a high endogenous secretion of ascorbic acid could explain the profound differences in iron assimilation in the two species. By obtaining a mutant rat strain unable to synthesize vitamin C, it was possible to induce severe ascorbic acid deficiency in rats. However, when radioisotopic measurements of food iron absorption were performed in these animals, absorption was only slightly reduced and was no more responsive to factors known to influence absorption in humans including isolated soy protein, meat, ascorbic acid, tea, and bran.

6. Drs. Cook, Skikne, and Baynes attended the Fourth International Congress of Hemochromatosis held in Jerusalem, Israel from April 27-30, 1993 and

the 11th International Conference on Iron and Iron Proteins, May 2-7, 1993. While these conferences have traditionally been devoted to problems of iron excess, efforts prior to, and at the time of, the meeting made it possible to introduce the topic of nutritional iron deficiency to the program. Dr. Cook presented a paper on the global issues relating to iron deficiency and stressed that more attention should be focussed on the problem of iron deficiency and on efforts to reconcile nutritional policies that would accommodate those concerned with iron excess and iron deficiency. The members of this conference agreed to change the title of the national meeting to "Conference on Clinical Disorders in Iron Metabolism". By this means, it is hoped that a much broader representation of investigators concerned with problems in iron metabolism will attend this meeting so that a meaningful dialogue could be held between those concerned with iron deficiency and those whose primary investigative focus is on problems of iron overload. The participants agreed with this title change. The next meeting of this group will be held in 1995 in the United States.

III. FIELD PROGRAMS

1. Efficacy of Iron Fortification of Wheat

INVESTIGATOR: William Simmons, Ph.D.

LOCATION: Grenada, W.I.

In view of the high prevalence of nutritional iron deficiency that was demonstrated in Grenada in the early 1980's, it was considered imperative to introduce some measures to reduce the prevalence of nutritional anemia which ranged, in prior surveys, from 14% in young men to as high as 74% in pregnant women. Because only one-half of the flour consumed in this country is iron fortified, the impact of fortifying all flour that is sold was examined. The details of the investigation are outlined in Appendix B-1.

In January of 1993 (January 20-23) a group including Mr. Richard Siefman, Dr. Patterson, Dr. Simmons, and Dr. Cook met in Grenada to evaluate the progress on this three year field trial. Meetings were held with a variety of government representatives who have provided logistical support for some aspects of this field trial and were generally pleased that Grenada had been selected as an area for more detailed evaluation of the nutritional impact of a fortified food. Visits were made to the local hospital where the samples obtained in the baseline survey to assess the prevalence of nutritional iron deficiency were obtained and processed. Overall, the impression of the local personnel is that anemia prevalence, which had been measured in the survey by the HemoCue® technology, was

somewhat lower than studies in the 1980's suggested. Some of the technical problems that were encountered during the conduct of this survey were discussed as well as the adequacy of the laboratory facilities for processing storage and transport of the specimens.

An interesting facet of this visit was a trip to a nutmeg factory in a region where nutritional anemia is believed to be even higher in prevalence than in Georgetown. During our visit to this facility, an opportunity to assess the effect of iron deficiency on skilled work performance became apparent. Women employed in this nutmeg processing facility had the task of sorting, according to grade, the quality of nutmegs transported to this facility. Because of the speed and intense concentration required for this activity, it was considered attractive to examine any difference in the productivity on this skilled work between normal and iron deficient women. Consequently, a study was discussed that will involve, initially, the assessment of some workers in this facility to determine the baseline prevalence of iron deficiency anemia.

The results of the baseline survey of iron status in Grenada performed in connection with the wheat fortification trial is still in progress although most of these samples have now been analyzed at CFNI. Overall, the results appear to indicate some decline in the prevalence of iron deficiency anemia although a relatively high proportion of samples are iron deficient based on

a reduced serum ferritin concentration. The analysis of this important field trial is now underway.

2. Adherence to Iron Supplementation During Pregnancy

INVESTIGATOR: Eva-Charlotte Ekström

LOCATION: Dar es Salaam, Tanzania

In this important field trial, the compliance rate with iron supplementation in pregnant women is being evaluated in conjunction with a study to determine whether compliance is improved by the use of GDS. The details of the investigation are outlined in Appendix B-2. The advantages of this preparation are once-a-day administration and reduction in gastrointestinal side effects. The trial in Tanzania is now concluded and samples are being transported by Ms. Ekström to the United States to perform the various analyses. In this trial, a total of about 200 women were given either conventional iron or the GDS preparation and compliance was monitored by a medication event monitor system (MEMS). The latter consisted of a microprocessor contained in a pill bottle cap that registers the time and date that each pill bottle is opened. A variety of technical problems were encountered with this new technology. The women broke several of the bottle caps and some became aware that their tablet use was being monitored electronically. Although the results have not yet been evaluated statistically, the impression is that there is a remarkably high rate of non-adherence to supplementation. There was also a significant improvement

in compliance rates in those given GDS and this was translated into an improved hematologic status. Although ICCNA had planned to perform the ferritin determinations in conjunction with this field trial, closing of funding will not permit this activity.

3. Serum Transferrin Receptor Measurements as an Index of Iron Status in Infants and Children

INVESTIGATOR: Tomas Walter, M.D.

LOCATION: Santiago, Chile

This extensive assessment of the utility of transferrin receptor measurements in infants and children has now concluded and preliminary manuscripts relating the results of this finding are being circulated. One important finding has been that the serum transferrin receptor, as in adults, is capable of distinguishing infants with true iron deficiency anemia from those with the anemia due to chronic infection. In one study, it was observed that following immunization and the associated inflammatory response, the serum ferritin was significantly influenced but little or no effect on serum transferrin receptor was observed. A large variety of iron-related measurements were performed in infants and school-aged children to examine the possible advantage of using serum transferrin receptor to evaluate iron status. The serum receptor levels in infants were surprisingly high as compared to measurements in adult subjects, suggesting that the effect of erythropoiesis per unit body weight is exaggerated during infancy.

This indicates that new criteria of normality need to be established in infancy for this new parameter as has been a requirement for most other measurements of iron status during infancy.

An important finding in this study is the fact that when iron status is expressed as the ratio of serum ferritin/serum transferrin receptor, a log normal distribution is obtained which is remarkably similar in all age groups. This provides additional evidence that iron status in a given population cannot be divided into normal and deficient groups but rather iron status is a continuum and is best expressed by the mean and standard deviation of the ferritin/receptor ratio. This analysis is still in progress.

4. Gastric Delivery System Iron Trial

INVESTIGATOR: Dr. Poedji Rochjati

LOCATION: Surabaya, Indonesia

This project, supported by MotherCare, is designed to assess the possible advantages of the GDS for iron supplementation of pregnant women. The rationale for this study is outlined in prior reports and in the appendix of the present report where the final project is summarized B-3a.

There are two trials in this program (Appendix B-3b). The first was conducted in pregnant women who were randomized to a group given 1 GDS capsule a day (50 mg iron) for 20 days during the last trimester of

pregnancy and a second group given standard ferrous sulfate (60 mg iron daily). As compared to the study recently concluded in Jamaica, the total amount of iron consumed in the two study groups in Indonesia is similar. Hemoglobin, ferritin, and serum transferrin measurements will be performed in blood samples obtained at monthly visits. The second trial will be performed in anemic, non-pregnant women, and is basically a trial of gastrointestinal side effects. In the first group, GDS iron is given for three months, in the second, ferrous sulfate for the same period of time, while the third receives a placebo.

Several problems have arisen in the field with this project. First, and most important, is a long delay in the conduct of the trial. It was initially believed that the first batch of samples from Indonesia would be received at ICCNA in December, 1992, whereas the first samples arrived six months later. In these preliminary measurements, it was noted that the serum transferrin receptor was far lower than in normal adults living in the United States and lower than in other samples that have been obtained during the last two years from developing countries. In an attempt to define the nature of this problem, Carol Flowers, from ICCNA, traveled to Surabaya for 10 days in July, 1993. It was learned that there were several difficulties during the conduct of the trial. First, because tubes were not available for both sampling and storage, the blood sample was initially placed in an unsterilized centrifuge tube prior to harvesting of the plasma. These tubes were not washed each day but simply rinsed under the tap until used for

the next sample. Consequently, the blood samples were not obtained under sterile conditions and were highly susceptible to bacterial growth during any period of storage at room temperature. The tubes were labelled with paper grade labels and a soluble marker. Consequently, a large proportion of the samples that were frozen and subsequently forwarded to Kansas did not have adequate identification. There were at least two, if not more, points when the samples were exposed to room temperature during the time that the samples were obtained and their receipt at ICCNA. One of these occurred in Indonesia due to a power failure over a weekend. The second was in transit to Kansas City. It is suspected that the markedly reduced levels of serum transferrin receptor in the samples received in the first batch of specimens was due to bacterial contamination of the sample. However, in rinsing the specimen tubes with water, exposure from minerals that interfere with the enzyme-linked assay is also a possibility. These problems have been outlined in a separate report by Carol Flowers (Appendix B-3c). It is hoped to complete the serum ferritin and serum transferrin receptor measurements performed on the remaining 1400 samples that were transported from Surabaya to Kansas City in the last week of July.

5. Joint Micronutrient Mission

LOCATION: Manila, Philippines

An extended effort has been made over the past six months to conduct a joint mission to a developing country in which the three micronutrients (vitamin A, iodine, and iron) are assessed simultaneously by a team approach. Several preliminary planning sessions for this mission have been held at the Nutrition Foundation in Washington, DC. These meetings were held to determine the commonality in micronutrients for such a visit, to select a country, and to define the priorities during such a visit. One of the earlier meetings was held October 21-22, 1992 and follow-up meetings were held in January, 1993 and again approximately six weeks prior to the visit to the Philippines (June 13-28, 1993).

The purpose of the Joint Micronutrient Mission (JMM) was to assess the problem of micronutrient malnutrition in the Philippines, to review existing information on prevalence and active and planned intervention programs, and to identify the areas for future coordination of programs to reduce the prevalence of micronutrient malnutrition whether by supplementation, fortification, or dietary modification. The primary goal of the mission was to evaluate the effectiveness of such a field evaluation by a team comprised of experts in each of the three micronutrients. Documents describing the iron-related aspects of the JMM are included in Appendix B-4a and b. A

merged overview and summary of the site visit is currently being drafted at AID Washington.

With regard to the iron component of the JMM, there were several key findings that were outlined in the executive summary (Appendix B-4a). One of the key findings in regard to the prevalence of iron deficiency anemia in the Philippines is that studies have been limited to measurements of hemoglobin concentration. There is little or no information on the extent to which malarial infection contributes to the prevalence of anemia although it is known that this is a significant problem in the Philippines. It is clear that additional information is needed to determine the appropriate hemoglobin cut-off levels for anemia in the Filipino population and whether WHO criteria, that have been derived from studies in industrialized countries, can be applied to this population. There is little evidence that iron supplementation of pregnant women, which has existed for at least two decades, has had any impact on prevalence. Although several fortification efforts are planned for the Philippines including fortified vitamin rice (FVR) as described in the appendix, all of these efforts are in a preliminary stage of development.

Overall, the JMM approach was instructive, if not tedious, and has the important advantage of increasing advocacy within a country to a greater extent than could be achieved by visiting teams concerned only with a single micronutrient deficiency. At the same time, complete integration of

the evaluation of all three micronutrients does not provide for the most effective use of time. Efforts to coordinate the three micronutrients should be limited to an initial period, at the outset of the visit, and again at the end. For the large intervening period, it would be more effective and efficient to examine the micronutrient problems in isolation.

IV. INTRAMURAL PROGRAM

1. Assessment of Iron Status

The major focus of the laboratory program at ICCNA has been to better define the utility of serum transferrin receptor measurements in the epidemiological assessment of iron status. Much of the effort in this area during the past year has been focussed on completion of the work on a filter paper or "blood spot" technique for assessing iron status. Initial studies showed that this technology for measuring hemoglobin offered no significant advantage and, in fact, gave results that were highly variable. Given the advent of the HemoCue® system which provides a highly reliable index of hemoglobin concentration at the time of sampling, the rationale for using blood spot measurements for hemoglobin is questioned.

Work on the paper method has continued on samples obtained from volunteers coming to the ICCNA laboratory. Determinations were performed on over 70 blood samples to determine the concentration of

ferritin and transferrin receptor in various fractions of blood. Measurements of these two iron determinants were performed on serum, whole blood before and following lysis of the sample, and washed isolated red cells. In addition, measurements were performed on whole blood and on serum placed on a filter paper that was then allowed to dry. These studies have shown that there is a large contribution to whole blood ferritin levels from the red cell compartment. Moreover, because the red cell ferritin increases with iron deficiency whereas the serum ferritin declines, the changes in ferritin with iron status are masked when whole blood measurements are performed.

During the past six months we have made an effort to further explore the use of the blood spot technology by drawing samples at the time of the Grenada baseline sample. In nearly 200 sampled individuals, blood was obtained for serum measurements of ferritin and transferrin receptor. At the same time, measured volumes of serum and whole blood were placed on a filter paper and stored under frozen conditions for later measurements at ICCNA. These measurements are just now being completed in the laboratory, a total of 6 measurements in over 150 patients: serum ferritin and serum transferrin receptor, paper serum ferritin and serum transferrin receptor, and paper whole blood ferritin and whole blood transferrin receptor. Analysis of these results is not yet available but they generally agree with the findings obtained with prior measurements at ICCNA, discussed above. However, there was a somewhat reduced recovery of

serum ferritin from the filter paper as compared to measurements at ICCNA, presumably related to the longer period of storage. Thus, serum ferritin determined by paper spot were about 30% less than the values obtained in serum. Even lower recovery rates from filter paper were observed for serum receptor measurements but, in these measurements, an additional problem of lower serum transferrin receptor measurements were observed. This presumably relates to the prolonged storage at 4°C when these samples were obtained initially. These preliminary results also indicated that whole blood ferritin eluted from paper was about 50% higher than the serum value and that the characteristic decline in ferritin levels in iron deficiency were masked when whole blood was used. In contrast, whole blood measurements of serum transferrin receptor gave results that were very similar to those obtained in serum.

Other activities during the past year in regard to the assessment of iron status are summarized elsewhere and much of this work is still in process. We have performed a large number of analyses in conjunction with the assessment of serum transferrin measurements in infants (Chile project). Unfortunately, our funding will close before there is an opportunity to perform the measurements in the Tanzania field study of iron supplementation. There will not be an opportunity to complete the field trial in Grenada to assess the effect of introducing fortified counter flour. In fact, all ongoing overseas collaborations are now closed.

2. Food Iron Availability

Under limited support by Nestlé, we are trying to conclude several studies of iron bioavailability, the focus of which are to develop more effective food vehicles for iron fortification of infants. During the past year, several phytate-free products prepared by Nestlé have been examined by performing radioisotopic measurements in human volunteer subjects. We have confirmed prior studies showing that a 2- to 3-fold increase in the absorption of cereals can be achieved by removal of phytate. In the past year, however, we have found that when these cereals are reconstituted with milk rather than water, much of this advantage is lost. We are attempting to determine whether the inhibiting effect of milk is due to the calcium content of milk, as recent studies in other laboratories would suggest. In another series of studies, we are attempting to define the potential use of *in vitro* measurements of iron bioavailability as a substitute for human measurements. In a wide range of complex meals, now totaling 12 to 15, we have completed measurements in human subjects of nonheme iron absorption. We will attempt, in the coming months, to complete *in vitro* measurements of the bioavailability of these meals to determine the adequacy of *in vitro* measurements as a predictor of food iron absorption in human subjects. Several other ongoing projects relating to fortification of food vehicles are still in progress with Nestlé. One important study is the comparison of iron absorption in infants as measured by stable isotopes compared with iron absorption in adults as measured by radioisotopes at

ICCNA. This series of measurements are still in progress but it is unlikely that they will be completed because of funding constraints.

APPENDIX A

Annu. Rev. Med. 1993. 44:63–74
Copyright © 1993 by Annual Reviews Inc. All rights reserved

SERUM TRANSFERRIN RECEPTOR

J. D. Cook, M.D., B. S. Skikne, M.D., and R. D. Baynes, M.D.

Division of Hematology, Department of Medicine, Kansas University Medical Center, Kansas City, Kansas 66160-7402

KEY WORDS: iron metabolism, erythropoiesis, anemia

ABSTRACT

The transferrin receptor plays a critical role in iron metabolism by precisely controlling the flow of transferrin iron into body cells. A soluble truncated form of the receptor can be detected in human serum using sensitive immunoassays, and the initial clinical experience with this new measurement indicates that it reflects the total body mass of tissue receptor. Serum receptor levels rise significantly with tissue iron deficiency and the heightened demand for iron associated with expansion of the erythroid marrow. The serum receptor provides a quantitative measure of functional iron deficiency and distinguishes the associated anemia from that of chronic disease. If iron deficiency is excluded, the serum receptor provides a quantitative measure of total erythropoiesis that is more sensitive and less invasive than bone marrow examination currently used to assess red cell precursor mass. Performed in conjunction with serum ferritin measurements, the serum receptor will be useful in establishing the true prevalence of iron deficiency anemia in population studies.

INTRODUCTION

The extracellular transport of iron within the body is accomplished by its binding to a specific and well-characterized carrier protein, transferrin. The structural and metal-binding properties of this glycoprotein have been known for many years, but it is only in the last decade that the mechanism

0066–4219/93/0401–0063$02.00

-30

for directing the flow of transferrin iron to cells in need has been fully defined. The movement of transferrin iron through the plasma compartment is controlled by the number of specific receptors on the surface of body cells. The highest concentration of transferrin receptor is in rapidly dividing cells, hemoglobin-synthesizing tissues, and the placenta. A reduction in cellular receptor prevents iron-replete tissues from receiving a harmful excess of iron.

Despite its critical role in regulating cellular iron supply, the transferrin receptor was of little clinical relevance until a group of investigators in Japan reported that small amounts of circulating receptor can be detected in human sera using a sensitive immunologic assay (1). There has since been a rapid growth in our awareness of the clinical value of serum receptor measurements for assessing iron status and clinical disorders of red cell production. This clinical experience is the focus of the present review.

CELLULAR TRANSFERRIN RECEPTOR

The physicochemical properties of human transferrin receptor and its role in cellular iron uptake have been the subject of several reviews (2–4). The primary structure of transferrin receptor has been deduced from the cloned gene (5). The receptor is comprised of two identical subunits, each with a molecular mass of 95,000 daltons. Each polypeptide subunit contains a transmembrane segment with 28 amino acid residues, an N-terminal cytoplasmic domain with 61 residues and a large C-terminal extracellular domain with 671 amino acids. The subunits are linked by disulfide bonds adjacent to the cell surface at cysteine 89 and 98. The genes for transferrin and its receptor are both located on chromosome 3 (6). Synthesis of transferrin receptor and the iron storage protein ferritin are reciprocally linked to cellular iron content (7). Untranslated regions of the mRNA for both proteins contain sequences termed the iron response element that interact with a cellular protein sensitive in its affinity to variations in cytosolic iron. Apart from the posttranscriptional regulation, transferrin receptor also appears to be transcriptionally regulated, at least in hemoglobin-synthesizing cells (8). Roughly one third of the total cellular content of receptor is located on the surface of the cell, and alterations in this distribution may be a further mechanism for regulating cellular iron uptake (9).

At physiological pH, the affinity of the transferrin receptor is highest for diferric transferrin, intermediate for monoferric transferrin, and least for the iron-free protein. This ensures that surface receptors are preferentially saturated with transferrin containing the needed iron. The complex is internalized by receptor-mediated endocytosis to form small non-

lysosomal vesicular structures termed endosomes. As the pH in these vesicles falls below 5.5, the affinity of transferrin is reduced and iron is transported to the cytosol by an undefined pathway. The apotransferrin remains bound to its receptor until the endosome is recycled to the cell surface, where it is released on return to physiological pH. Less than 10 minutes are required for one complete cycle. Other receptors, including those for epidermal growth factor and low density lipoprotein, share this pathway, but unlike transferrin, their ligands are released and degraded in lysosomes.

CIRCULATING TRANSFERRIN RECEPTOR

The first quantitative measurements of receptor in serum were performed with a two-site immunoradiometric assay (IRMA) established with the commercially available monoclonal antibodies, OKT9 and B3/25, produced against cell surface receptor (1, 10). Normal values of 251 ± 94 μg/liter (mean $\pm$ 1 SD) in 38 healthy males and 256 ± 99 μg/liter in 54 healthy females were reported. Subsequently, monoclonal antibody reagents developed against intact placental receptor were employed in an enzyme-linked immunosorbent assay (ELISA), which gave normal values nearly 20-fold higher of 5.63 ± 1.42 mg/liter in 82 normal male and female subjects (11). By interchanging the monoclonal antibodies in the two assays, we showed the disparity to result from a difference in the sensitivity of the IRMA to free receptor used as standard and the complexed receptor in serum. An ELISA was also developed for the serum receptor using rabbit polyclonal antisera developed against the placental receptor-transferrin complex and absorbed with transferrin (12). Normal values of 8279 ± 1261 μg/liter whole blood in 56 subjects (12) and 5000 ± 1100 μg/liter serum in 165 normal subjects have been reported (13). This polyclonal ELISA was standardized in relation to transferrin-receptor complex rather than the free receptor. At least some of the disparity between existing receptor assays could be eliminated by using a common standard.

The physicochemical nature of circulating receptor was recently defined. It was initially reported that the serum receptor is a dimeric fragment of intact receptor (10), but Huebers and coworkers later concluded that the circulating receptor is intact receptor (12). The molecular identity of serum receptor has now been established by purifying it from human serum using immunoaffinity chromatography with a monoclonal antibody (14). The serum receptor exhibits a molecular mass of 85,000 daltons on SDS-PAGE under both nonreducing and reducing conditions, whereas placental receptor displays values of 190,000 and 95,000 daltons, respectively. Amino-terminal amino acid sequence analysis revealed that residues 1–19 of the

serum receptor are identical to residues 101–119 of intact receptor, which indicates that the circulating receptor is a truncated form lacking the cytoplasmic and transmembrane domains of intact receptor.

Because the concentration of serum transferrin is a thousand times higher than the monomeric receptor fragments in serum, the latter must circulate bound to transferrin. This agrees with evidence that $95 \pm 3\%$ of receptor immunoreactivity is removed by precipitating antibodies against transferrin (12). It must be assumed that the receptor circulates as a complex of one transferrin and one receptor fragment, corresponding to a molecular mass of 165,000 daltons. It is unknown whether the affinity of the fragment, like the intact cellular receptor, varies with the iron content of transferrin.

The identification of circulating receptor was originally prompted by in vitro studies with maturing sheep reticulocytes showing that intact transferrin receptor is released in small vesicles termed exosomes in which the extracellular domain is positioned externally (15, 16). The relationship between the soluble fragment in serum and the particulate form of intact receptor is unclear, but exosomal receptor is not detected with the ELISA for serum receptor (17). Culture studies with K562 cells in log phase growth have shown that $<30\%$ of receptor is released to the supernatant as exosomes while the remainder is the same truncated fragment identified in human serum (17). Using antibodies against peptides in the intracellular and extracellular domain, we recently demonstrated that over 99% of serum receptor contains only the extracellular domain (Y. J. Shih et al, unpublished observations). However, in certain hemolytic states such as sickle cell anemia and autoimmune hemolytic anemia, up to 4% of the total circulating receptor is consistent with a particulate form based on measurement of the solubilized pellet obtained by ultracentrifugation. It remains to be determined whether serum receptor is derived directly from cellular receptor or generated before or after the release of exosomal particles from the cell.

CLINICAL ASSESSMENT OF IRON STATUS

In cell culture studies, a reduction in iron supply from the media is associated with a sharp increase in transferrin receptor synthesis (18–20). Thus, it is not surprising that the serum receptor is significantly elevated in patients with iron deficiency. In the original report, the serum receptor level was 2.8 times normal in 41 patients with iron deficiency anemia (21), very similar to the increase of 3.20 times normal reported later in 19 patients (11). A value of 4.1 times normal was reported using the polyclonal ELISA, the highest value being exceeded only in patients with thalassemia

(12). Thus, despite differences in the normal mean and range of different serum receptor assays, the relative increase in iron deficiency anemia reported by different laboratories is very similar.

The changes in serum receptor during iron deficiency have a different pattern than the alterations in serum ferritin. The serum receptor is nearly identical in normal men and women (11), whereas the serum ferritin shows a striking sex difference. Iron absorption in iron-replete normal subjects correlates closely with serum ferritin levels but not with serum receptor (22). The degree of iron deficiency required to produce an elevation in serum receptor levels has been established by repeatedly phlebotomizing normal subjects to induce mild iron deficiency (23). There is a rapid and proportional fall in serum ferritin during the phase of storage iron depletion but only a minimal change in serum receptor. With the onset of tissue iron deficiency, the serum receptor rises in direct proportion to the induced deficit in functional iron, increasing to a mean of about 10 mg/liter or roughly two-fold when the tissue iron deficit is 5 mg iron/kg body weight, the equivalent of one to two units of blood. The enhanced erythropoiesis associated with repeated phlebotomies in this study had a minimal effect on serum receptor levels as compared to the rise associated with iron deficiency. A comparison of these results with the three- to four-fold elevation in patients with advanced iron deficiency anemia indicates that the level of serum transferrin receptor changes in proportion to the degree of iron deficiency over a wide range. In the phlebotomy study, other iron status measurements including erythrocyte protoporphyrin, mean red cell volume, and variation in red cell diameter changed more slowly and less dramatically than the serum receptor.

The importance of recognizing iron deficiency anemia in pregnant women has been highlighted by recent studies showing that it is associated with an increased rate of premature delivery and low birth weight (24, 25). Most of the existing laboratory measurements to identify iron deficiency are less reliable in pregnancy. Anemia due to iron deficiency is difficult to distinguish from the dilutional anemia due to an increase in plasma volume during the second trimester. The serum ferritin falls dramatically as iron stores of the mother are mobilized and is therefore a less reliable index of iron deficiency. Red cell measurements such as the mean corpuscular volume (MCV) or erythrocyte protoporphyrin may change too slowly to detect iron deficiency that develops during the latter half of pregnancy.

The ability to recognize iron deficiency during pregnancy can be improved with serum receptor measurements. It was initially reported that immunoreactive receptor begins to rise after 20 weeks of gestation to a level of about twice normal at the time of delivery (26). The increase was attributed to the high concentration of transferrin receptor in the

syncytiotrophoblast of the placenta. In a subsequent study in 176 women in third trimester pregnancy, the frequency distribution of serum receptor was nearly identical to that observed in normal controls, although there was a slight rise in serum receptor from 5.36 ± 2.67 mg/liter at 28–32 weeks to 6.21 ± 2.14 mg/liter during the last month of pregnancy (27). A high serum receptor was observed only in women with storage iron depletion as defined by a serum ferritin concentration of < 16 μg/liter, which indicates that the elevation in serum receptor is due to iron deficiency. While iron deficiency anemia was relatively uncommon in this sample, 11 of 13 women in whom this diagnosis was based on multiple laboratory criteria had elevated serum receptor levels. Because the serum receptor may change more rapidly than other red cell measurements of iron deficiency, it will be useful for detecting iron deficiency in pregnancy and monitoring the response to iron therapy.

One of the difficult problems in identifying iron deficiency anemia in a clinical setting is in distinguishing it from the hypoproliferative anemia that accompanies infection, inflammation, or malignancy, termed the anemia of chronic disease. Most of the available laboratory measurements of iron status, including the serum iron, red cell size heterogeneity, mean corpuscular volume, and erythrocyte protoporphyrin, do not distinguish these common causes of iron-deficient erythropoiesis from true iron deficiency (28). The distinction can be made if the serum ferritin is reduced, but many clinical disorders, including liver disease, infection, and alcohol abuse, elevate the serum ferritin independently of iron status. Consequently, a bone marrow examination for stainable iron is often needed to distinguish iron deficiency from the anemia of chronic disease.

Our initial experience indicates that the serum receptor will be of major value in separating these two common causes of anemia (29). In 17 patients with iron deficiency anemia, the serum receptor was increased to 13.9 ± 4.6 mg/liter and all but two patients had values above the upper normal limit of 8.5 mg/liter. In 15 patients with acute bacterial infection and 5 with acute viral hepatitis, the serum receptor was invariably within the normal range. In 41 patients with the anemia of chronic disease, the serum receptor averaged 5.65 ± 1.91 mg/liter, nearly identical to the mean of 5.36 ± 0.82 mg/liter in 17 normal individuals. Four of the patients with the anemia of chronic disease had a slight elevation in serum receptor but it remained lower than in all but four patients with iron deficiency anemia. Because the serum receptor increased with the severity of the anemia in patients with iron deficiency but not in those with chronic disease, there was complete separation of the two groups when the reduction in hemoglobin concentration from normal was greater than 25 g/liter. Only two of ten patients with chronic liver disease had a minimal elevation in serum recep-

tor, whereas the serum ferritin was elevated in all. Although this study did not assess serum receptor in patients with combined iron deficiency and chronic inflammation, it is likely that the serum receptor will rise only when iron deficiency is the major component. These studies indicate that the serum receptor rather than bone marrow examination can be used to identify iron deficiency anemia.

CLINICAL ASSESSMENT OF ERYTHROPOIESIS

The contribution of different body tissues to the circulating receptor level has not been determined in humans, although it is known from ferrokinetic studies that over two thirds of circulating transferrin iron is directed to developing red cells. This is in reasonable agreement with a decrease of 55 to 60% in serum receptor levels reported in patients with aplastic anemia (11, 12) and following marrow ablation for a bone marrow transplant (11, 30). Because all body cells contain transferrin receptor, it can be assumed that roughly one third of circulating receptor reflects production from nonerythroid tissues. While this limits the use of the serum receptor in the detection of bone marrow failure, elevated serum receptor levels provide a valuable measure of enhanced erythropoiesis.

An elevation in serum receptor in patients with hemolytic anemia has been described in several reports. An average increase of 3–5 times normal was reported in patients with autoimmune hemolytic anemia (12, 21), and a similar four-fold rise was noted in patients with hereditary spherocytosis (12). We have observed a six-fold increase to 18.0 mg/liter in 11 patients with sickle cell anemia, a finding consistent with their more severe degree of lifelong hemolysis (11). Even higher elevations have been reported in patients with ineffective erythropoiesis. In 72 patients with β-thalassemia/hemoglobin E disease, the mean circulating receptor was increased more than eight-fold over normal (12). These workers obtained evidence that the elevation in circulating receptor is directly proportional to the increase in erythroid precursor mass by comparison with the erythron transferrin uptake, a ferrokinetic measure of total erythropoiesis. A high correlation was observed with the circulating receptor level in 148 patients who had a wide range of hematological disorders, indicating that the relative increase in serum receptor can be used as a quantitative measure of total erythropoiesis.

Because the cellular receptor density is increased in rapidly proliferating cells, the possibility that serum receptor levels are elevated in hematologic malignancies independently of erythropoiesis was examined (30). A significant two- to three-fold increase was observed in patients with myeloproliferative disorders, whereas no elevation was found in patients with

chronic granulocytic leukemia or acute nonlymphocytic leukemia. The mean value in 12 patients with myelodysplastic syndrome was not significantly higher than normal, although seven of these patients had an elevated level. Of the lymphoproliferative disorders, normal values were observed in most patients with lymphoma, multiple myeloma, and hairy cell leukemia, but a significant rise to 14.17 mg/liter in 19 patients with chronic lymphocytic leukemia was observed: levels were elevated in eight of the 19 patients. Higher levels were observed in patients with more advanced disease, with splenomegaly, and with higher lymphocyte counts. Thus, of the various hematologic malignancies, only in patients with chronic lymphocytic leukemia is the level increased independently of the erythroid precursor mass.

One of the more durable classifications of anemia for clinical purposes is that based on pathophysiology and derived originally from ferrokinetic measurements (31, 32). The three major categories are hypoproliferative in which an appropriate erythroid marrow response to anemia has failed to occur, maturation disorders characterized by ineffective erythropoiesis, and hemolytic anemia with enhanced effective red cell production. Because of the cost and time required to perform ferrokinetic measurements, their use cannot be justified for routine clinical purposes. Consequently, the pathophysiological classification of anemia is based clinically on the reticulocyte index as a measure of effective erythropoiesis and erythroid cellularity on bone marrow examination as a measure of total erythropoiesis. One of the important applications of the serum receptor is to provide a less invasive and more reliable quantitative guide to total erythropoiesis than presently obtained by bone marrow examination. Because serum receptor is increased in iron deficiency independently of total erythroid precursor mass, it is useful to alter the classification of anemia by placing iron deficiency anemia in a separate category (Table 1). The changes in serum ferritin, serum receptor, and reticulocyte index provide a clear distinction between the four major categories of anemia. Iron deficiency is recognized by a low serum ferritin, an elevated serum receptor, and a normal reticulocyte index. Hypoproliferative anemia is identified by a reciprocal change in serum ferritin and receptor. Maturation abnormalities are recognized by a high serum ferritin and serum receptor with a normal reticulocyte index, while hemolytic disorders are associated with an elevation in all three laboratory indices.

The serum receptor will also be valuable in assessing changes in erythropoiesis over time within the same patient and in evaluating erythropoiesis when conventional assessment by marrow examination is precluded. For example, in patients with myelofibrosis, a two-fold rise in serum receptor has been reported, which indicates that this measurement can be used to

31

Table 1 Laboratory assessment of anemia

	Serum ferritin	Serum receptor	Reticulocyte index
Iron deficiency	low	high	normal
Hypoproliferative	high[a]	normal	normal
Maturation disorders	high[a]	high	normal
Hemolytic	high[a]	high	high

[a] Elevated above patient's baseline value but may remain below the upper cutoff level of 300 μg/liter.

assess the degree of extramedullary hematopoiesis (12). Furthermore, when reliable estimates of erythropoiesis cannot be obtained by marrow examination, as in patients who have received extensive radiation for the treatment of a malignancy, the serum receptor provides a reliable index. The serum receptor can also be used to assess the response to recombinant erythropoietin, especially in patients with chronic renal disease (B. S. Skikne et al, unpublished observations). Finally, by comparing serum erythropoietin levels with the serum receptor, it should be possible to assess the appropriateness of the erythroid response to a given level of erythropoietic stimulation in various hematological disorders.

EPIDEMIOLOGICAL ASSESSMENT OF ANEMIA

It is presently estimated that 25% of the world population is anemic (33). Iron deficiency is believed to be the primary cause of this anemia, but it is seldom identified in population surveys by specific laboratory testing. Depending on the particular geographic region, hemoglobinopathies such as thalassemia, chronic infections such as malaria, and inadequate intake of dietary protein or folic acid all contribute to the anemia seen in population surveys. Because of mounting concern that an excess of body iron may be harmful, it is also important to obtain precise estimates of the prevalence of iron deficiency in relatively iron-replete populations and especially in countries where iron fortification is legally enforced.

Performing measurements of serum ferritin and serum receptor in tandem offers a major advantage in population studies of anemia and iron status. In healthy subjects, these measurements provide a precise quantitative measure of body iron over a broad range of iron status, the serum ferritin indicating the level of storage iron and the serum receptor indicating the degree of tissue iron deficiency. It has been shown that the ferritin/receptor ratio can be used to estimate body iron in each sampled individual (23). In contrast to existing laboratory iron measurements, most

of which require a venous blood sample, the serum ferritin and receptor require only a few microliters of blood that can be readily obtained by capillary sampling. In field surveys conducted in remote geographic areas, a spot of blood or serum can be placed on a filter paper to facilitate storage and transport of specimens (J. D. Cook, unpublished observations). Since only the immunological reagents differ in these two assays, their laboratory measurement is efficient when performed together.

In developing countries, simultaneous measurements of serum ferritin and receptor provide a means of distinguishing iron deficiency anemia from other causes of anemia. Microcytic hypochromic anemia due to thalassemia, which is often confused with iron deficiency, can be recognized by an elevation in both ferritin and receptor. A normal serum receptor serves to identify anemia due to chronic infection. The use of these measurements will also be of advantage in monitoring the impact of intervention programs to combat iron deficiency. In a recent study to determine the efficacy of a new form of iron supplement for pregnant women in Kingston, Jamaica, the serum receptor proved to be a more reliable index of hemopoietic response than other iron measurements including the serum iron, iron-binding capacity, erythrocyte protoporphyrin, MCV, or variation in red cell size (W. K. Simmons et al, unpublished observations). Combined serum ferritin and serum receptor measurements will assist in identifying the cause of the anemia detected in population surveys.

CONCLUSIONS

The serum transferrin receptor is a soluble truncated form of intact cellular receptor that can be detected in human serum by sensitive immunological methods. The serum receptor provides a valuable clinical index of tissue iron needs. Progressive tissue iron deficiency causes a proportional elevation in serum receptor that serves to distinguish true iron deficiency from the anemia of chronic disease. Progressive expansion of erythroid precursors causes a similar elevation that provides a reliable quantitative measure of total erythropoiesis. The measurement of serum transferrin receptor is an important new laboratory tool for evaluating the anemic patient.

ACKNOWLEDGMENTS

This work was supported by NIH grant DK39246 and AID Cooperative Agreement DAN-5115-A-00-7908-00.

Literature Cited

1. Kohgo, Y., Nishisato, T., Kondo, H., Tsushima, N., Niitsu, Y., Urushizaki, I. 1986. Circulating transferrin receptor in human serum. *Br. J. Haematol.* 64: 277–81
2. Huebers, H. A., Finch, C. A. 1987. The physiology of transferrin and transferrin receptors. *Physiol. Rev.* 67: 520–82
3. Trowbridge, I. S. 1988. Transferrin receptor as a potential therapeutic target. *Prog. Allergy* 45: 121–46
4. Ward, J. H. 1987. The structure, function, and regulation of transferrin receptors. *Invest. Radiol.* 22: 74–83
5. Schneider, C., Owen, M. J., Banville, D., Williams, J. G. 1984. Primary structure of human transferrin receptor deduced from the mRNA sequence. *Nature* 311: 675–78
6. Miller, Y. E., Jones, C., Scoggin, C., Morse, H., Seligman, P. 1983. Chromosome 3q(22-ter) encodes the human transferrin receptor. *Am. J. Hum. Genet.* 35: 573–83
7. Cazzola, M., Bergamaschi, G., Dezza, L., Arosio, P. 1990. Manipulations of cellular iron metabolism for modulating normal and malignant cell proliferation: achievements and prospects. *Blood* 75: 1903–19
8. Chan, L.-N. L., Gerhardt, E. M. 1992. Transferrin receptor gene is hyperexpressed and transcriptionally regulated in differentiating erythroid cells. *J. Biol. Chem.* 267: 8254–59
9. Bomford, A. B., Munro, H. N. 1985. Transferrin and its receptor: their roles in cell function. *Hepatology* 5: 870
10. Kohgo, Y., Niitsu, Y., Nishisato, T., Kato, J., Kondo, H., et al. 1988. Quantitation and characterization of serum transferrin receptor in patients with anemias and polycythemias. *Jpn. J. Med.* 27: 64–70
11. Flowers, C. H., Skikne, B. S., Covell, A. M., Cook, J. D. 1989. The clinical measurement of serum transferrin receptor. *J. Lab. Clin. Med.* 114: 368–77
12. Huebers, H. A., Beguin, Y., Pootrakul, P., Einspahr, D., Finch, C. A. 1990. Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood* 75: 102–7
13. Cazzola, M., Beguin, Y. 1992. New tools for clinical evaluation of erythron function in man. *Br. J. Haematol.* 80: 278–84
14. Shih, Y. J., Baynes, R. D., Hudson, B. G., Flowers, C. H., Skikne, B. S., Cook, J. D. 1990. Serum transferrin is a truncated form of tissue receptor. *J. Biol. Chem.* 265: 19077–81
15. Pan, B. T., Johnstone, R. M. 1983. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell* 33: 967–78
16. Pan, B. T., Teng, K., Wu, C., Adam, M., Johnstone, R. M. 1985. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *J. Cell. Biol.* 101: 942–48
17. Baynes, R. D., Shih, Y. J., Hudson, B. G., Cook, J. D. 1991. Characterization of transferrin receptor released by K562 erythroleukemia cells. *Proc. Soc. Exp. Biol. Med.* 197: 416–23
18. Baynes, R. D., Shih, Y. J., Cook, J. D. 1991. Production of soluble transferrin receptor by K562 erythroleukemia cells. *Br. J. Haematol.* 78: 450–55
19. Bridges, K. R., Cudkowicz, A. 1984. Effect of iron chelators on the transferrin receptor in K562 cells. *J. Biol. Chem.* 259: 12970–77
20. Rao, K. K., Shapiro, D., Mattia, E., Bridges, K., Klausner, R. 1985. Effects of alterations in cellular iron on biosynthesis of the transferrin receptor in K562 cells. *Mol. Cell. Biol.* 5: 595–600
21. Kohgo, Y., Niitsu, Y., Kondo, H., Kato, J., Tsushima, N., et al. 1987. Serum transferrin receptor as a new index of erythropoiesis. *Blood* 70: 1955–58
22. Cook, J. D., Dassenko, S., Skikne, B. S. 1990. Serum transferrin receptor as an index of iron absorption. *Br. J. Haematol.* 75: 603–9
23. Skikne, B. S., Flowers, C. H., Cook, J. D. 1990. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. *Blood* 75: 1870–76
24. Lieberman, E., Ryan, K. J., Monson, R. R., Schoenbaum, S. C. 1988. Association of maternal hematocrit with premature labor. *Am. J. Obstet. Gynecol.* 159: 107–14
25. Scholl, T. O., Hediger, M. L., Fischer, R. L., Shearer, J. W. 1992. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. *Am. J. Clin. Nutr.* 55: 985–88
26. Kohgo, Y., Niitsu, Y., Nishisato, T., Kondo, H., Kato, J., et al. 1988. Immunoreactive transferrin receptor in sera of pregnant women. *Placenta* 9: 523–31
27. Carriaga, M. T., Skikne, B. S., Finley,

B., Cutler, B., Cook, J. D. 1991. Serum transferrin receptor for the detection of iron deficiency in pregnancy. *Am. J. Clin. Nutr.* 54: 1077–81

28. Cook, J. D. 1982. Clinical evaluation of iron deficiency. *Semin. Hematol.* 19: 6–18
29. Ferguson, B. J., Skikne, B. S., Simpson, K. M., Baynes, R. D., Cook, J. D. 1992. Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. *J. Lab. Clin. Med.* 119: 385–90
30. Klemow, D., Einsphar, D., Brown, T. A., Flowers, C. H., Skikne, B. S. 1990. Serum transferrin receptor measurements in hematologic malignancies. *Am. J. Hematol.* 34: 193–98
31. Bothwell, T. H., Charlton, R. W., Cook, J. D., Finch, C. A. 1979. *Iron Metabolism in Man.* Oxford: Blackwell Scientific. 576 pp.
32. Hillman, R. S., Finch, C. A. 1985. *Red Cell Manual.* Philadelphia: Davis. 5th ed.
33. DeMaeyer, E., Adiels-Tegman, M. 1985. The prevalence of anaemia in the world. *World Health Stat. Q.* 38: 302–16

EVALUATION OF A GASTRIC DELIVERY SYSTEM FOR IRON SUPPLEMENTATION IN PREGNANCY

William K. Simmons*, James D. Cook†, Keith C. Bingham*, Marjorie Thomas*, Jean Jackson*, Maria Jackson*, Namanjeet Ahluwalia†, Samuel G. Kahn‡, A. W. Patterson*

*Caribbean Food and Nutrition Institute, Kingston, Jamaica; †Division of Hematology, Department of Medicine, Kansas University Medical Center, Kansas City, Kansas, USA; ‡Office of Nutrition, USAID, Washington DC.

Address reprint requests to James D. Cook, M.D., Division of Hematology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160-7402, USA.

The field study, carried out by the Caribbean Food and Nutrition Institute, was supported by the International Center for Research on Women through AID Cooperative Agreement DAN-1010-A-00-7061-00. The work conducted at the International Center for the Control of Nutritional Anemia, Kansas University Medical Center, was supported by AID Cooperative Agreement DAN-5115-A-00-7908-00.

Running head: Iron Supplementation in Pregnancy

The GDS capsules were kindly supplied by F. Hoffman-La Roche Ltd., Basel, Switzerland.

ABSTRACT

The present investigation was undertaken to assess the efficacy of oral iron supplementation during pregnancy using a gastric delivery system (GDS). Three hundred and seventy-six pregnant women between 16-35 years of age and 14-22 weeks gestation were selected if mild anemia was present (hemoglobin concentration 80-110 g/L), The participants were randomized to one of three study groups given either no iron, two $FeSO_4$ tablets (100 mg iron) daily, or one GDS capsule (50 mg iron) daily. Blood was obtained initially and after 6 and 12 weeks for measurement of red blood cell and iron parameters including serum transferrin receptor. There was a significant and comparable improvement in hematologic and iron status measurements in the two groups of women given iron whereas iron deficiency evolved in women given no iron supplement. We conclude that by eliminating gastrointestinal side effects and reducing the administration frequency of an iron supplement to once daily, GDS offers significant advantages for iron supplementation of pregnant women.

Key Words: Iron deficiency; Pregnancy; Iron supplementation

INTRODUCTION

It is presently estimated that over a billion people in the world are anemic (1) and that the major cause of this anemia is a deficiency in the supply of dietary iron relative to the iron needs of a population. The population segment most vulnerable to this nutritional iron deficiency is pregnant women (2). The global prevalence of nutritional anemia during pregnancy exceeds 50% and although this figure is much higher in Third World countries, no region or society is exempt. Recent studies have highlighted the significant consequences of iron deficiency anemia during pregnancy. Mild anemia is associated with an increase in the rate of premature delivery and low birth weight (3-5) whereas more severe anemia is estimated to account for up to 20% of maternal deaths during parturition. In light of the dismal success in reducing the global prevalence of anemia in this highly vulnerable segment of the population, there is a need to develop novel methods to improve the iron status of pregnant women.

For the past several decades, the provision of oral iron supplements to pregnant women has been the primary method to decrease the prevalence of anemia. A commonly cited reason for the ineffectiveness of iron supplementation in developing countries is the reluctance of women to adhere to a regimen that is often associated with gastrointestinal side effects. One way of circumventing this problem is to supply iron in a slow release form while retaining it in the stomach proximal to the iron absorbing region of the small intestine, a formulation referred to as a gastric delivery system (GDS). Recent studies indicate that this preparation not only eliminates the

side effects of oral iron but also results in a more efficient assimilation of the nutrient (6). The present investigation was undertaken to evaluate the hematological efficacy of GDS in a field setting.

MATERIALS AND METHODS

The primary objective of this trial was to compare the efficacy of the officially recommended program for iron supplementation of pregnant women in Jamaica with a regimen employing GDS. Women were recruited from eight maternal and child-health (MCH) centers in Kingston, St. Andrews, and Spanish Town, Jamaica, West Indies. All pregnant women who agreed to participate in the investigation were assigned randomly to one of three study groups, a no iron group given one placebo tablet daily, a $FeSO_4$ group given the recommended program for iron supplementation in Jamaica consisting of two $FeSO_4$ tablets or 100 mg elemental iron daily, and one GDS capsule or 50 mg elemental iron daily. All participants were given 400 mg folic acid daily. This study was approved by the Ethics Committee at the University of the West Indies and conducted by personnel of the Caribbean Food and Nutrition Institute.

The selection criteria for participation in the investigation were a maternal age 16-35 years, gestational age 14-22 weeks, and hemoglobin concentration between 80-110 g/L as determined by the Hemocue system (DeCentech Inc., St. Paul, MN). The randomization procedure was as follows. Using a random number table, cards

designating the assignment to one of the three study groups were placed in sealed envelopes and distributed to one of the eight maternal and child-health (MCH) centers. In women who fulfilled the entry criteria and gave written, informed consent to participate in the trial, an additional 5 mL of blood was drawn into EDTA for further hematological and biochemical laboratory measurements. The envelope was then opened to determine the assigned study group. Women were given 16-18 days supply of tablets and were requested to return at two week intervals for the duration of the 12 week trial. It should be noted that this was not a double blind study because the placebo, $FeSO_4$ tablets, and GDS capsules differed in their appearance. However, the women were not told which of these preparations contained iron. All women were given a small travel allowance to defray the cost of returning to the prenatal clinic for their biweekly visit.

At each return visit during the 12 week trial, a questionnaire was administered to assess the number of supplements taken and recorded any associated side effects. The women were requested to return any unused tablets which were then counted to provide an independent measure of adherence to the supplementation program. Additional blood samples were obtained at the 6 and 12 week follow-up visit to monitor the hematological response. If the hemoglobin concentration at the 6 week follow-up visit fell below 70 g/L, the participant was removed from the study, given full therapeutic doses of oral iron, and referred to a physician for follow-up medical care.

At the conclusion of the 12 week trial, all participants were given sufficient iron tablets to continue iron supplementation until parturition.

Blood samples were processed on the same day that they were obtained for measurement of zinc protoporphyrin using an Aviv hematofluorometer (Aviv Biomedical Inc., Lakewood, NJ) and for electronic measurements of hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), red cell distribution width (RDW), and white blood count (WBC) using a model Coulter S Plus IV Counter (Coulter Electronics Inc., Hialeah, FL). Plasma was distributed in small iron-free plastic vials and stored at -20°C for later measurement of serum iron (7), total iron binding capacity (TIBC) (8), and enzyme-linked immunosorbent assays established with monoclonal antibodies for measurement of serum ferritin (9) and serum transferrin receptor (10) as described previously. The immunoassays were deferred until the conclusion of the trial to permit measurements on the baseline, 6 week, and 12 week samples in each individual to be performed in the same assay plate.

All samples were screened for a hemoglobinopathy using established methods (11). All participants found to have sickle cell disease or thalassemia were later rejected. These included all patients with SS, SC (2 subjects), α-chain or β-chain variants (3 subjects), hemoglobin Lepore (1 subject), and β-thalassemia (6 subjects). Of 363 women who were evaluated at the initial clinic visit, 305 (84%) had a normal (AA) hemoglobin phenotype, 47 (12.9%) had sickle cell trait (AS), and 11 (3.0%) had

hemoglobin C trait (AC). Subsequent analysis of hematological and iron measurements failed to reveal significant differences in women with sickle cell trait or hemoglobin C trait.

Statistical analyses involved repeated measures analysis of variance (ANOVA). Serum ferritin values were analyzed after logarithmic transformation (12). Significant group effects were followed by Scheffe's post-hoc test at 6 and 12 weeks. For laboratory measurements where the groups differed significantly at baseline, as determined by one way ANOVA, repeated measures analysis of covariance (ANCOVA) was carried out using the baseline value for the corresponding laboratory parameter as the covariate (13). Significant group effects were followed by testing the statistical significance of differences on adjusted means (values adjusted for the covariate).

RESULTS

The combined total of women initially registered in the trial was 376. Their mean age was 23 years and was virtually identical in the three study groups (Table 1). In the baseline sample, 31.9% of the women were below 20 years of age, and 10.4% were 30 years or older. Sixty-nine percent were not employed outside of the home and of the remainder, 9.7% were employed in domestic work, 8.1% in factory work, 5.9% as street vendors, and 7.3% in miscellaneous jobs. Fifty-nine percent of the women

completed primary education and 42% of the sample completed secondary education. The mean body weight at the time of entry to the study was 61.2 kg and was similar in the three study groups as was the average parity. The mean gestational age was the approximate mid-point of the second trimester and was five days later in the iron supplemented groups than in the group receiving no iron. None of the sample characteristics listed in Table 1 were significant when analyzed by analysis of variance.

There was a progressive reduction in all three study groups throughout the 12 week trial in the number of women who returned for their biweekly clinic visit. Twenty-eight women (7.5%) did not return for the initial two week follow-up and the initial participants were reduced to 85.5% at the midpoint of the investigation. Two-hundred and seventy-five women (73.1%) completed the trial with a relatively even distribution of the drop-outs among the three study groups ranging from 23% in the no iron group to 30% in the GDS group. These differences were not statistically significant when tested by chi-square ($p>0.10$). In order to permit comparison of the laboratory results within each subject at the initial, 6 week, and 12 week follow-up visits, a further 27 women were excluded because of incomplete laboratory information on one of these occasions. The final sample, for which all hematological and biochemical data were available, was comprised of 86 women in the no iron group, 79 in the $FeSO_4$ group, and 83 in the GDS group.

Adherence to the supplementation program exceeded 90% in all study groups during the 12 week trial as determined by tablet counts and questionnaires. The adherence rate did not differ significantly among the three study groups. Side effects were relatively common in the no iron group and included abdominal pain (22%), heartburn (16%), nausea (10%), constipation (8%), appetite loss (5%), vomiting (3%), and diarrhea (2%). The frequency of these side effects did not differ among the three study groups when tested by X^2 analysis indicating that they were related more to pregnancy than iron supplements. The symptoms diminished with time in all three study groups.

There were significant differences for certain laboratory measurements among the three study groups at the time of initial registration (Table 2). The hemoglobin concentration was significantly higher in the no iron group than in the GDS group ($p<0.001$) and the difference in hemoglobin concentration between the no iron and $FeSO_4$ group also approached statistical significance ($0.05<p<0.10$). Several of the other iron parameters also indicated that the iron status of the no iron group was slightly better than the GDS but not the $FeSO_4$ group. Thus, the MCV and transferrin saturation were both significantly higher and the TIBC and serum transferrin receptor significantly lower in the no iron group as compared with the GDS group. None of the hematological or biochemical laboratory measurements differed between the two groups given iron supplements.

One way ANOVA at baseline showed that the three groups differed significantly with respect to all variables examined except RDW and FEP (Table 2). Subsequent analyses using repeated measures ANOVA for RDW and FEP and repeated measure ANCOVA for the rest of the laboratory indices showed significant group X time interaction ($p<0.001$) for all variables except serum ferritin, serum iron, TIBC, and transferrin saturation.

The results for all laboratory measurements at 6 and 12 weeks, after adjusting for the inter-group differences at baseline for all measurements except RDW and FEP, are presented in Table 3 and 4, respectively. Table 3 clearly shows that the no iron group had impaired iron status at 6 weeks as compared to the groups receiving either GDS or $FeSO_4$, as reflected in significantly lower hemoglobin, MCV, serum iron, transferrin saturation, and ferritin values and significantly higher TIBC and serum transferrin receptor. It is noteworthy that none of the iron status measurements differed in the two groups receiving iron at 6 weeks.

A comparison of the laboratory data at the conclusion of the three month trial showed a further progression of iron deficiency in women receiving no iron (Table 4). The adjusted hemoglobin concentration in the no iron group was more than 10 g/L lower than in the iron-supplemented groups ($p<0.001$) reflecting a further fall as compared to the 6 week examination. The unadjusted values for hemoglobin concentration were very similar to the values adjusted for the initial group differences (Figure 1). A similar

degree of difference was observed with the majority of the remaining laboratory parameters including significantly lower values for the MCV, serum ferritin, serum iron, and transferrin saturation and significantly higher values for the FEP, TIBC, and serum transferrin receptor (Figure 2). Only the RDW showed no significant differences between the three treatment groups at 6 and 12 weeks. The two groups receiving iron were similar at 12 weeks except for serum ferritin ($p=0.039$), serum iron ($p=0.030$), and transferrin saturation ($p=0.017$) which were higher in the women given $FeSO_4$.

DISCUSSION

The measures presently available for reducing the prevalence of iron deficiency anemia during pregnancy are limited both in number and effectiveness. Improving the diet with respect to either the total iron content or, more importantly, the bioavailability of food iron should improve the iron status of a population over time and thereby influence the iron status of women entering pregnancy. However, the duration of gestation is too short and the benefit of modifying the diet too limited to have a meaningful impact on the evolution of iron deficiency anemia during pregnancy. The provision of a highly fortified food supplement to pregnant women offers more promise but the inhibiting effect of food on iron absorption remains an important constraint when attempting to modify iron balance in a limited time period. These considerations have led to a focus in the past several decades on the provision of a medicinal iron

supplement to pregnant women as the only reasonable intervention. Even in the US where the prevalence of gestational iron deficiency is lower than in most regions of the world, it is recommended that women consume a supplement containing at least 30 mg elemental iron daily separate from meals during the second and third trimesters to diminish the risk of developing iron deficiency anemia (14).

The efficacy of iron supplementation in pregnancy has been repeatedly demonstrated in controlled trials both in highly industrialized and lesser developed regions of the world. At the same time, it is widely believed that when undertaken as a public health measure, iron supplementation has had little impact on the prevalence of anemia in pregnant women. Of various reasons that have been cited to explain the marginal effectiveness of iron supplementation at the population level, limited coverage of national primary health care systems is considered important in most Third World countries (15). Problems in the procurement and distribution of iron tablets restrict the supply to pregnant women and even when tablets are available at MCH centers, primary care workers often fail to instruct or encourage women to take their supplement regularly.

The extent to which the major gastrointestinal side effects of medicinal iron such as nausea, vomiting, and epigastric pain, account for the diminished effectiveness of iron supplementation in pregnancy is unclear. However, these side effects are a well known obstacle in conducting field trials in which the motivation of those administering

the supplements is high. When problems in the procurement and distribution of iron tablets are eliminated, it is highly likely that constraints imposed by the side effects of oral iron will again emerge as the major difficulty with iron supplementation.

The GDS employed in the present trial has the major advantage that upper gastrointestinal side effects are eliminated (6). Although delayed release forms of iron have been provided by pharmaceutical companies for several decades, their ineffectiveness is well recognized by medical practitioners because the release of iron in the intestinal tract is delayed until the tablet is beyond the maximal absorbing area in the proximal small bowel. With the GDS, $FeSO_4$ is incorporated into a hydrocolloid matrix that becomes buoyant on exposure to gastric secretions and is thereby retained for prolonged periods in the acidic environment of the stomach where iron solubility is favored. All excipients in the GDS are GRAS (Generally Recognized As Safe) substances (6) and are presently used in other pharmaceutical preparations in the United States. Upper gastrointestinal symptoms are eliminated because the iron is released slowly over a period of hours. Of equal importance is the fact that iron absorption in this form may be 2- to 4-fold higher than absorption of $FeSO_4$ when given with food, presumably because the GDS retains iron in the stomach until the bulk of the meal and its absorption inhibitors has emptied. The present trial was undertaken in part because of this absorptive advantage. We reasoned that because of the higher absorption of iron in GDS, a single capsule daily containing 50 mg elemental iron would be equivalent to a standard $FeSO_4$ tablet taken twice daily.

In the present study, a progressive deterioration in iron status occurred in women who received no iron supplements as compared to those given iron whereas the response in the women given $FeSO_4$ or GDS was similar at the 6 week and 12 week examination. When the change in hemoglobin concentration within each subject between baseline and 12 weeks was compared, a fall in mean hemoglobin of 5.7 ± 2.4 g/L in those given no iron was seen as compared with mean increases of 6.6 ± 2.8 and 9.4 ± 2.8 g/L, in women given $FeSO_4$ and GDS, respectively (Figure 3). The changes in serum transferrin receptor within each subject at 12 weeks were similarly dramatic (Figure 4). The transferrin receptor increased by an average of 3.27 ± 0.73 mg/L in the no iron group as compared with slight declines of 0.49 ± 0.68 and 0.30 ± 0.68 in women given $FeSO_4$ and GDS, respectively. The only differences seen between the supplemented groups were observed at 12 weeks when the serum iron, transferrin saturation, and serum ferritin showed a modest difference in the direction of better iron status in those given $FeSO_4$. The differences in serum iron and transferrin saturation might be explained by the fact that these women were not instructed to omit their iron supplement on the day of their clinic visit. A prompt elevation in serum iron occurs following $FeSO_4$ but the rise with GDS occurs more slowly (Skikne et al., unpublished observations) which could explain higher serum iron and transferrin saturation values in the $FeSO_4$ group. However, the difference in serum ferritin cannot be explained on this basis and is consistent with slightly higher iron stores in women given twice the amount of iron as $FeSO_4$.

It remains to be established that GDS can significantly improve the adherence to an iron supplementation program but there are several reasons to believe that it will. A single GDS tablet daily is as effective as two $FeSO_4$ tablets and compliance is likely to be improved by reducing the administration frequency. Another major advantage is that the troublesome upper gastrointestinal side effects are eliminated with GDS but persist in a substantial portion of women taking a conventional $FeSO_4$ tablet. The present investigation was not designed optimally to assess compliance because it was not possible to obtain the placebo, $FeSO_4$ tablets, and GDS in an indistinguishable form. Even if this had been possible, differences would have existed in the frequency of tablet administration in the two iron supplemented groups. Our evaluation of compliance and side effects do indicate an advantage of the GDS tablets (Simmons et al., unpublished observations) but there is a need for further field studies performed in a double blind fashion.

ACKNOWLEDGEMENTS

The authors wish to thank the clinic nurses and field workers who so willingly collaborated in assisting in the clinics. We also wish to thank Graham Serjeant and his staff at the Sickle Cell Unit, Medical Research Council for their help in laboratory analysis and Leslie Kuharich for her assistance in the preparation of this manuscript.

REFERENCES

1. DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. Wld Hlth Statist Quart 1985;38:302-316.

2. Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron Metabolism in Man. Oxford: Blackwell Scientific, 1979.

3. Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1986;1:992-994.

4. Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Association of maternal hematocrit with premature labor. Am J Obstet Gynecol 1988;159:107-114.

5. Scholl TO, Hediger ML, Fischer RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr 1992;55:985-988.

6. Cook JD, Carriaga M, Kahn SG, Schalch W, Skikne BS. Gastric delivery system for iron supplementation. Lancet 1990;335:1136-1139.

7. International Committee for Standardization in Haematology . Recommendations for measurement of serum iron in human blood. Br J Haematol 1978;38:291-294.

8. Cook JD. An evaluation of adsorption methods for measurement of plasma iron-binding capacity. J Lab Clin Med 1970;76:497-506.

9. Flowers CA, Kuizon M, Beard JL, Skikne BS, Covell AM, Cook JD. A serum ferritin assay for prevalence studies of iron deficiency. Am J Hematol 1986;23:141-151.

10. Flowers CH, Skikne BS, Covell AM, Cook JD. The clinical measurement of serum transferrin receptor. J Lab Clin Med 1989;114:368-377.

11. Morris J, Dunn D, Beckford M, et al. The haematology of homozygous sickle cell disease after the age of 40 years. Br J Haematol 1991;77:382-385.

12. Lipschitz DA, Cook JD, Finch CA. A clinical evaluation of serum ferritin as an index of iron stores. N Engl J Med 1974;290:1213-1216.

13. SAS User's Guide: Basics, Version 5. Cary, NC: SAS Institute Inc., 1985.

14. Guidelines for the Assessment and Management of Iron Deficiency in Women of Childbearing Age. Bethesda, MD: Life Sciences Research Office, FASEB, 1991.

15. Controlling Iron Deficiency - A Report based on a ACC/SCN Workshop. Suffolk, England: Lavenham Press Ltd., 1991.

Table 1. Characteristics of study groups at baseline

	No Iron	$FeSO_4$	GDS
Sample Size			
Baseline	121	123	132
6 weeks (% of baseline)	102 (83)	105 (85)	115 (87)
12 weeks (% of baseline)	93 (77)	89 (72)	93 (70)
Age (years)*	23.3±5.0	23.4±4.4	23.0±4.9
Weight (kg)	61.1±11.7	62.3±13.4	60.4±8.7
Parity	1.10±1.24	1.20±1.37	1.27±1.41
Gestational age (days)	135±17	140±22	141±18

* Mean±1SD

Table 2. Laboratory parameters at baseline

Laboratory Measurement	No Iron (N)	$FeSO_4$ (F)	GDS (G)	Intergroup Comparisons (p values) N:F	N:G	G:F
N	86	79	83			
Hemoglobin (g/l)*	105.0±2.0	101.4±2.2	98.9±2.4	0.073	<0.001	ns‡
MCV (fl)	88.1±1.6	87.0±1.6	85.1±1.9	ns	0.036	ns
RDW (%)	14.0±0.3	14.0±0.3	14.5±0.4	ns	ns	ns
FEP (µmol/L RBC)	64.5±5.3	68.2±8.0	75.7±9.3	ns	ns	ns
Serum ferritin (µg/L)†	13.9 (11.2-17.2)	10.7 (8.4-13.5)	9.4 (7.4-11.9)	ns	0.051	ns
Serum iron (µg/100 mL)	83.1±7.1	77.5±8.6	69.6±7.6	ns	0.051	ns
TIBC (µg/100 mL)	416±15	435±17	453±17	ns	0.006	ns
Saturation (%)	20.8±2.1	19.0±2.4	16.3±2.1	ns	0.017	ns
Transferrin receptor (mg/L)	7.24±0.54	7.81±0.83	8.63±0.83	ns	0.028	ns

* Mean±2SE
† Geometric mean (±2SE)
‡ Not significant (p>0.05)

Table 3. Laboratory parameters at 6 week follow-up

Laboratory Measurement	No Iron (N)	$FeSO_4$ (F)	GDS (G)	Intergroup Comparisons (p values) N:F	N:G	G:F
N	86	79	83			
Hemoglobin (g/l)*	99.1±1.9	105.2±2.0	104.5±1.9	<0.001	<0.001	ns‡
MCV (fl)	86.0±0.8	89.0±0.8	88.6±0.8	<0.001	<0.001	ns
RDW (%)	13.8±0.38	15.1±0.8	15.4±0.8	0.027	0.003	ns
FEP (µmol/L RBC)	68.9±6.5	62.7±6.1	69.4±8.9	ns	ns	ns
Serum ferritin (µg/L)†	7.4 (6.4-11.5)	17.0 (14.7-19.8)	14.7 (12.7-17.0)	<0.001	<0.001	ns
Serum iron (µg/100 mL)	64.2±8.6	94.3±8.9	94.1±8.6	<0.001	<0.001	ns
TIBC (µg/100 mL)	480±12	432±12	443±12	<0.001	<0.001	ns
Saturation (%)	13.6±2.0	22.3±2.0	22.0±2.0	<0.001	<0.001	ns
Transferrin receptor (mg/L)	9.19±0.44	7.56±0.46	7.86±0.46	<0.001	<0.001	ns

* Mean±2SE
† Geometric mean (±2SE)
‡ Not significant (p>0.05)

Table 4. Laboratory parameters at 12 week follow-up

Laboratory Measurement	No Iron (N)	$FeSO_4$ (F)	GDS (G)	Intergroup Comparisons (p values) N:F	N:G	G:F
N	86	79	83			
Hemoglobin (g/l)*	97.8±2.3	108.2±2.4	109.6±2.3	<0.001	<0.001	ns‡
MCV (fl)	83.5±1.0	88.7±1.1	88.5±1.1	<0.001	<0.001	ns
RDW (%)	14.2±3.9	14.6±5.3	15.0±5.9	ns	ns	ns
FEP (µmol/L RBC)	91.4±9.3	64.5±5.4	74.7±7.2	<0.001	0.007	ns
Serum ferritin (µg/L)†	8.2 (6.9-9.6)	18.9 (15.9-22.5)	14.8 (12.5-17.5)	<0.001	<0.001	0.039
Serum iron (µg/100 mL)	62.9±9.3	100.2±9.6	85.6±9.3	<0.001	<0.001	0.030
TIBC (µg/100 mL)	502±14	437±14	451±14	<0.001	<0.001	ns
Saturation (%)	13.4±2.4	23.6±2.4	19.6±2.4	<0.001	<0.001	0.017
Transferrin receptor (mg/L)	10.89±0.64	7.38±0.66	7.85±0.64	<0.001	<0.001	ns

* Mean±2SE
† Geometric mean (±2SE)
‡ Not significant (p>0.05)

LEGENDS

Figure 1. Mean hemoglobin concentration at baseline, 6 weeks, and 12 week follow-up in groups receiving no iron (O), 100 mg iron as $FeSO_4$ daily (●), or 50 mg iron as GDS daily (□). The vertical bars represent ±2SE.

Figure 2. Mean serum transferrin receptor concentration at baseline, 6 weeks, and 12 week follow-up in groups receiving no iron (O), 100 mg iron as $FeSO_4$ (●), or 50 mg iron as GDS daily (□). The vertical bars represent ±2SE.

Figure 3. Change in hemoglobin concentration within each subject between baseline and 12 week follow-up in groups receiving no iron, 100 mg iron as $FeSO_4$, or 50 mg iron as GDS daily.

Figure 4. Change in serum transferrin receptor concentration within each subject between baseline and 12 week follow-up in groups receiving no iron, 100 mg iron as $FeSO_4$, or 50 mg iron as GDS daily.

Figure 1

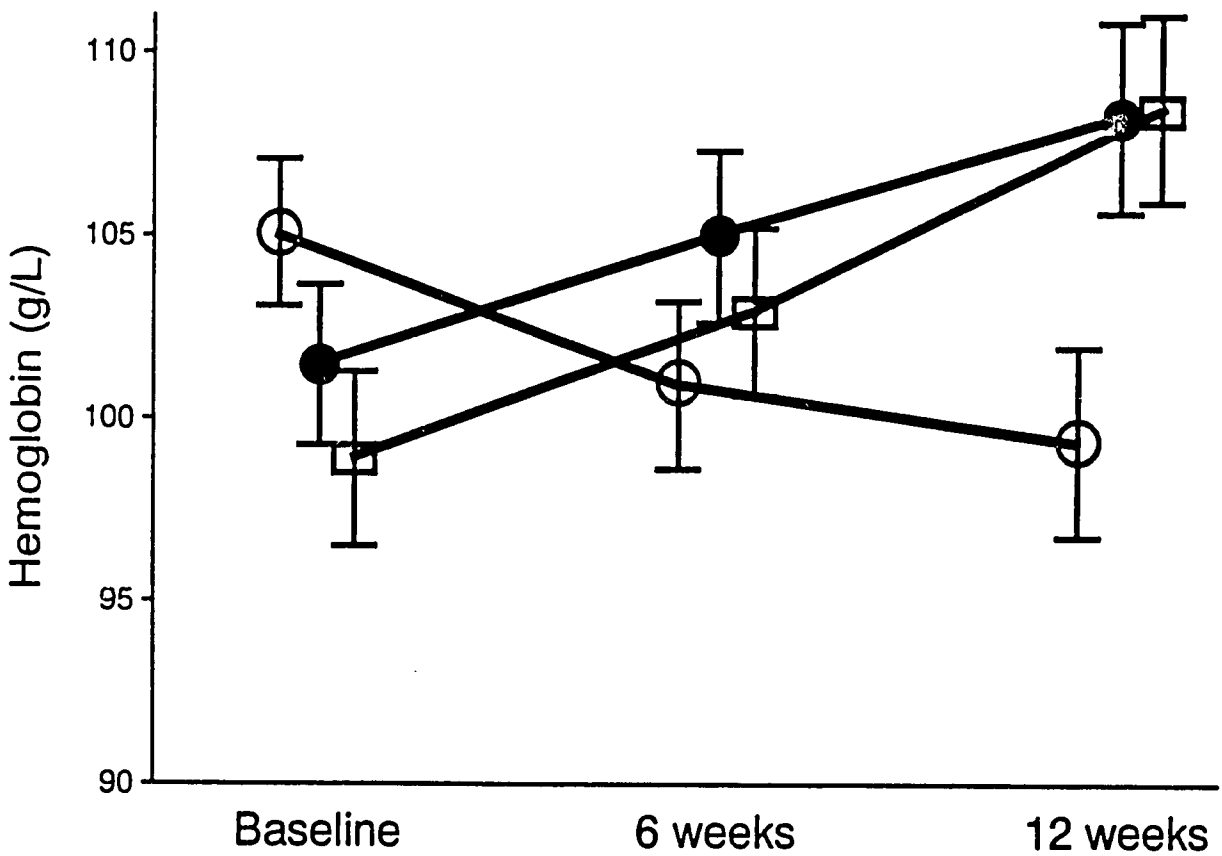


Figure 2

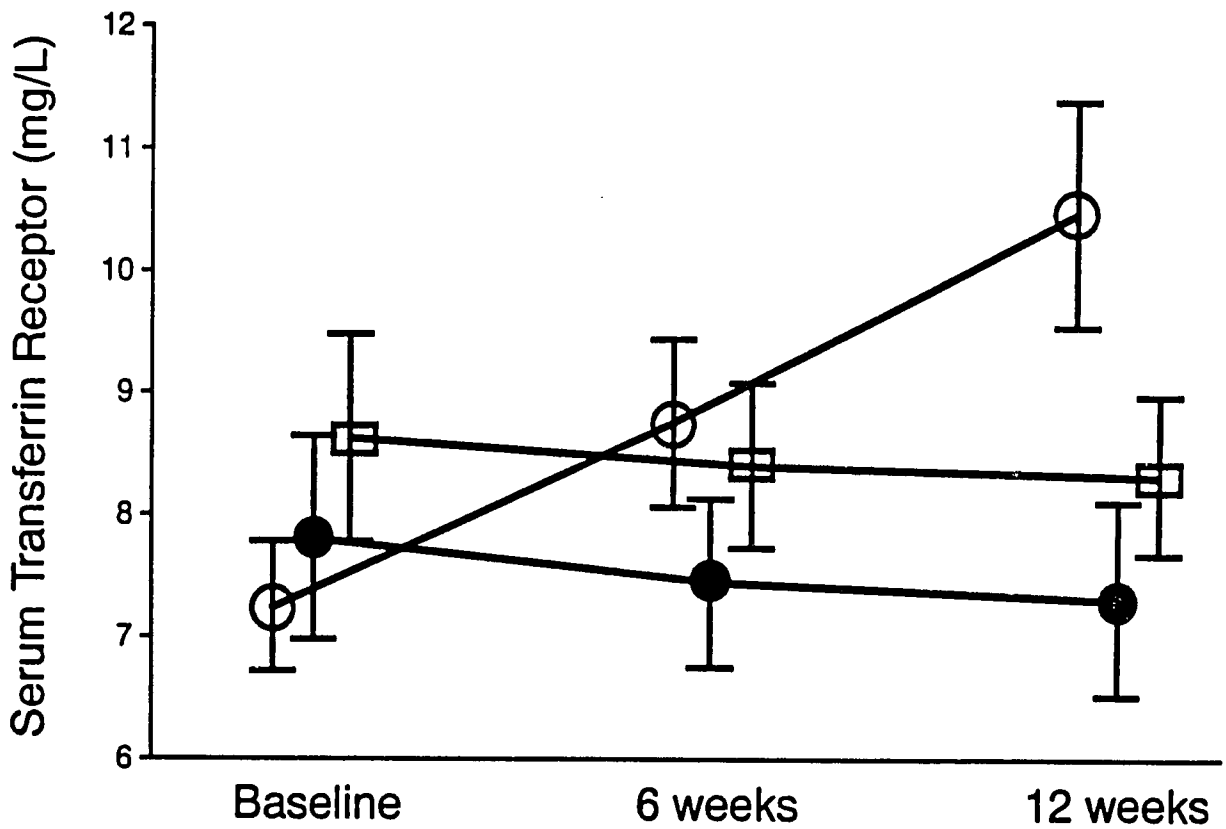

Serum Transferrin Receptor (mg/L)
12
11
10
9
8
7
6
Baseline
6 weeks
12 weeks

Figure 3

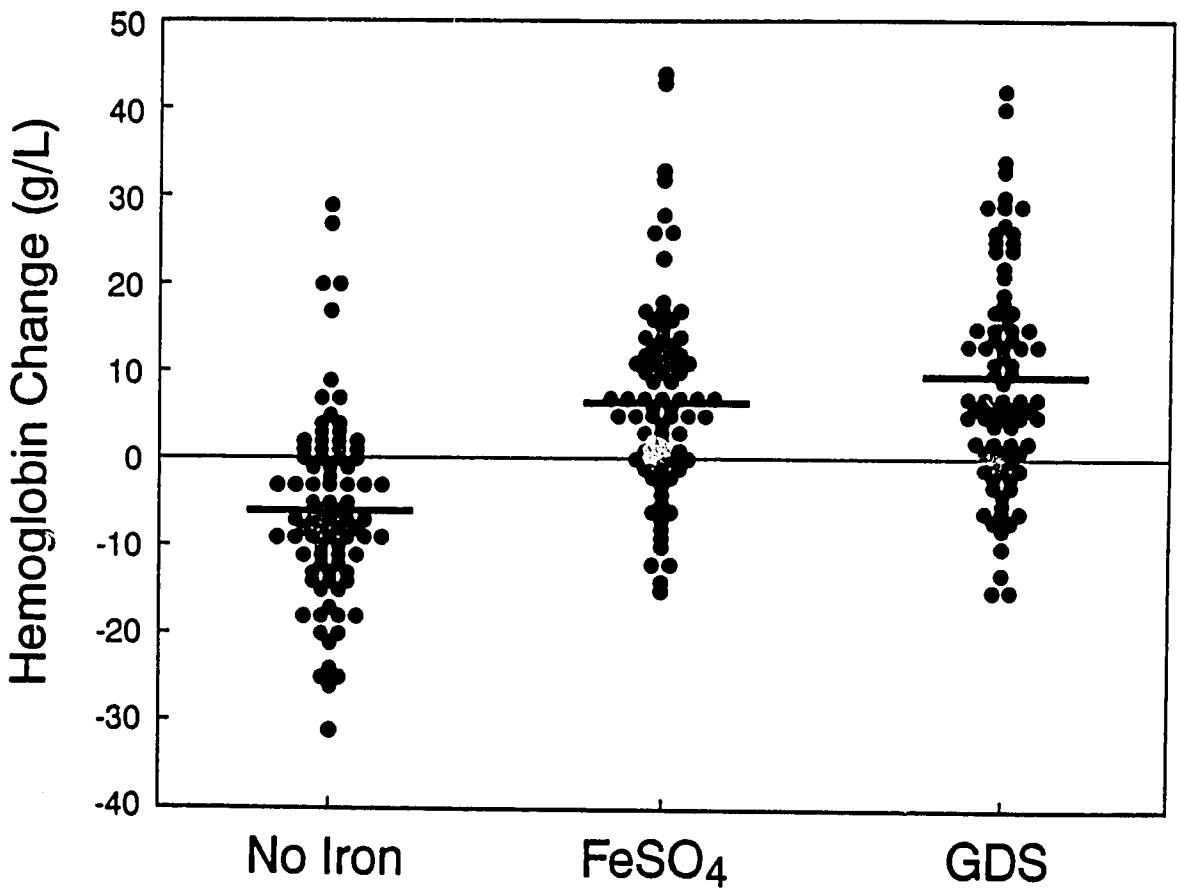
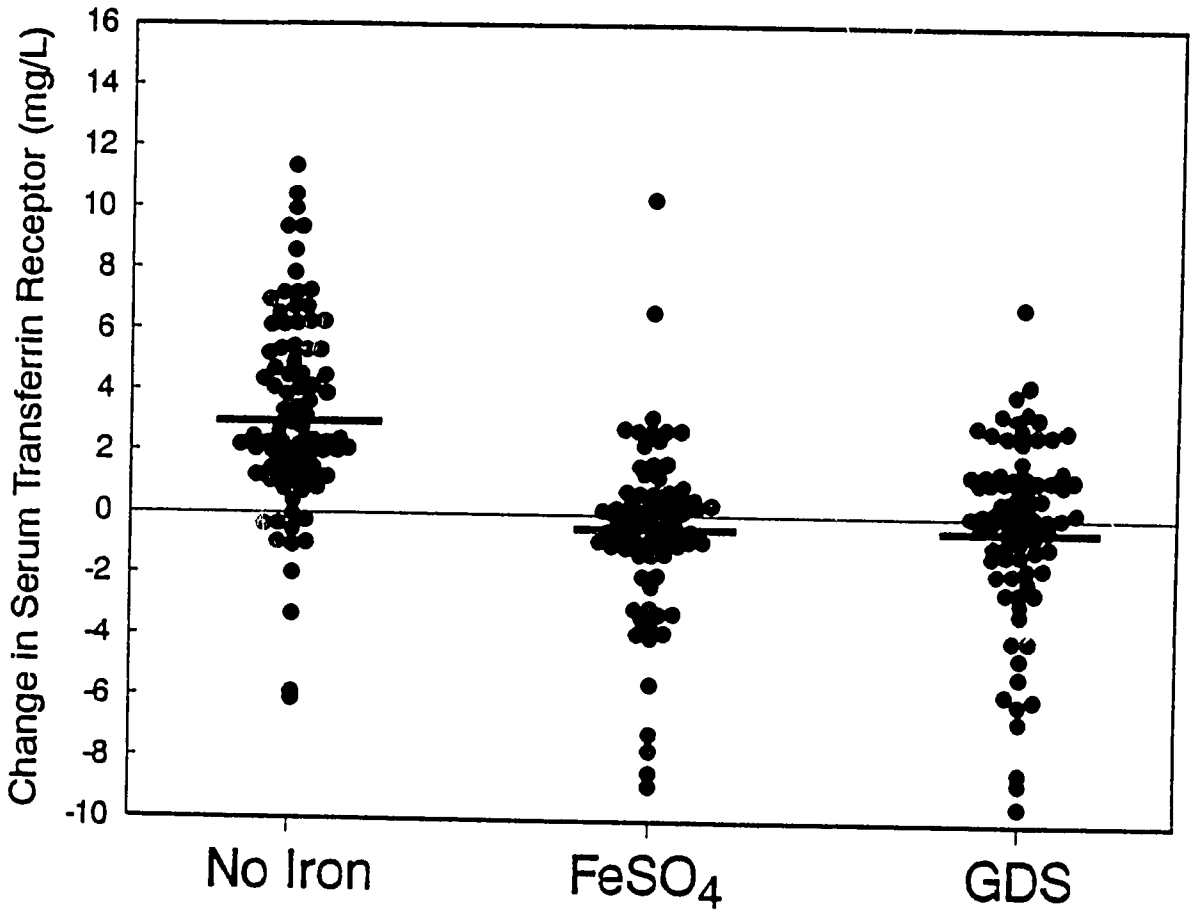

Hemoglobin Change (g/L)
50
40
30
20
10
0
-10
-20
-30
-40
No Iron
FeSO4
GDS

Figure 4


Change in Serum Transferrin Receptor (mg/L)
16
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
No Iron
FeSO4
GDS

British Journal of Haematology, 1993, 84, 301–304

The clinical significance of serum transferrin receptor levels in sickle cell disease

ATUL SINGHAL, JAMES D. COOK,* BARRY S. SKIKNE,* PETER THOMAS, BERYL SERJEANT AND GRAHAM SERJEANT *Medical Research Council Laboratories (Jamaica), University of the West Indies, Kingston, Jamaica, and *Division of Hematology, Department of Medicine, Kansas University Medical Center, U.S.A.*

Received 20 October 1992; accepted for publication 30 December 1992

Summary. Serum transferrin receptor (TfR) levels were measured in 182 children with homozygous sickle cell (SS) disease, 47 with sickle cell–haemoglobin C (SC) disease and 41 normal (AA) controls on their eighth birthday. Highly significant elevations occurred in SS compared to SC disease and in SC disease compared to AA controls. Females had higher levels than males in controls but lower levels than males in SS and SC disease. In SS disease, serum TfR levels tended to rise with age from 2 to 8 years, the change within individuals correlating with a change in reticulocyte count ($r=0{\cdot}38$, $P=0{\cdot}017$) and fall in fetal haemoglobin levels ($r=-0{\cdot}51$, $P=0{\cdot}004$). Serum TfR levels did not change with infection or painful crisis but were markedly elevated in hypersplenism and fell following splenectomy in these subjects. In the aplastic crisis, serum TfR levels tended to rise following clinical presentation and then fall, reflecting the reticulocyte counts. These observations are consistent with serum TfR levels being a useful indicator of the degree of erythropoietic expansion in sickle cell disease.

Transferrin receptors (TfRs) control the uptake of circulating transferrin and iron into cells (Huebers & Finch, 1987). High concentrations occur on the membrane of cells with high iron requirements such as red cell precursors and other rapidly dividing tissues (Kozlowski *et al.* 1988). As the cells mature, transferrin receptors are cleaved from the cell surface and are detectable in the serum (Kohgo *et al.* 1986). Serum TfR levels, measured by immunoassay, correlate with ferrokinetic measures for erythropoiesis (Huebers *et al.* 1990), are elevated in iron deficiency anaemia, sickle cell disease and thalassaemia, and reduced in aplastic anaemia (Kohgo *et al.* 1987, 1988; Flowers *et al.* 1989; Huebers *et al.* 1990). The present study measured serum TfR levels in normal children, in patients with steady state homozygous sickle cell (SS) and sickle cell–haemoglobin C (SC) disease, and in SS disease with a variety of complications, to assess the determinants of serum TfR levels and explore the clinical value of serum TfR levels as an index of erythropoietic expansion.

PATIENTS AND METHODS

The patients attended the Sickle Cell Clinic at the University of the West Indies, Kingston, Jamaica, and were mostly drawn from a cohort study which has been described previously (Serjeant *et al.* 1974; Hayes *et al.* 1985).

The distribution of serum TfR levels was measured in 270 children (41 AA, 182 SS, 47 SC) representing all available sera taken on the eighth birthday from cohort children and stored at −70°C. The relationship of serum TfR values to total haemoglobin (Hb), fetal haemoglobin (HbF), mean cell volume (MCV), absolute and percentage reticulocyte counts measured on a simultaneous blood sample was assessed by correlation coefficient analysis after restricting to steady state values (routine clinic visits in clinically well subjects not transfused in the preceding 3 months). The relationship between serum TfR levels and growth was assessed in 28 cohort SS children (14 M, 14 F) with the greatest height increment and a similar group with the lowest height increment between 3 and 9 years. Longitudinal trends in this group were assessed in all stored sera in three age bands (1–3 years, 4–6 years, and 7–9 years).

The influence of painful crisis on serum TfR was assessed by comparing levels in 30 SS patients (11 M, 19 F: median age 17·6 years, range 2·3–43·9 years) during bone pain with levels in the closest steady state stored serum sample in the same patient. The effect of gross erythropoietic expansion on serum TfR was assessed in eight SS patients with hypersplenism (Emond *et al.* 1984) before and after splenectomy. The effect of erythroid maturation arrest on serum TfR was

Correspondence: Dr Atul Singhal, Medical Research Council Laboratories, University of the West Indies, Kingston 7, Jamaica, W.I.

Table I. Serum transferrin receptor levels (mg/l) in AA controls, and patients with SS and SC disease measured at age 8 years.

	AA controls			SS disease			SC disease		
	n	Mean (range)	SD	*n*	Mean (range)	SD	*n*	Mean (range)	SD
Males	16	5·9 (1·9–9·2)	1·7	100	40·4 (12·4–83·9)	12·8	25	26·1 (2·8–45·6)	10·1
Females	25	7·2 (2·9–12·4)	1·8	82	35·9 (15·9–75·0)	12·3	22	20·5 (4·9–37·8)	8·5
Total	41	6·7 (1·9–12·4)	1·9	182	38·3 (12·4–83·9)	12·7	47	23·4 (2·8–45·6)	9·7

All genotype differences were highly significant ($P<0·001$).

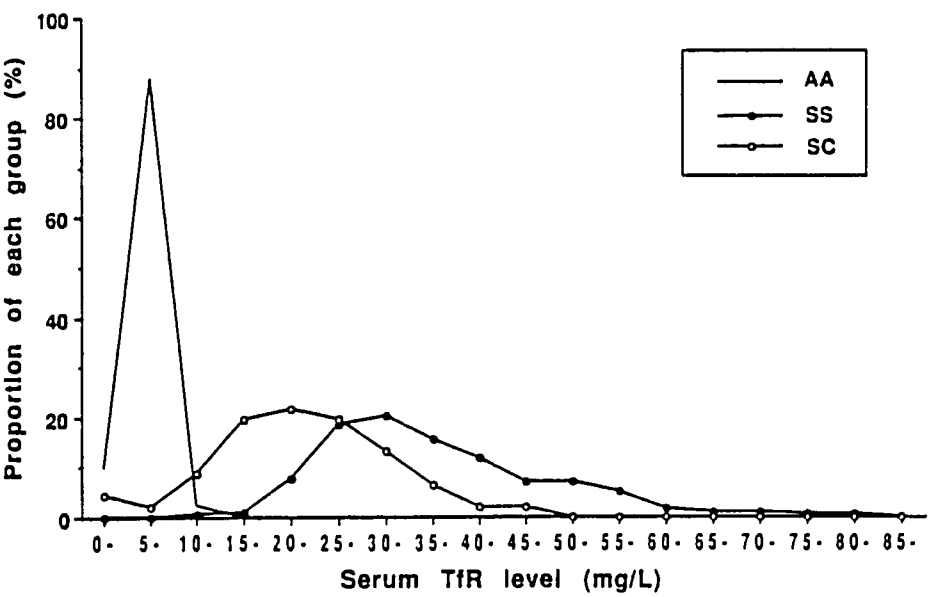


Fig 1. Distribution of serum TfR levels according to genotype.

assessed by longitudinal observations in seven patients (three SS, four SC) in aplastic crisis, as previously defined (Goldstein *et al*, 1987), and during the recovery phase. Iron deficiency, defined as a serum transferrin saturation below 15% or a serum ferritin level <12 μg/l, occurred in 34 SS patients.

The diagnosis of SS and SC disease was based on standard criteria (Serjeant, 1992). Haematological indices were measured in a Coulter S plus 4 (Coulter Electronics, Hialeah, Florida) and fetal haemoglobin by alkali denaturation (Betke *et al*, 1959). Serum TfR levels were measured by a sandwich, enzyme linked, immunoabsorbant assay (Flowers *et al*, 1989) in the laboratories of Dr James Cook on frozen serum samples shipped in dry ice from Jamaica. All determinations were performed in triplicate after dilution of all sera to 1:5000 because of the elevated TfR levels in patients with SS disease. Since some of the sera were stored at −70°C for up to 10 years, sample age was plotted against TfR level and failed to show any relationship suggestive of deterioration of samples.

RESULTS

Serum TfR levels and haemoglobin genotype

Serum TfR levels at the age of 8 years were markedly elevated in SC disease compared to AA controls ($P<0·001$) and in SS disease compared to SC disease ($P<0·001$) (Table I; Fig 1). Values were normally distributed and the means were compared by the unpaired *t* test using separate variance estimates when necessary.

Serum TfR levels and sex

Serum TfR levels were higher in females in AA controls ($t=-2·24$, $P=0·03$) and in males with SS disease ($t=2·41$, $P=0·02$) and SC disease ($t=2·03$, $P=0·048$). There was no significant sex difference in ferritin levels in SS disease ($P=0·86$) or AA controls ($P=0·41$). Ferritin levels were not available in SC disease.

Table II. Correlation between serum TfR levels and haematological indices in SS disease.

		Log_{10} (HbF)	Hb	MCV	Reticulocytes (%)	Reticulocytes Absolute
Males	*r*	−0·32	−0·40	−0·31	0·15	0·12
	n	58	66	66	66	66
	P	0·02	0·001	0·01	0·23	0·33
Females	*r*	−0·38	−0·27	−0·13	0·25	0·23
	n	51	52	51	51	51
	P	0·006	0·055	0·34	0·08	0·11

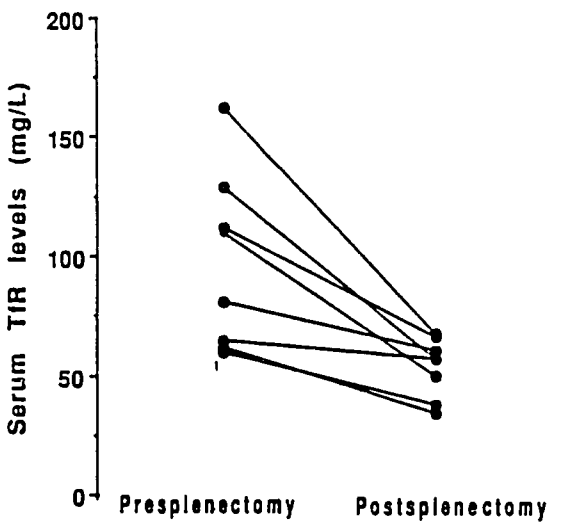


Fig 2. Serum TfR levels in eight hypersplenic SS patients before and after splenectomy.

Serum TfR levels and haematological indices in SS disease
Serum TfR level correlated negatively with HbF in both sexes, with haemoglobin and MCV in males only (Table II), but not with reticulocytes (proportional or absolute counts). Serum TfR levels did not differ between patients with or without iron deficiency either in males (mean, SD: 39·6, 14·8 *v* 40·6, 12·2; $P=0·74$) or females (37·6, 10·4 *v* 35·6, 12·6; $P=0·63$).

Serum TfR levels and age in SS disease
The effect of age was assessed in the 56 SS cohort patients in the growth subgroup in whom, contrary to the whole population, serum TfR values were highly skewed and required transformation (log_e serum TfR levels). Serum TfR levels were available in all three age bands (1–3, 4–6 and 7–9 years) in 34 subjects who showed higher values in the oldest compared to the youngest group (paired *t*-test, $P=0·035$) but not compared to the middle group. The age related change in serum TfR within individuals correlated with a change in reticulocyte count (Spearman rank correlation: $r=0·38$, $n=32$, $P=0·017$) and with a fall in HbF levels ($r=-0·51$, $n=26$, $P=0·004$), but not with changes in other haematological indices. Thus subjects with greater rises in serum TfR tended to manifest larger increases in reticulocyte count and smaller falls in HbF level.

Serum TfR levels and height velocity
In the growth subgroup of 34 subjects with serum TfR levels measured at each age, values at 7–9 years were compared in 18 and 16 patients with the greatest and lowest height velocities between 3 and 9 years. Serum TfR levels did not differ between patients with the greatest (median, [range]: 56·5, [35·5–90·9]) and the least growth (58·3, [34·5–114·4], the *t*-test on the log_e transformed data gave $P=0·58$).

Variability in serum TfR levels
Analysis of between- and within-patient variation was confined to 34 SS patients with multiple assays ($n=109$) in subjects aged 4 years and above, in whom no age related variation was demonstrable. In these, intra-individual differences accounted for 47%, and inter-individual differences 53% of the variability in serially measured (log_e transformed) serum TfR values compared to corresponding figures for log_e reticulocyte counts of 86% and 14%. Serum TfR levels in comparison to reticulocyte counts thus tended to be characteristic for individual patients.

Serum TfR levels and clinical complications
Serum TfR levels in 63 SS patients aged 8 years with a variety of inflammatory complications (25 upper respiratory tract infections, 15 painful crises, seven fever, five skin infections, and 11 other inflammatory events or infections) did not differ from levels in 119 age matched steady state SS patients (mean, SD: 37·7, 13·5 *v* 38·7, 12·4; $P=0·61$).

During painful crises serum TfR levels in 30 SS patients (median, [range]; 48·3, [20·8–125·8]) did not differ from steady state values in the same patients (44·8, [18·0–88·8]) using the Wilcoxon matched pairs test ($P=0·78$).

Serum TfR levels were markedly elevated in eight patients with chronic hypersplenism, values in the seven of these patients aged 7–9 years (median [range]: 110·0, [59·7–162·6]) tending to be higher than in 34 steady state cohort SS children of comparable age (57·1, [34·3–114·2]) (Mann-Whitney U-test gave $P=0·0069$). Following splenectomy (Fig 2), serum TfR fell in all patients (Wilcoxon matched pairs test comparing pre- and post-splenectomy levels, $P=0·028$). Post-splenectomy levels in the seven patients aged 7–9 years (57·0, [34·1–67·4]) were similar to those in the 34 SS children in the steady state.

Serum TfR levels during the early recovery phase of an aplastic crisis (6–13 d from presentation) (46·1, [19·3–66·8]) were greater than at 25–40 d (26·7, [14·2–44·1], $P=0·028$), as were percentage reticulocyte counts (10·0, [2·0–24·0] at 6–13 d and 4·0, [2·0–14·0] at 25–40 d, $P=0·035$).

DISCUSSION

Serum transferrin receptor levels are markedly elevated in subjects with SS disease (Flowers *et al.* 1989) consistent with the increases observed in other haemolytic anaemias (Kohgo *et al.* 1987, 1988; Huebers *et al.* 1990). Levels in the present study were lower in SC disease which has a moderate increase in haemolysis and varied widely between SS subjects, consistent with the variable haemolytic rate in this condition (Bensinger & Gillette, 1974). Both observations

support the hypothesis that serum TfR levels may indicate the degree of erythropoietic expansion. The negative correlation of HbF and serum TfR levels further supports this hypothesis since high levels of HbF inhibit sickling and haemolysis and might be expected to be associated with lesser erythropoietic activity.

The lack of a significant relationship between serum TfR levels and reticulocyte counts was not unexpected. A relationship has been reported in autoimmune haemolytic anaemia (Kohgo *et al.* 1987), but reticulocyte counts are very variable in SS disease and correlate only poorly with HbF levels (Maude *et al.* 1987) or other indices of haemolysis such as red cell survival (Serjeant *et al.* 1969). The density of surface transferrin receptors is high in immature erythroblasts and falls as the cells mature through the reticulocyte stage (Huebers & Finch. 1987: Parmley *et al.* 1983). Furthermore, the response to haemolytic stress includes not only an increase in production of erythrocyte precursors, but also a shift of precursors normally retained within the bone marrow. The overall effect of these factors is difficult to quantitate but would make unlikely a simple relationship between reticulocyte count and serum TfR level.

Iron deficiency in otherwise normal subjects increases the serum TfR level three to four fold (Kohgo *et al.* 1987, 1988: Flowers *et al.* 1989; Huebers *et al.* 1990). No increase was demonstrable in SS disease in the present study possibly because any effect of iron deficiency was obscured by the greater increase caused by the disease itself. Longitudinal studies within SS patients during iron deficient and iron replete phases may provide a more sensitive indicator of the effect of iron deficiency on serum TfR levels in SS disease.

Serum TfR levels increased with age in children with SS disease, suggesting an age related increase in erythropoietic activity. Greater erythropoietic activity should attend the rise in HbS levels and sickling consequent on the fall in HbF levels, so the negative correlation between change in serum TfR and fall in HbF was unexpected. This association is difficult to explain but stimulation of erythropoiesis may increase HbF production in animals (DeSimone *et al.* 1981) and this mechanism may also occur in patients.

Levels of serum TfR in complications of SS disease are consistent with its role as an indicator of bone marrow activity. The lack of a change in serum TfR levels during painful crises was not unexpected and was consistent with observations that the haemolytic rate is unchanged (Diggs, 1956). High serum TfR levels in the recovery phase of aplastic crisis reflected the intense erythropoietic activity during recovery and levels paralleled changes in reticulocyte counts. In hypersplenism, where a marked erythropoietic expansion is largely relieved by splenectomy, high serum TfR levels fell to values in the normal range for SS disease following splenectomy reflecting the expected change in erythropoietic activity. Serial measurement of serum TfR levels may therefore help to distinguish chronic hypersplenism from simple splenomegaly and identify children that could benefit from splenectomy.

Serum TfR levels show less intra-individual variability than reticulocyte counts, and measurement of serum TfR may be a reliable indicator of bone marrow activity useful in clinical decisions.

ACKNOWLEDGMENT

This study was supported in part by AID Cooperative Agreement DAN-5115-A-00-7908-00.

REFERENCES

Bensinger, T.A. & Gillette, P.N. (1974) Hemolysis in sickle cell disease. *Archives of Internal Medicine*, 133, 624–631.

Betke, K., Marti, H.R. & Schlicht, I. (1959) Estimation of small percentages of foetal haemoglobin. *Nature*, 184, 1877–1878.

DeSimone, J., Heller, P., Biel, M. & Zwiers, D. (1981) Genetic relationship between fetal haemoglobin levels in normal and erythropoietically stressed baboons. *British Journal of Haematology*, 49, 175–183.

Diggs, L.W. (1956) The crisis in sickle cell anemia. Hematologic studies. *American Journal of Clinical Pathology*, 26, 1109–1118.

Emond, A.M., Venugopal, S., Morais, P., Carpenter, R.G. & Serjeant, G.R. (1984) Role of splenectomy in homozygous sickle cell disease in childhood. *Lancet*, i, 88–90.

Flowers, C.H., Skikne, B.S., Covell, A.M. & Cook, J.D. (1989) The clinical measurement of serum transferrin receptor. *Journal of Laboratory and Clinical Medicine*, 114, 368–377.

Goldstein, A.R., Anderson, M.J. & Serjeant, G.R. (1987) Parvovirus associated aplastic crisis in homozygous sickle cell disease. *Archives of Disease in Childhood*, 62, 585–588.

Hayes, R.J., Beckford, M., Grandison, Y., Mason, K., Serjeant, B.E. & Serjeant, G.R. (1985) The haematology of steady state homozygous sickle cell disease: frequency distributions, variations with age and sex, longitudinal observations. *British Journal of Haematology*, 59, 369–382.

Huebers, H.A. Beguin, Y., Pootrakul, P., Einspahr, D. & Finch, C.A. (1990) Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood*, 75, 102–107.

Huebers, H.A. & Finch, C.A. (1987) The physiology of transferrin and transferrin receptors. *Physiological Reviews*, 67, 520–582.

Kohgo, Y., Niitsu, Y., Kondo, H., Kato, J., Tsushima, N., Sasaki, K., Hirayama, M., Numata, T., Nishisato, T. & Urushizaki, I. (1987) Serum transferrin receptor as a new index of erythropoiesis. *Blood*, 70, 1955–1958.

Kohgo, Y., Niitsu, Y., Nishisato, T., Kato, J., Kondo, H., Sasaki, K. & Urushizaki, I. (1988) Quantitation and characterization of serum transferin receptor in patients with anaemias and polycythaemias. *Japanese Journal of Medicine*, 27, 64–70.

Kohgo, Y., Nishisato, T., Kondo, H., Tsushima, N., Niitsu, Y. & Urushizaki, I. (1986) Circulating transferrin receptor in human serum. *British Journal of Haematology*, 64, 277–281.

Kozlowski, R., Reilly, I.A.G., Sowter, D., Robins, R.A. & Russell, N.H. (1988) Transferrin receptor expression on AML blasts is related to their proliferative potential. *British Journal of Haematology*, 69, 275–280.

Maude, G.H., Hayes, R.J. & Serjeant, G.R. (1987) The haematology of steady state homozygous sickle cell disease: interrelationships between haematological indices. *British Journal of Haematology*, 66, 549–558.

Parmley, R.T., Hajdu, I. & Denys, F.R. (1983) Ultrastructural localization of the transferrin receptor and transferrin on marrow cell surfaces. *British Journal of Haematology*, 54, 633–641.

Serjeant, G.R. (1992) *Sickle Cell Disease*, 2nd edn. Oxford University Press.

Serjeant, B.E., Forbes, M., Williams, L.L. & Serjeant, G.R. (1974) Screening cord bloods for detection of sickle cell disease in Jamaica. *Clinical Chemistry*, 20, 666–669.

Serjeant, G.R., Serjeant, B.E. & Milner, P.F. (1969) The irreversibly sickled cell: a determinant of haemolysis in sickle cell anaemia. *British Journal of Haematology*, 17, 527–533.

IRON DEFICIENCY: THE GLOBAL PERSPECTIVE

James D. Cook, Barry S. Skikne, Roy D. Baynes

Division of Hematology
Department of Medicine
University of Kansas Medical Center
Kansas City, KS 66160-7402

INTRODUCTION

Micronutrient deficiency is increasingly recognized as a priority nutritional problem in the developing world; at least 20% of the world's population is at risk of a deficiency in vitamin A, iodine, or iron (Trowbridge et al. 1993). Vitamin A deficiency is the most important cause of preventable blindness whereas iodine deficiency is a significant cause of permanent brain damage, mental retardation, and reproductive failure. A deficiency of either of these nutrients is associated with a decrease in childhood survival.

The focus of the present review is iron deficiency, the primary cause of nutritional anemia and the most common nutritional deficiency in the world today. In the present review of iron deficiency anemia (IDA), emphasis will be placed on the liabilities of iron lack, newer methods for its identification in population studies, and a review of available methods for its control.

LIABILITIES OF IRON DEFICIENCY

Mental and Motor Development

The most compelling reason for reducing the high prevalence of IDA is the defect in learning capacity and cognitive performance that is associated with IDA during the first few years of life. The seminal observation by Oski and coworkers (1978;1983) who demonstrated that a significant improvement in tests of mental development occur when infants with IDA are given iron, have been confirmed in larger field studies in Guatemala (Lozoff et al. 1982), Costa Rica (Lozoff et al. 1987), and Chile (Walter et al. 1983; Walter et al. 1989). In addition to impaired mental performance, abnormalities in psychomotor development have also been demonstrated in most of these reports. Infants between 9 and 12 months of age appear to be particularly susceptible (Walter et al. 1989).

One of the most significant findings in relation to IDA in infancy is that abnormalities

in tests of intellectual and motor performance have persisted when examined several years later despite full correction of the IDA (Lozoff et al. 1991; Walter, 1992). It is possible that other factors such as maternal education, socioeconomic status, or low birth weight may have explained some of these persistent abnormalities but, it is believed that these background factors were statistically controlled in these studies. Other workers have observed a complete reversal of abnormalities associated with IDA in infancy following treatment with iron. In a recent randomized double-blind clinical trial in 12 to 18 months infants living in Bandung, Indonesia (Idjradinata and Pollitt, 1993), the mean Bayley scores of mental and motor development in 50 infants with IDA were significantly lower than in 47 iron replete infants and in 29 infants with less severe iron deficiency without anemia. When each of these three categories of iron status were randomized to receive a placebo or ferrous sulfate and examined four months later, a highly significant improvement in mental and psychomotor development scores was observed in the infants with IDA given iron. It is clear that additional studies are needed to define the full impact of IDA in infancy, particularly in regard to the period of maximum susceptibility and the persistence of the impairment following correction of the iron deficiency. Nevertheless, there is compelling evidence that IDA in infancy can result in significant handicaps in mental and motor performance that may have lasting effects on an individuals' ability to achieve his or her full potential.

Work Capacity

A second important consequence of iron deficiency is an impairment in work capacity that has been repeatedly demonstrated in regions where IDA is common and in situations where work productivity can be accurately measured. In one of the better known studies, the effect of iron supplementation on the work output of latex tappers and weeders working on a rubber plantation in Indonesia was examined (Basta et al. 1979). Following a period of iron supplementation, there was a dramatic increase of nearly 50% in work output in those with IDA and a significant increase was also seen in those who were originally iron deficient but not anemic. Similar findings have been reported when iron supplements were given to tea pickers in Sri Lanka (Ohira et al. 1979). These findings have major economic significance for the developing world. In the study by Basta, for example, it was calculated that for every dollar invested in the purchase of ferrous sulfate, an economic return of US $250 was realized from the increased production of latex. The cost benefit of alleviating iron deficiency in the developing world has been analyzed by Levin, who estimated that with each 10% increase in hemoglobin concentration, a 15% improvement in worker productivity of anemic laborers can be expected (Levin, 1986). These calculations leave little doubt about the economic benefit of reducing the prevalence of IDA in regions where the disorder is prevalent. Additional studies are needed to determine whether a similar impairment exists for tasks that are less physically demanding and more dependent on sustained cognitive or coordinated motor function.

Premature Birth

A third important liability of iron deficiency that has been better defined in recent years is the consequence of IDA during pregnancy. Several reports have demonstrated a increase in the rate of premature delivery and low birth weight when significant anemia develops during the last trimester. In one of the more extensive studies involving more than 7000 pregnant women in the Boston area, a remarkably high prematurity rate of 30% was recorded at a relatively modest hemoglobin concentration of <100 g/L (Lieberman et al. 1988). These findings may be partly explained by gestational changes in hemoglobin concentration, which are lower in women who deliver earlier in pregnancy. Nevertheless,

it is unlikely the relationship between prematurity and hematocrit can be fully explained on this basis. In other studies relating maternal hemoglobin levels to birth weight, the relationship has been shown to be J-shaped with an increase in prematurity at hemoglobin concentrations above 120 g/L (Murphy et al. 1986; Garn et al. 1981). The increased risk of prematurity at higher hemoglobin concentrations is associated with hypertension and an assumed decline in circulating blood volume due to increased production of angiotensin II (Hallberg, 1992). This is a different phenomenon and should not detract from the importance of the effect of IDA during pregnancy in relation to increased prematurity, low birth weight, and increased fetal mortality.

One of the issues arising from studies of anemia and birth weight is whether the association is due to anemia from any cause or specifically to IDA. This question was answered in a recent perspective study of maternal growth and iron nutrition during pregnancy in 826 women from an inner-city minority population (Scholl et al. 1992). At the time of entry to care, roughly one-third of the women were anemic. Although less than half of the women who were anemic at entry to the study met the criteria for IDA based on the combination of a low hemoglobin and serum ferritin concentration, the odds of low birth weight were trebled and of premature delivery doubled in women with IDA but were not increased in women with anemia from other causes. Vaginal bleeding at or before entry to prenatal care also increased the risk of premature birth and the effects of bleeding and IDA were additive, increasing the risk 5-fold. A low concentration of ferritin reflecting reduced iron stores did not, in itself, influence the risk of premature delivery. Although the prevalence of IDA in this study was lower than anticipated (3.5%), the results leave little doubt that the risk of premature delivery and low birth weight is specifically related to IDA.

PREVALENCE OF IRON DEFICIENCY

Despite the unquestioned frequency with which iron deficiency occurs in many countries throughout the world, there was only fragmented evidence on global prevalence until the publication of DeMaeyer and Adiels-Tegman (1985). These investigators reviewed the published literature on anemia prevalence throughout the world; the mid-point of the publications reviewed was around 1980. It was estimated that 30% of the world's population is anemic or approximately 1.3 billion individuals. The authors estimated that slightly less than half, or 500-600 million people, had IDA, although most reports did not provide specific iron-related measurements. There was a striking relationship between anemia and both age and sex, with peak frequencies occurring in childhood and in pregnancy. A marked variation was also observed between different geographic regions. For example, the prevalence in women ranged from a low of 8% in North America to 44% and 58% in Africa and South Asia, respectively.

While this study provided the first clear view of the global magnitude of IDA, the use of isolated hemoglobin measurements as an index of iron lack has major limitations. The first is the low sensitivity of the hemoglobin concentration because of the marked overlap in values between normal and IDA populations. When a single cut-off level of hemoglobin was used to define anemia in pregnancy in one study, as many as one-third of normal subjects were classified as anemic while a similar proportion of anemic subjects are incorrectly considered normal (Cook et al. 1971). An even more important limitation of isolated hemoglobin or hematocrit measurements is their inability to distinguish IDA from other causes of anemia. The most important cause of non-iron deficiency anemia, especially in regions where the intake of many nutrients is marginal, is chronic infection that characteristically suppresses hemoglobin production. In certain regions, malaria and HIV infection are more important causes of anemia than IDA. Genetic abnormalities in hemoglobin structure or synthesis, such as sickle cell disease or thalassemia, are also

important causes of non-iron deficiency anemia in certain regions of the world.

Significant progress towards reducing the global prevalence of iron deficiency will require the introduction of simple, inexpensive, and specific laboratory indices of iron deficiency that are suited to field use. One of the most important additions to the field assessment of iron status during the past two decades is the serum ferritin assay. Because the measurement requires only a few microliters of serum, fingerstick specimens can be obtained and stored in the frozen state for determinations months, or even years, later. The most important attribute of the serum ferritin is its high specificity. A value <12 ng/L is only seen with depleted iron stores and provides unequivocal evidence of IDA when associated with a significant reduction in hemoglobin or hematocrit levels. The serum ferritin is particularly valuable when assessing the impact of an intervention because it portrays changes in iron status in those who were not initially iron deficient.

The major limitation of serum ferritin measurements is that they provide no information about the severity of tissue iron deficiency in individuals with absent iron stores. Most of the iron measurements which provide this information are not suitable for field use. For example, the serum iron and total iron-binding capacity have been used extensively in epidemiological studies but the derived parameter of transferrin saturation (serum iron/total iron binding capacity) lacks specificity because it is similarly affected by iron deficiency and the anemia of chronic disease. Moreover, these determinations require more blood than can be obtained by capillary sampling. Measurements relating to the circulating red cell, such as the mean corpuscular volume (MCV) and red cell distribution width (RDW), are used frequently in a clinical setting but entail the use of costly electronic equipment which is seldom available in developing countries. These techniques also require same-day analysis which is impractical in many field situations. The portable instruments that are available for the measurement of erythrocyte protoporphyrin offer a more practical option in field studies but this equipment is not inexpensive and its standardization and long-term reliability remains uncertain.

An important new assay, termed the serum transferrin receptor (TfR), shows promise in providing the needed information about the severity of tissue iron deficiency. The cellular form of the TfR is a transmembrane glycoprotein comprised of two identical subunits of 95,000 kDa mass linked by two disulfide bridges. Over 80% of the protein is contained in the extracellular domain. The cellular form of TfR has emerged, in recent years, as playing a key role in the acquisition of transferrin iron by all body cells (Huebers and Finch, 1987; Ward, 1987). Following binding of the TfR at the cell surface by diferric transferrin, the iron is carried into the cell in small vesicles termed endosomes. The iron is then released as the pH of the endosome falls. The apotransferrin remains bound to the TfR and is returned to the cell surface at which point transferrin is released to obtain additional iron from the storage compartment.

Kohgo and coworkers were the first to identify TfR in human serum using sensitive immunological techniques (Kohgo, 1986; Kohgo et al. 1986) and this important observation has been confirmed subsequently in other laboratories (Flowers et al. 1989; Huebers et al. 1990). It was originally suggested that circulating TfR is intact receptor (Huebers and Finch, 1987), but more recent studies in which the serum form was purified using immunoaffinity chromatography with monoclonal antibodies, have demonstrated that circulating TfR is truncated at amino acid residue 100 and thereby lacks the cytoplasmic and transmembrane domains of intact receptor (Shih et al. 1990). Because the concentration of transferrin in serum is far higher than that of serum TfR, it is assumed that the serum TfR circulates as a complex with a molecular mass of 160 kDa containing one TfR subunit for each molecule of transferrin.

The salient features of serum TfR measurements have been recently reviewed (Beguin, 1992; Cook et al. 1993). The mean and range of values in normal subjects reported by different laboratories varies widely although the relative changes in different clinical

disorders are roughly comparable. At least some of the differences in values reported by different laboratories appears to relate to immunologic reagents and assay standards (Flowers et al. 1989). In various hematological disorders, the concentration of serum TfR varies in proportion to the number of erythroid precursors. The serum TfR increases by a factor of 10 in patients with ineffective erythropoiesis and rises to a lesser extent in patients with compensated hemolytic states such as autoimmune hemolytic anemia or sickle cell disease. In patients with aplastic anemia or complete marrow ablation, the level falls by only 50% of normal indicating that nonerythroid tissues contribute significantly to the concentration of serum TfR. An excellent correlation has been demonstrated between the erythron iron turnover, a ferrokinetic measurement of total erythropoiesis, and serum TfR in a wide range of hematologic disorders (Huebers et al. 1990).

The value of serum TfR in the epidemiologic assessment of iron nutriture is based on the observation that the concentration increases progressively with advancing tissue iron deficiency, reaching a level of roughly 3-times normal in patients with overt IDA (Flowers et al. 1989). The effects of evolving IDA on the serum TfR have been defined by monitoring normal subjects who underwent serial phlebotomies to induce a wide spectrum of iron deficiency (Skikne et al. 1990). Little or no change in the serum TfR concentration occurred during the period of storage iron depletion whereas a predictable and dramatic fall in serum ferritin levels occurred. When iron stores approached full depletion, the serum TfR began to rise and continued to increase in direct proportion to the degree of tissue iron deficiency. By calculating the ratio of the receptor/ferritin concentration in serum, a single parameter was obtained, the log of which was directly proportional to the body iron level over a wide spectrum ranging from normal iron stores to advanced iron deficiency. The ability to characterize iron status quantitatively in each sampled individual offers an important advantage in epidemiological studies in developing countries. Although the technology is complex and the commercial availability of immunologic kits limited, serum obtained by capillary sampling in the field can be frozen and assayed later in a central laboratory using automated systems. The investment in this technology is warranted because of the high specificity and sensitivity of this new index of body iron status.

A key advantage of the serum TfR is its ability to distinguish the anemia of chronic disease from IDA. In a recent study addressing this question, the mean serum TfR in 17 normal controls was 5.36 ± 0.82 mg/L as compared to 13.91 ± 4.63 mg/L in 17 patients with IDA (Ferguson et al. 1992). The mean serum TfR was normal in all 20 patients with acute infection and was also normal in 8 of 10 anemic patients with chronic liver disease. In 41 patients with the anemia of chronic disease, only 4 had elevated serum TfR levels and the mean TfR of 5.65 ± 1.91 mg/L in this group was comparable to the values in normal subjects. When the deficit in hemoglobin concentration was >25 g/L, there was complete separation of serum TfR levels in patients with the anemia of chronic disease from those with IDA.

The serum TfR is valuable in determining the iron status of pregnant women. Although it might be anticipated that the high concentration of transferrin receptor in human placental tissue would contribute to the serum concentration during gestation, we have observed that the frequency distributions of serum TfR in normal subjects and in pregnant women are very similar (Carriaga et al. 1991). Serum TfR elevations in pregnancy were observed only in those women with a serum ferritin concentration <16 µg/L. In 13 women in whom IDA was diagnosed by a combination of iron-related measurements, the serum TfR level was increased significantly in all but 2. In a recent study to determine the efficacy of iron supplementation in over 300 pregnant women living in Kingston, Jamaica, the serum TfR was the most reliable laboratory index of iron status (W.K. Simmons, unpublished observations).

INTERVENTION METHODS

The optimal method for reducing the prevalence of iron deficiency in developing countries should be based on knowledge about the primary cause but there is relatively little information about the factors that lead to nutritional IDA in these areas. The high prevalence of anemia in infants, pre-schoolers, and pregnant women indicate that the primary mechanism is an inadequate absorption of dietary iron to offset the enhanced iron requirements associated with growth and gestation. One method of intervention that has been proposed is to alter the diet to improve the bioavailability of dietary iron. While this remains a theoretical possibility, economic restraints usually limit the introduction of the most important foods such as meat and animal tissue. Not only are most individuals unwilling to alter their traditional diet appreciably but there is little or no evidence that dietary modifications, other than increased meat intake, have a significant impact on iron balance. In certain regions of the world, excessive blood loss due to parasitic infection, such as hookworm, is known to be of key importance and in these regions, efforts to reduce parasitism should be a prominent feature of an intervention program. In the present review, emphasis will be placed on two established interventions: iron supplementation or the provision of medicinal iron, and food fortification or the enhancement of the dietary content of iron.

Iron Supplementation

The medical practitioner has been aware for many decades that when medicinal iron is taken regularly by a patient with IDA, a complete hematologic response invariably occurs within a few weeks. This has prompted efforts, over the past several decades, to apply this approach at a population level by supplying iron supplements to groups as high risk of iron deficiency. In pregnancy, for example, when iron demands are large in amount and brief in duration, iron supplementation has been the traditional approach to preventing IDA. The supplementation of pregnant women with iron is the only intervention used in many developing countries despite a paucity of evidence that the supplementation program have a sustained efficacy.

The reason for the failure of iron supplementation programs was the subject of a recent conference in which the experience of several different countries was reviewed (Gillespie et al. 1991). The reasons for failed programs were as numerous as the countries reporting their experience. In some countries, economic constraints limited the regular procurement of the iron tablets or prompted the use of less expensive and less effective forms of iron. In other instances, a break-down in the delivery system at some point between the procurement of iron supplements by the government and their regular supply to maternal health clinics was the major difficulty. Poor adherence of the women to regular supplementation usage is undoubtedly a major factor but the nature of poor compliance remains uncertain. In some instances, women do not receive proper instruction about the importance of taking their iron tablets regularly and do not obtain adequate follow-up at their regular visits about use of their iron supplements. The problem of non-compliance in established programs of iron supplementation and pregnancy was highlighted by a recent study in Jakarta, Indonesia (Schultink et al. 1993). The baseline prevalence of anemia in 50 women in second trimester pregnancy (42%) did not decrease after 2 months of iron supplementation with 60 mg elemental iron daily. Of 33 women who completed the study, adherence to the iron supplementation program was confirmed by analysis of stool iron in only 12 women although there was a significant improvement in both serum ferritin ($p<0.05$) and hematocrit ($p<0.01$) in the total sample. It was concluded that poor compliance was the major factor accounting for inadequate response to iron supplements although gastrointestinal side effects were reported only rarely as a reason. Most women

said that they simply forgot to take their iron tablets on a regular basis.

Another important factor that unquestionably contributes to poor compliance is the well-known gastrointestinal side effects of medicinal iron. The more distressing of these are the nausea, epigastric discomfort, and occasional vomiting that occurs within an hour or two of taking iron. The frequency and severity of these symptoms vary with the amount of iron taken and its relationship to food. Lower intestinal symptoms such as diarrhea or constipation are apparently less troublesome and occur independently of the amount of iron consumed. The upper intestinal symptoms can often be circumvented in clinical practice because the patient can be instructed to make adjustments in the dose, form, or timing of iron administration. However, health care workers in large maternal clinics have limited time available to provide individual counselling. In small rural communities, women are often reluctant to use iron tablets when they learn that others have had gastrointestinal symptoms.

One approach to enhancing the effectiveness of iron supplementation programs is to use iron preparations that reduce the frequency of nausea and epigastric discomfort. A number of delayed release iron preparations have been marketed but iron absorption from these formulations is usually lower than standard iron tablets because the iron is not released in the gastrointestinal tract until the preparation is beyond the duodenum where iron assimilation is maximal. A newer preparation, termed a gastric delivery system (GDS), circumvents this problem by mixing ferrous sulfate with a colloid suspension that becomes buoyant on exposure to gastric secretion (Cook et al. 1990). The preparation is retained within the stomach for 2 to 3 hours, while slowly releasing the iron to the acidic milieu of the stomach. This preparation has two major advantages. First, when taken with food, which strongly inhibits iron absorption, the bulk of the meal passes out of the stomach before the GDS iron is released. As a consequence, iron absorption is 2- to 3-times better than conventional ferrous sulfate tablets taken with food. This feature of GDS allows less frequent administration while providing the same amount of iron. An equally important advantage of the GDS is complete elimination of the gastrointestinal side effects associated with iron. The GDS is now undergoing field trials in Kingston, Jamaica and rural Tanzania with promising early findings. In the former trial, the same hematological efficacy of a single GDS iron tablet taken once daily was obtained as twice the amount of iron as conventional ferrous sulfate tablets (Simmons et al. 1993).

While newer technology such as the GDS will eventually enhance the effectiveness of iron supplementation programs, iron supplementation has many limitations. Even if compliance and efficacy can be improved, this intervention is likely to have little influence on the overall prevalence of iron deficiency in the total population.

Food Fortification

The only practical means of achieving a significant reduction in IDA is to increase iron in the food supply by fortifying one or more food vehicles with iron. Fortification of wheat and bakery products was first introduced in industrialized countries during World War II and currently provides between 10% (United Kingdom) and 40% (Sweden) of the dietary iron intake; about 20% of dietary iron is derived from fortification in North America. Iron fortification of milk formulas and infant cereals has also been used widely in industrialized countries. The iron status of both children and adults has improving progressively in recent decades in countries where foods are enriched with iron but it has not been possible to determine the extent to which this improvement is due to iron fortification.

The extension of iron fortification programs to developing countries is beset by several obstacles. The use of cereal-based programs depends not only on identifying a suitable vehicle but on a means of adding iron in a bioavailable form and in a manner that does not alter the color or taste of the food. Rice is the staple food for a large proportion of the

world population in regions where iron deficiency is prevalent but despite efforts for many years, a fully satisfactory method for fortifying this cereal has not been developed. With most cereals, a major problem is the lack of central milling facilities. When cereals are grown in rural areas, and milled locally or in the home, it is difficult to introduce the fortification iron. Nevertheless, given the apparent success of fortifying infant cereals and wheat-based foods in industrialized countries, fortification of cereals remains an important strategy for many regions of the world.

Because of these problems with cereal fortification, efforts have been made to fortify other vehicles such as salt, sugar, or condiments that are usually produced in a limited number of facilities. Salt is especially attractive as a vehicle because it has been used effectively for iodine fortification in most countries. The technology of salt fortification with iron has been studied extensively in India and has been shown, in pilot studies, to reduce the prevalence of IDA significantly (Working Group on Fortification of Salt with Iron., 1982). Efforts are currently underway to doubly fortify salt with both iodine and iron.

One of the major constraints with iron fortification in developing countries is the inhibiting effect of the cereal-based diets on the absorption of nonheme dietary iron and added fortification iron. Indeed, it is the inhibitory nature of many diets that is believed to account for the high prevalence of iron deficiency in the Third World. Even when highly available forms of fortification iron are used, such as ferrous sulfate, absorption never exceeds that of the common pool of dietary nonheme iron which is usually low. One important development in recent years is the use of a chelated form of iron, NaFeEDTA, that has several important advantages. The most important is its higher absorption than non-chelated iron sources, particularly when the diet is strongly inhibitory. Another important advantage of NaFeEDTA is that by maintaining iron in a less reactive form, problems in alteration of taste or appearance of the food vehicle or foods with which the fortified product is mixed, is greatly reduced. Extensive field trials with this fortification strategy using sugar (Viteri et al. 1983), fish sauce (Garby and Areekul, 1974), or condiments (Ballot et al. 1989), have all shown a significant improvement in iron status.

One of the most important questions in regard to iron fortification policies is whether it is better to select a vehicle that reaches all segments of the population in rough proportion to their caloric intake or whether it is better to target the fortified vehicle to the populations in greatest need of iron. Recent studies have suggested that genetically normal individuals with higher iron stores may be at increased risk of common but serious disorders, such as cancer (Stevens et al. 1988) or heart disease (Salonen et al. 1992). While much more work will be needed to confirm these suspicions, a conservative approach would be to use targeted fortification such as weaning foods in infancy or fortified foods provided in a school lunch program. The disadvantage of this approach is that the prevalence of iron deficiency in women of the childbearing age would not. The advantage is that any untoward, and yet undefined, liabilities of providing iron to those who do not require it would be avoided.

SUMMARY

The prevalence of IDA in industrialized countries has declined in recent decades, but there has been little change in the worldwide prevalence. IDA is currently estimated to affect more than 500 million people. Recent studies have indicated that anemia *per se*, the most common manifestation of iron deficiency, is less important from a public health standpoint than liabilities associated with tissue iron deficiency. The most important of the latter are an impairment in psychomotor development and cognitive function in infants and pre-schoolers, a deficit in work performance in adults, and an increase in the frequency of low birth weight, prematurity, and perinatal mortality in pregnancy. There have been several

recent advances in combatting nutritional iron deficiency. One of the major problems has been in distinguishing iron deficiency from other causes of anemia seen epidemiologically such as malaria, HIV infection, chronic inflammation, hemoglobinopathies, and protein energy malnutrition. When combined with serum ferritin and hemoglobin determinations, the serum transferrin receptor assay is a valuable addition in epidemiologic surveys because it provides a quantitative measure of functional iron deficiency and it distinguishes true IDA from the anemia of chronic disease. The most difficult challenge is to develop effective methods of supplying iron to large segments of a population. Supplementation with iron tablets is suitable for only brief periods of need such as during pregnancy. The poor compliance with existing supplementation programs is believed to be due mainly to the gastrointestinal side effects of oral iron which can be eliminated by the use of a gastric delivery system. The most effective long-term strategy is to increase the intake of bioavailable iron in the diet. The customary approach has been to fortify a food staple such as wheat, rice, sugar, or salt, and thereby increase the iron intake of the entire population. However, because of concerns about the risk of cancer and heart disease in individuals with high iron stores, there is an increasing reluctance to supply iron to individuals who do not require it. A more effective strategy is to fortify food vehicles that are targeted to segments of the population at greatest risk of iron deficiency such as infants and school children. Because of the strong inhibitory properties of diets in regions of the world where iron deficiency is most prevalent, the use of NaFeEDTA has important advantages for food fortification.

REFERENCES

Ballot, D.E., MacPhail, A.P., Bothwell, T.H., Gillooly, M. and Mayet, F.G. 1989, Fortification of curry powder with NaFe(III)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. *Am J Clin Nutr* 49:162.

Basta, S.S., Soekirman, M.S., Karyadi, D. and Scrimshaw, N.S. 1979, Iron deficiency anemia and the productivity of adult males in Indonesia. *Am J Clin Nutr* 32:916.

Beguin, Y. 1992, The soluble transferrin receptor: Biological aspects and clinical usefulness as quantitative measure of erythropoiesis. *Haematologica* 77:1.

Carriaga, M.T., Skikne, B.S., Finley, B., Cutler, B. and Cook, J.D. 1991, Serum transferrin receptor for the detection of iron deficiency in pregnancy. *Am J Clin Nutr* 54:1077.

Cook, J.D., Alvarado, J., Gutniskey, A., Jamra, M., Labardini, J., Layrisse, M., Linares, J., Loria, A., Maspes, V., Restrepo, A., Reynafarje, C., Sanchez-Medal, L., Velez, H. and Viteri, F. 1971, Nutritional deficiency and anemia in Latin America: a collaborative study. *Blood* 38:591.

Cook, J.D., Carriaga, M., Kahn, S.G., Schalch, W. and Skikne, B.S. 1990, Gastric delivery system for iron supplementation. *Lancet* 335:1136.

Cook, J.D., Skikne, B.S. and Baynes, R.D. 1993, Serum transferrin receptor. *Annu Rev Med* 44:63.

DeMaeyer, E. and Adiels-Tegman, M. 1985, The prevalence of anaemia in the world. *Wld Hlth Statist Quart* 38:302.

Ferguson, B.J., Skikne, B.S., Simpson, K.M., Baynes, R.D. and Cook, J.D. 1992, Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. *J Lab Clin Med* 119:385.

Flowers, C.H., Skikne, B.S., Covell, A.M. and Cook, J.D. 1989, The clinical measurement of serum transferrin receptor. *J Lab Clin Med* 114:368.

Garby, L. and Areekul, S. 1974, Iron supplementation in Thai fish-sauce. *Ann Trop Med Parasitol* 68:467.

Garn, S.M., Ridella, S.A., Tetzold, A.S. and Falkner, F. 1981, Maternal haematological levels and pregnancy outcomes. *Semin Perinatol* 5:155.

Gillespie, S., Kevany, J. and Mason, J. 1991, *Controlling Iron Deficiency - A Report based on a ACC/SCN Workshop*, Lavenham Press Ltd., Suffolk, England.

Hallberg, L. 1992, Iron balance in pregnancy and lactation. in: *Nutritional Anemias, Nestle Nutrition Workshop Series, Vol. 30*, S.J. Fomon and S. Zlotkin. eds., Nestec Ltd., Vevey/Raven Press, New York.

Huebers, H.A., Beguin, Y., Pootrakul, P., Einspahr, D. and Finch, C.A. 1990, Intact transferrin receptors

in human plasma and their relation to erythropoiesis. *Blood* 75:102.

Huebers, H.A. and Finch, C.A. 1987, The physiology of transferrin and transferrin receptors. *Physiol Rev* 67:520.

Idjradinata, P. and Pollitt, E. 1993, Reversal of developmental delays in iron-deficient anaemic infants treated with iron. *Lancet* 341:1.

Kohgo, Y. 1986, Structure of transferrin and transferrin receptor. *Acta Haematol Jpn* 49:1627.

Kohgo, Y., Nishisato, T., Kondo, H., Tsushima, N., Niitsu, Y. and Urushizaki, I. 1986, Circulating transferrin receptor in human serum. *Br J Haematol* 64:277.

Levin, H.M. 1986, A benefit-cost analysis of nutritional programs for anaemia reduction. *Res Observ* 1:219.

Lieberman, E., Ryan, K.J., Monson, R.R. and Schoenbaum, S.C. 1988, Association of maternal hematocrit with premature labor. *Am J Obstet Gynecol* 159:107.

Lozoff, B., Brittenham, G.M., Viteri, F.E., Wolf, A.W. and Urrutia, J.J. 1982, The effects of short-term oral iron therapy on developmental deficits in iron-deficient anemic infants. *J Pediatr* 100:351.

Lozoff, B., Brittenham, G.M., Wolf, A.W., McClish, D.K., Kuhnert, P.M., Jimenez, E., Jimenez, R., Mora, L.A., Gomez, I. and Krauskoph, D. 1987, Iron deficiency anemia and iron therapy: Effects on infant developmental test performance. *Pediatrics* 79:981.

Lozoff, B., Jimenez, E. and Wolf, A.W. 1991, Long-term developmental outcome of infants with iron deficiency. *N Engl J Med* 325:687.

Murphy, J.F., O'Riordan, J., Newcombe, R.G., Coles, E.C. and Pearson, J.F. 1986, Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. *Lancet* 1:992.

Ohira, Y., Edgerton, V.R., Gardner, G.W., Senewiratne, B., Barnard, R.J. and Simpson, D.R. 1979, Work capacity, heart rate and blood lactate responses to iron treatment. *Br J Haematol* 41:365.

Oski, F.A., Honig, A.S., Helu, B. and Howanitz, P. 1983, Effect of iron therapy on behavior performance in nonanemic, iron-deficient infants. *Pediatrics* 71:877.

Oski, F.A. and Honig, A.S. 1978, The effects of therapy on the developmental scores of iron-deficient infants. *J Pediatr* 92:21.

Salonen, J.T., Nyyssonen, K., Korp[illegible] Tuomilehto, J., Seppanen, R. and Salonen, R. 1992, High stored iron levels are associa[illegible] excess risk of myocardial infarction in Eastern Finnish men. *Circulation* 86:803.

Scholl, T.O., Hediger, M.L., Fischer, R.L. and Shearer, J.W. 1992, Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. *Am J Clin Nutr* 55:985.

Schultink, W., van der Ree, M., Matulessi, P. and Gross, R. 1993, Low compliance with an iron-supplementation program: A study among pregnant women in Jakarta, Indonesia. *Am J Clin Nutr* 57:135.

Shih, Y.J., Baynes, R.D., Hudson, B.G., Flowers, C.H., Skikne, B.S. and Cook, J.D. 1990, Serum transferrin is a truncated form of tissue receptor. *J Biol Chem* 265:19077.

Simmons, W.K., Cook, J.D., Bingham, K.C., Thomas, M., Jackson, J., Jackson, M., Ahluwalia, N., Kahn, S.G. and Patterson, A.W. 1993, Evaluation of a gastric delivery system for iron supplementation in pregnancy. *Am J Clin Nutr* (In Press)

Skikne, B.S., Flowers, C.H. and Cook, J.D. 1990, Serum transferrin receptor: A quantitative measure of tissue iron deficiency. *Blood* 75:1870.

Stevens, R.G., Jones, D.Y., Micozzi, M.S. and Taylor, P.R. 1988, Body iron stores and the risk of cancer. *N Engl J Med* 319:1047.

Trowbridge, F.L., Harris, S.S., Cook, J., Dunn, J.T., Florentino, R.F., Kodyat, B.A., Mannar, M.G.V., Reddy, V., Tontisirin, K., Underwood, B.A. and Yip, R. 1993, Coordinated strategies for controlling micronutrient malnutrition: A technical workshop. *J Nutr* 123:775.

Viteri, F.E., Alvarez, E., Pinedor, O. and Torun, B. 1983, Prevention of iron deficiency by means of iron fortification of sugar. in: *Nutrition Intervention Strategies in National Development*, B.A. Underwood. ed., Academic Press, New York.287

Walter, T., Kovalskys, J. and Stekel, A. 1983, Effect of mild iron deficiency on infant mental development scores. *J Pediatr* 102:519.

Walter, T., De Andraca, I., Chadud, P. and Perales, C.G. 1989, Iron deficiency anemia: Adverse effects on infant psychomotor development. *Pediatrics* 84:7.

Walter, T. 1992, Early and long-term effect of iron deficiency anemia on child development. in: *Nutritional Anemias, Nestle Nutrition Workshop Series, Vol. 30*, S.J. Fomon and S. Zlotkin. eds., Nestec Ltd., Vevey/Raven Press, Ltd., New York.81

Ward, J.H. 1987, The structure, function, and regulation of transferrin receptors. *Invest Radiol* 22:74.

Working Group on Fortification of Salt with Iron. 1982, Use of common salt fortified with iron in the control and prevention of anemia -- a collaborative study. *Am J Clin Nutr* 35:1442.

MECHANISM OF PRODUCTION OF THE SERUM TRANSFERRIN RECEPTOR

Roy D. Baynes, Yuan J. Shih, James D. Cook

Division of Hematology
Department of Medicine
University of Kansas Medical Center
Kansas City, KS 66160-7402, USA

INTRODUCTION

The detection of a soluble form of the transferrin receptor (TfR) in human sera has been a major advance in hematological and ferrobiological research. Since the predominant expression of TfR is on the erythroid colony forming unit and late erythroid progenitors, the serum TfR would be expected to provide a quantitative measure of the size of the late progenitor pool. This has been confirmed by its excellent correlation with the size of this pool as determined ferrokinetically (Huebers et al. 1990). The other determinant of tissue receptor mass is the cellular iron content. A raised serum TfR is the earliest biochemical indicator of depletion of the functional iron pool (Skikne et al. 1990) and the most reliable indicator of iron deficiency in pregnancy where other measurements, including hemoglobin concentration, serum iron, percentage saturation, and serum ferritin, are often not helpful (Carriaga et al. 1991). Serum TfR is the only non-invasive measure to reliably distinguish the anemia of chronic disease from that of iron deficiency (Ferguson et al. 1992). Given these attributes, this measurement is becoming a crucial component in the evaluation of population iron nutriture (Cook et al. 1992; Cook et al. 1993a; Cook et al. 1993b).

Early studies employing sequential imaging of gold-labelled transferrin after endocytosis (Harding et al. 1983) along with electron microscopic and immunochemical evaluation (Pan and Johnstone, 1983; Pan and Johnstone, 1984; Pan et al. 1985; Johnstone et al. 1987; Johnstone et al. 1989; Johnstone et al. 1991) have established a pathway of cellular TfR loss in terminally maturing erythroid elements. This is characterized by a process in which TfR-ligand bearing endocytic vesicle membrane undergoes intravesicular outpouching resulting in a large vesicle (multivesicular body) containing multiple 50 nm vesicles (exosomes). These are expelled from the cell by exocytosis. The demonstration of serum TfR has been interpreted by some as reflecting this process (Kohgo, 1986; Kohgo et al. 1986; Kohgo et al. 1987a; Kohgo et al. 1987b; Beguin et al. 1988; Huebers et al. 1990; Chitambar et al. 1991). This notion appeared untenable, however, since ready quantitation of a particulate form of receptor in a sandwich ELISA (Flowers et al. 1989) would *a priori* seem unlikely.

BIOCHEMICAL IDENTIFICATION OF THE SERUM TfR

To facilitate biochemical identification, the serum TfR was successfully isolated by monoclonal antibody affinity chromatography. The antibodies had been produced in this laboratory against purified intact receptor from human placenta as antigen for use in a monoclonal ELISA (Flowers et al. 1989). Analysis of this purified serum TfR revealed that it is always isolated as a complex with transferrin. It has a molecular mass of 85 kDa as compared to the intact receptor monomer of 95 kDa. It reacts with the monoclonal antibodies and is glycosylated as evidenced by positive PAS staining and a reduction in molecular mass after treatment with N glycanase. Its biochemical identity based on amino terminal amino acid sequence analysis is shown below and is compared with residues 95-119 of the intact TfR (Shih et al. 1990).

Serum TfR											L	A	G	X	E	S	P	V	X	E	E	P	G	E	D	F	P	A	A	***
Intact TfR	L	T	E	C	E	R	L	A	G	T	E	S	P	V	R	E	E	P	G	E	D	F	P	A	A					***

X indicates non-identified residues

It is therefore clear that the serum TfR is an abbreviated receptor ectodomain with the truncation event occurring between arginine (100) and leucine (101). The non-identification of residue 104 in the serum TfR may relate to glycosylation. In this regard, threonine (104) has been identified as the only site of O-linked glycosylation in the TfR molecule (Hayes et al. 1992; Do and Cummings, 1992).

DOMAIN SPECIFIC ANTI-PEPTIDE ANTIBODIES

To facilitate the evaluation of the mechanism of production of the serum TfR, polyclonal antibodies were produced to specific peptide sequences in the endo- and ectodomains of the TfR on either side of the biologically relevant truncation site. The sequences employed are shown below.

31	G	D	N	S	H	V	E	M	K	L	A	V	D	E	E	E	N	A	D	N	N	T	K	A	N	V	T	K	P	P
61	R	C	S	G	S	I	C	Y	G	T	I	A	V	I	Y	F	F	L	I	G	F	M	I	G	Y	L	G	Y	C	K
91	G	V	E	P	L	T	E	C	E	R	L	A	G	T	E	S	P	V	R	E	E	P	G	E	D	F	P	A	A	R
											↑																			
121	R	L	Y	W	D	D	L	K	R	K	L	S	E	K	L	D	S	T	D	F	T	S	T	I	K	L	L	N	E	N
	⇑																													

40-54 - endodomain peptide; 107-120 - ectodomain peptide; bold - membrane spanning region; ↑ - truncation site (Shih et al. 1990); ⇑ - putative trypsin cleavage site (Turkewitz et al. 1988)

These sequences were selected on account of their strategic location, their hydrophilicity, proline richness, and paucity of intra-sequence lysines. The synthesis of these peptides, their purification, linkage to keyhole limpet hemocyanin, production of polyclonal antibodies, and their reactivity have been fully described previously (Shih et al. 1993). To summarize, the endodomain peptide antibody reacts with purified intact TfR and would be expected to react with the cytoplasmic TfR remnant remaining after truncation. It does not react with purified serum TfR. The ectodomain peptide antibody reacts with

both intact and serum TfR but would not be expected to react with the cytoplasmic remnant.

The biologically relevant truncation site (Shih et al. 1990) is clearly distinct from the previously reported trypsin cleavage site between arginine (121) and leucine (122)(Turkewitz et al. 1988). The ectodomain peptide sequence situated as it is between the biologically relevant truncation site and the trypsin cleavage site would be expected to distinguish between a soluble ectodomain resulting from biological truncation as opposed to trypsin cleavage.

To verify this, the time dependent reactivity of receptor fragments with the ectodomain and monoclonal antibodies, after exposure of purified intact TfR to trypsin, were evaluated. The incubation of intact TfR, purified from human placenta, with trypsin was terminated by the addition of soybean trypsin inhibitor. The receptor fragments and their reactivities were evaluated by Western blotting. The results of Western blotting with ectodomain antibody after electrophoresis under non-reducing conditions are shown in Figure 1.

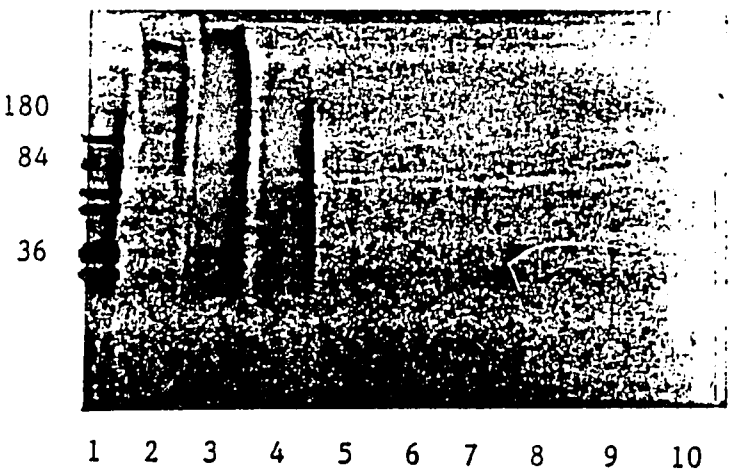


Figure 1. Western blot with ectodomain antibcdy of the time dependent product of tryptic hydrolysis of intact TfR. The duration of incubations were 0 (lane 2), 1 (lane 3), 5 (lane 4), 15 (lane 5), 30 (lane 6), 60 (lane 7), 90 (lane 8), 150 (lane 9), and 300 minutes (lane 10). Material was electrophoresed under non-reducing conditions. Purified intact TfR is present in lane 2 and molecular weight indicators in lane 2.

It can be seen that the 85 kDa protein is transiently detected (lanes 3, 4, and 5). By contrast, Western blot of the identical material with monoclonal antibody (Flowers et al. 1989) reveals the 85 kDa moiety only transiently and very faintly (lane 3) while at subsequent times large amounts of proteins slightly smaller than 85 kDa are detectable (Figure 2).

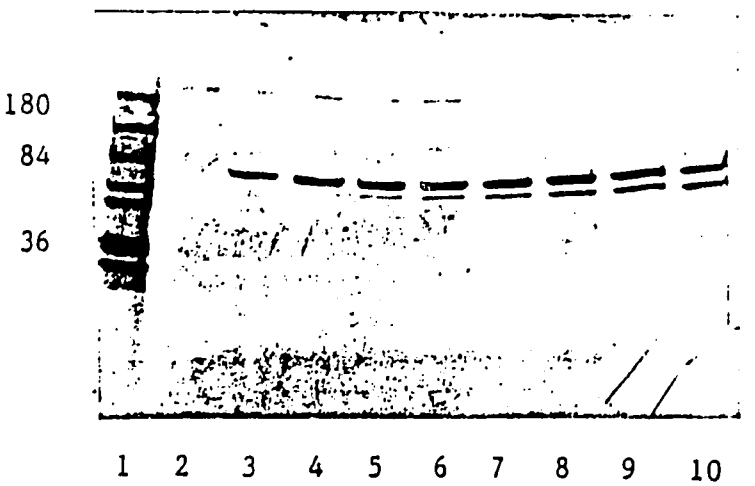


Figure 2. Western blot with monoclonal anti-TfR antibody of the identical material used in Figure 1.

Three conclusions can be drawn from this study. Trypsin digestion transiently yields an 85 kDa soluble TfR but the limit product is significantly smaller than serum TfR, compatible with the previously reported trypsin cleavage site (Turkewitz et al. 1988). The ectodomain peptide antibody detects the transient 85 kDa moiety but, as predicted, not the limit product of trypsin digestion. The epitope present on the TfR which is detected by the monoclonal antibody must not include the region between amino acid positions 100 and 122.

EVALUATION OF HUMAN SERA WITH DOMAIN SPECIFIC ANTIBODIES

Development of domain specific anti-peptide antibodies facilitated confirmation of the biochemical identity of the serum TfR and exclusion of the possibility that a significant intact TfR population bound to exosomes was not being detected by the monoclonal antibody affinity isolation. These investigations employed competitive binding ELISAs to measure ecto- and endodomain content of human sera after ultracentrifugation and of the resultant pellets after they had been solubilized in detergent (Shih et al. 1993). To summarize these findings, in normal subjects exosomes account for <1% of circulating non-cell associated TfR with >99% in the form of the truncated ectodomain. In subjects with the enhanced erythropoiesis of sickle cell disease, exosomes account for <3% of circulating non-cell associated TfR. A finding of potential significance is that in detectable exosomes there appears to be an excess of endodomains over ectodomains.

VALIDATION OF AN *IN VITRO* MODEL OF SOLUBLE RECEPTOR PRODUCTION

To extend the evaluation of mechanisms of serum TfR production, it was necessary to establish *in vitro* models. The initial *in vitro* system evaluated was the K562 erythroleukemic cell line in culture. Soluble TfR was detected in culture supernatant using our monoclonal antibody ELISA (Baynes et al. 1991a). To biochemically identify this soluble TfR it was isolated by monoclonal antibody affinity chromatography by methods employed to purify the serum TfR (Shih et al. 1990). It was characterized electrophoretically, immunologically, by glycoprotein staining, and by means of amino terminal amino acid sequencing (Baynes et al. 1991c). Like serum TfR soluble TfR is an 85 kDa glycoprotein which results from truncation of the intact TfR between arginine (100) and leucine (101)(Baynes et al. 1991c). Like serum TfR, soluble TfR in culture supernatant varies in direct proportion to cellular TfR mass which, in turn, is inversely related to the cellular iron status (Baynes et al. 1991a). Culture supernatant was further evaluated in a number of ways for the presence of exosomes. Pellets obtained by ultracentrifugation were subjected to electron microscopy, monoclonal antibody ELISA after solubilization in detergent, and Western blot analysis (Baynes et al. 1991c; Baynes et al. 1993a). In addition, the difference between TfR assayed in supernatant before and after solubilization in detergent was shown to be closely related to the exosome TfR measured in the pellet obtained by ultracentrifugation. Based on these analyses, approximately 20% of the non-cell associated TfR in K562 supernatant is accounted for by exosomes.

A second cell culture system employing the HL60 promyelocytic cell line was also studied. The process of soluble TfR release from these cells is qualitatively the same as K562 cells. However, analysis employing the domain specific peptide antibodies reveals that HL60 cells release the same 85 kDa soluble TfR but at 2-3 times the rate of K562 cells. Exosomes in this system account for <5% of the non-cell associated TfR (Baynes et al. 1991b; Baynes et al. 1993a).

These two well-validated cell culture systems producing, as they do, quantitatively dominant amounts of soluble TfR and minor amounts of intact TfR bound to exosomes were employed to establish the predominant site of receptor truncation.

THE SUBCELLULAR SITE OF TfR TRUNCATION

Having validated *in vitro* models of serum TfR production and developed domain specific antibodies on either site of the biologically relevant truncation site, these were employed to study the subcellular components of the TfR. More specifically, solubilized cells, solubilized exosomes, and post-ultracentrifugation supernatant were evaluated by Western blotting with endo- and ectodomain peptide antibodies. Solubilized cell membranes contain predominantly intact TfR of 190 kDa under non-reducing conditions detected by both antibodies. They also contain minor amounts of a 105 kDa TfR detected by both antibodies but predominantly the one against the ectodomain. This is compatible with a monomeric truncation of the intact TfR. The culture supernatant contains predominantly an 85 kDa moiety detected only by ectodomain antibody compatible with serum TfR. The solubilized exosomes contain intact 190 kDa TfR and the same 105 kDa moiety found in cell membranes. There appears to be more 105 kDa moiety in the exosome fraction. In addition, the solubilized exosomes contain a 20 kDa protein which reacts only with ectodomain antibody compatible with the cytoplasmic remnant of TfR after truncation of both ectodomain monomers (Baynes et al. 1993a). Intrinsic to the interpretation of these findings is the assumption that the intact cell does not clear endodomain remnants at an accelerated rate. That this assumption is valid is suggested by the finding that production of endodomain remnant dimer by treatment of cells with trypsin does not modify the rate of its cellular catabolism (Rutledge et al. 1991).

These findings, therefore, suggest that truncation by proteolysis of TfR is most complete at the exosome surface as compared to the cell surface. This does not, however, preclude a significant role for cell surface truncation.

A MEMBRANE ASSOCIATED SERINE PROTEASE IS CAPABLE OF PRODUCING SERUM TfR

The production of the ectodomain peptide antibody provided an excellent assay for the detection of the biologically relevant serum TfR ectodomain and for distinguishing it from the ectodomain resulting from trypsin hydrolysis. This antibody was used in Western blot analysis after incubation of cell culture fractions at 37°C with purified intact TfR. The fractions studied included concentrated supernatant, density separated post-nuclear cell membrane fractions, and cytoplasm. Surface membranes were identified by enrichment for 5' nucleotidase activity. Because HL60 cells produced more soluble TfR than K562 cells, these were studied first.

A synopsis of the results of these studies is that culture supernatant and cytoplasm lacked the appropriate proteolytic activity whereas the surface membrane fraction with the highest 5' nucleotidase to protein ratio contained proteolytic activity capable of producing the biologically relevant soluble TfR (Baynes et al. 1993b). Using various protease inhibitors, this activity was identified as being mediated by a serine protease.

These studies indicate that a surface membrane associated serine protease is present in HL60 cells which has the ability to produce the serum TfR. Since the multivesicular body is a structure where surface derived membrane associated protease, both on the exosomes and the limiting membrane of the vesicle are in close proximity to membrane bound intact TfR on both structures this would be a logical site for receptor truncation.

This would also be in accord with our demonstration that the exosome appears to be the subcellular site of most complete TfR truncation.

THE SUBCELLULAR SITE OF EXOSOME TfR TRUNCATION

The data presented thus far indicate that the TfR on the exosome surface is subject to the most complete truncation. Further, culture supernatant lacks the protease to effect the biologically relevant truncation suggesting that this does not occur at the exosome surface once released by exocytosis. To confirm that soluble TfR is not released from exosomes liberated from cells, studies were conducted in which post-cell culture supernatant was incubated at 37°C and evaluated at various times for exosome and soluble TfR content. These studies employed the monoclonal antibody ELISA. A typical example is shown in Figure 3 where it can be seen that over 22 hours of incubation TfR content of soluble and exosome fractions remained unchanged. This work was conducted with HL60 cell cultures. The same result was obtained with K562 cell cultures except that the ratio of exosome to soluble TfR was slightly higher in keeping with our prior quantitative data.

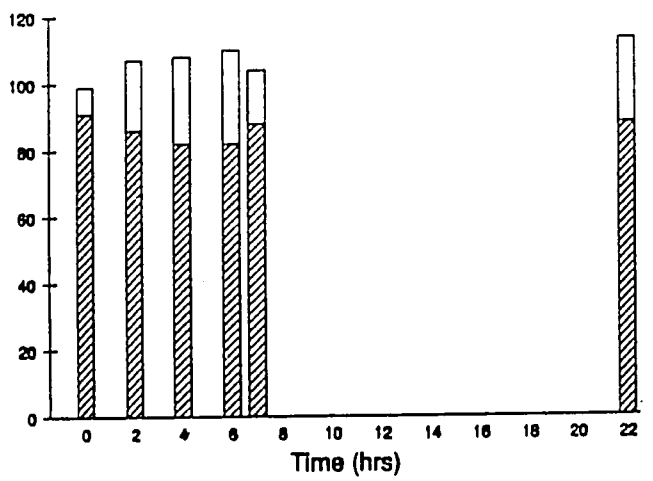


Figure 3. The soluble (hatched bars) and exosome (open bars) TfR content of HL60 culture supernatant during incubation at 37°C. The values are expressed as a percentage of the starting receptor content.

DISCUSSION

These data lead to the model that serum TfR is produced by proteolysis predominantly at the exosome surface and to a lesser extent at the cell surface. This proteolysis appears to be mediated by a membrane associated serine protease. Proteolysis at the exosome surface appears to occur prior to exocytosis, probably within the multivesicular body.

Our data on the tracking of the receptor remnants with the domain specific peptide antibody have been presented in abstract and communicated in full (Baynes et al. 1992; Baynes et al. 1993a). Subsequently, work has appeared using a similar approach and reports to find evidence for cytoplasmic domain remnants of the TfR in exosomes obtained from *ex vivo* matured sheep and human reticulocytes (Ahn and Johnstone, 1993). While superficially confirmatory, there are certain important aspects in which this work differs from the current study. This work (Ahn and Johnstone, 1993) reports that *ex vivo* matured sheep reticulocytes release more exosome TfR than soluble TfR, whereas both our *in vitro* and *in vivo* data reveal the opposite. These workers report that sheep soluble TfR is a truncated dimer of 160 kDa whereas our serum TfR is clearly an 85 kDa monomer. They indicate that cytoplasmic TfR remnant on sheep exosomes consists, under reducing conditions, of a predominantly 17 kDa moiety but with significant amounts of a 25 kDa

protein and material between 17 and 25 kDa and that human exosomes show the identical remnant cytoplasmic domains, whereas the current data report a single remnant of 20 kDa under non-reducing conditions. They theorize that truncation occurs at amino acid position 130 whereas our sequence data clearly establish truncation as occurring at position 100. They indicate that intact TfR is equally evident on a Western blot of a single human serum sample as compared to the serum TfR whereas exosome TfR in our assay studies is trivial in comparison to serum TfR. Clearly, these numerous differences may relate, in part, to species differences and to differences in the cellular models. In this regard, it remains to be seen whether *ex vivo* reticulocyte maturation (a terminal process) is reflective of serum TfR production by all cells *in vivo*. Another possible explanation may relate to the immunologic reagents used. In this regard, it should be noted that their data in sheep are based on cross species immunological reagents. It would also be important to know whether the monoclonal antibody employed detects TfR equally regardless of whether it is free or bound to ligand. The question of the specificity and sensitivity of the reagents is highlighted by their stated inability to detect exosomal material in HL60 cells whereas it is readily detectable with our reagents. This potential problem is further emphasized by these workers' observations that serum TfR could not be detected in rats. In unpublished experiments using immunological reagents developed in this laboratory to the intact rat TfR we find, analogous to the human situation that serum TfR is the predominant circulating non-cell associated TfR. Clearly, further evaluation of these reported data is indicated.

WORKING MODEL

Serum TfR is a truncated ectodomain monomer resulting from proteolytic cleavage between arginine 100 and leucine 101. The predominant site of receptor proteolysis appears to be at the surface of the exosome. The cell surface may be a secondary site of proteolysis. Cleavage is mediated by a surface membrane associated serine protease. Exosome TfR proteolytic cleavage appears to occur intracellularly, probably within the multivesicular body. The factors predisposing TfR to inclusion within the exosome and to proteolytic cleavage remain important questions for further investigation.

REFERENCES

Ahn, J. and Johnstone, R.M. 1993, Origin of a soluble truncated transferrin receptor. *Blood* 81:2442.

Baynes, R.D., Shih, Y.J. and Cook, J.D. 1991a, Production of soluble transferrin receptor by K562 erythroleukemia cells. *Br J Haematol* 78:450.

Baynes, R.D., Shih, Y.J. and Cook, J.D. 1991b, In vitro and vivo quantitation of soluble and exosome forms of the transferrin receptor. *Blood* 78:89a.

Baynes, R.D., Shih, Y.J., Hudson, B.G. and Cook, J.D. 1991c, Characterization of transferrin receptor released by K562 erythroleukemia cells. *Proc Soc Exp Biol Med* 197:416.

Baynes, R.D., Shih, Y.J. and Cook, J.D. 1992, Mechanism of soluble transferrin receptor production as determined by domain specific antibodies. *Blood* 80:278a.

Baynes, R.D., Shih, Y.J., Hudson, B.G. and Cook, J.D. 1993a, Identification of the membane remnants of transferrin receptor using domain specific antibodies. *J Lab Clin Med* (Communicated).

Baynes, R.D., Shih, Y.J., Hudson, B.G. and Cook, J.D. 1993b, Production of the serum form of the transferrin receptor by a cell membrane associated serine protease. *Proc Soc Exp Biol Med* (In Press)

Beguin, Y., Huebers, H.A., Josephson, B. and Finch, C.A. 1988, Transferrin receptors in rat plasma. *Proc Natl Acad Sci USA* 85:637.

Carriaga, M.T., Skikne, B.S., Finley, B., Cutler, B. and Cook, J.D. 1991, Serum transferrin receptor for the detection of iron deficiency in pregnancy. *Am J Clin Nutr* 54:1077.

Chitambar, C.R., Loebel, A.L. and Noble, N.A. 1991, Shedding of transferrin receptor from rat reticulocytes during maturation in vitro: Soluble receptor is derived from receptor shed in vesicles. *Blood* 78:2444.

Cook, J.D., Baynes, R.D. and Skikne, B.S. 1992, Iron deficiency and the measurement of iron status. *Nutr Res Rev* 5:189.

Cook, J.D., Skikne, B.S. and Baynes, R.D. 1993a, Screening strategies for nutritional iron deficiency. in: *Nutritional Anemias*, S. Fomon and S. Zlotkin. eds., Nestec and Raven Press, New York.

Cook, J.D., Skikne, B.S. and Baynes, R.D. 1993b, Serum transferrin receptor. *Annu Rev Med* 44:63.

Do, S.I. and Cummings, R.D. 1992, Presence of O-linked oligosaccharide on a threonine residue in the human transferrin receptor. *Glycobiology* 2:345.

Ferguson, B.J., Skikne, B.S., Simpson, K.M., Baynes, R.D. and Cook, J.D. 1992, Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. *J Lab Clin Med* 119:385.

Flowers, C.H., Skikne, B.S., Covell, A.M. and Cook, J.D. 1989, The clinical measurement of serum transferrin receptor. *J Lab Clin Med* 114:368.

Harding, C., Heuser, J. and Stahl, P. 1983, Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J Cell Biol* 97:329.

Hayes, G.R., Enns, C.A. and Lucas, J.J. 1992, Identification of the O-linked glycosylation site of the human transferrin receptor. *Glycobiology* 2:355.

Huebers, H.A., Beguin, Y., Pootrakul, P., Einspahr, D. and Finch, C.A. 1990, Intact transferrin receptors in human plasma and their relation to erythropoiesis. *Blood* 75:102.

Johnstone, R.M., Adam, M., Hammond, J.R., Orr, L. and Turbide, C. 1987, Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *J Biol Chem* 262:9412.

Johnstone, R.M., Bianchini, A. and Teng, K. 1989, Reticulocyte maturation and exosome release: Transferrin receptor containing exosomes shows multiple plasma membrane functions. *Blood* 74:1844.

Johnstone, R.M., Mathew, A., Mason, A.B. and Teng, K. 1991, Exosome formation during maturation of mammalian and avian reticulocytes: evidence that exosome release is a major route for externalization of obsolete membrane proteins. *J Cell Physiol* 147:27.

Kohgo, Y. 1986, Structure of transferrin and transferrin receptor. *Acta Haematol Jpn* 49:1627.

Kohgo, Y., Nishisato, T., Kondo, H., Tsushima, N., Niitsu, Y. and Urushizaki, I. 1986, Circulating transferrin receptor in human serum. *Br J Haematol* 64:277.

Kohgo, Y., Niitsu, Y., Kondo, H., Kato, J., Tsushima, N., Sasaki, K., Hirayama, M., Numata, T., Nishisato, T. and Urushizaki, I. 1987a, Serum transferrin receptor as a new index of erythropoiesis. *Blood* 70:1955.

Kohgo, Y., Niitsu, Y., Nishisato, T., Kato, J., Sasaki, K., Tsushima, N., Hirayama, M., Kondo, H. and Urushizaki, I. 1987b, Externalization of transferrin receptor in established human cell lines. *Cell Biol Int Rep* 11:871.

Pan, B.T., Teng, K., Wu, C., Adam, M. and Johnstone, R.M. 1985, Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *J Cell Biol* 101:942.

Pan, B.T. and Johnstone, R. 1984, Selective externalization of the transferrin receptor by sheep reticulocytes in vitro. Response to ligands and inhibitors of endocytosis. *J Biol Chem* 259:9776.

Pan, B.T. and Johnstone, R.M. 1983, Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. *Cell* 33:967.

Rutledge, E.A., Mikoryak, C.A. and Draper, R.K. 1991, Turnover of the transferrin receptor is not influenced by removing most of the extracellular domain. *J Biol Chem* 266:21125.

Shih, Y.J., Baynes, R.D., Hudson, B.G., Flowers, C.H., Skikne, B.S. and Cook, J.D. 1990, Serum transferrin is a truncated form of tissue receptor. *J Biol Chem* 265:19077.

Shih, Y.J., Baynes, R.D., Hudson, B.G. and Cook, J.D. 1993, Characterization and quantitation of the circulating forms of serum transferrin receptor using domain specific antibodies. *Blood* 81:234.

Skikne, B.S., Flowers, C.H. and Cook, J.D. 1990, Serum transferrin receptor: A quantitative measure of tissue iron deficiency. *Blood* 75:1870.

Turkewitz, A.P., Amatruda, J.F., Borhani, D., Harrison, S.C. and Schwartz, A.L. 1988, A high yield purification of the human transferrin receptor and properties of its major extracellular fragment. *J Biol Chem* 263:8318.

Coordinated Strategies for Controlling Micronutrient Malnutrition: A Technical Workshop[1,2]

FREDERICK L. TROWBRIDGE, SUZANNE S. HARRIS,*[3] JAMES COOK,† JOHN T. DUNN,‡ RUDOLFO F. FLORENTINO,** BENNY A. KODYAT,†† M. G. VENKATESH MANNAR,‡‡ VINODINI REDDY,*** KRAISID TONTISIRIN,††† BARBARA A. UNDERWOOD††† AND RAY YIP

Division of Nutrition, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control, Atlanta, GA 30333; *Human Nutrition Institute, International Life Sciences Institute Research Foundation, Washington, DC 20036; †Division of Hematology, University of Kansas Medical Center, Kansas City, KS 66103; ‡International Council for Control of Iodine Deficiency Disorders, University of Virginia School of Medicine, Charlottesville, VA 22908; **Food and Nutrition Research Institute, Manila 1000, Philippines; ††Directorate of Nutrition, Ministry of Health, Jakarta, 12950, Indonesia; ‡‡87 Hillcroft Drive, Etobicoke, Ontario M9B 4X8, Canada; ***National Institute of Nutrition, Tarnaka, Hyderabad, Andhra Pradesh 500 007, India; †††Institute of Nutrition, Mahidol University at Salaya, Nakorn Chaisri, Nakorn Pathom 73170, Thailand; ‡‡‡National Eye Institute, National Institutes of Health, Bethesda, MD 20892

ABSTRACT Participants in a November 1991 workshop concluded that coordinated strategies for controlling malnutrition due to iodine, iron, vitamin A and other micronutrients deficiencies are technically feasible and should be given consideration in planning control efforts. Coordinated surveys involving clinical, biochemical and dietary assessment of multiple micronutrients are feasible. Multiple fortification is also possible using such vehicles as salt, processed rice or sugar. Supplementation efforts can be integrated with existing health care programs. Food-based strategies are also effective. The best examples have been community-based and have included a strong nutrition and health education component designed to change food consumption patterns, improve food preservation and preparation practices, and link income-generating activities with food production activities. Successful coordinated efforts will require a strong political commitment and a supportive infrastructure. Specific recommendations include the formation of national coordinating bodies for micronutrient deficiency control, establishment of a micronutrient information network and expansion of technical exchange and training. J. Nutr. 123: 775–787, 1993.

INDEXING KEY WORDS:

- *micronutrient malnutrition* • *iodine*
- *iron* • *vitamin A* • *humans*

BACKGROUND

Twenty percent of the world's population, primarily in developing countries, is at risk for micronutrient malnutrition. Iodine, iron, and vitamin A deficiencies are of great public health importance because of their high prevalence, related morbidity and mortality, and preventability. About 16% to 18% of the world's population (or approximately 1 billion people) live in iodine-deficient areas, placing them at risk for permanent brain damage, mental retardation, reproductive failure, decreased child survival, goiter and socioeconomic stagnation (Task Force 1992).

Iron deficiency is the most prevalent micronutrient deficiency of public health importance in the world and the most common cause of nutritional anemia in

[1]Summary of a workshop held November 7–9, 1991, in Atlanta, GA, sponsored by the International Life Sciences Institute in collaboration with the Centers for Disease Control and the Program Against Micronutrient Malnutrition. Guest editor for this publication was Suzanne S. Harris, Human Nutrition Institute, ILSI Research Foundation, Washington, DC.

[2]The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC Section 1734 solely to indicate this fact.

[3]To whom correspondence should be addressed.

0022-3166/93 $3.00 © 1993 American Institute of Nutrition.

children and women of reproductive age (15 to 49 years) (De Maeyer 1989). The primary consequences of iron deficiency anemia are impaired child development and reduced work capacity and economic output. In developing regions, 51% of children under 4 years of age and 59% of women of childbearing age are estimated to have anemia. Worldwide, approximately 1 billion people are anemic due to iron deficiency.

Vitamin A deficiency, the most preventable cause of irreversible blindness, affects nearly 14 million children clinically and 50 million more children subclinically in developing countries. Approximately 190 million children are at risk for vitamin A deficiency. Each year, at least 350,000 new cases of irreversible blindness in children are caused by vitamin A deficiency; more than 60% of these children die within one year of diagnosis. Children with subclinical cases of vitamin A deficiency are at increased risk of dying from infections (World Hunger Program 1989).

Prevention and control of micronutrient malnutrition could substantially affect worldwide socioeconomic development by increasing child survival, learning potential, productivity, self-reliance and quality of life. Effective control strategies have been proposed for each micronutrient deficiency, but the opportunity for coordinating these strategies has not yet been fully explored. Various factors make such exploration imperative:

- Population groups at risk for vitamin A, iron, and iodine deficiencies frequently overlap.
- Surveillance mechanisms used to assess one micronutrient may be adaptable for others.
- Intervention delivery systems and facilities, as well as technical skills and education for control of one micronutrient deficiency, may be the same as or compatible with those for controlling other micronutrient deficiencies.
- Coordinated control strategies could reduce duplication of effort and costs and thereby improve the effectiveness and efficiency of programs for controlling micronutrient malnutrition.

The importance of and opportunities for coordinated control have received increasing international support during the past few years. During the 1990 World Summit for Children, sponsored by the United Nations Children's Fund (UNICEF), seventy heads of state endorsed the World Declaration and Plan of Action on the Survival, Protection, and Development of Children, which calls for elimination of vitamin A deficiency and iodine deficiency disorders and for significant reduction in iron deficiency anemia. Country representatives at this meeting committed themselves to developing national plans for coordinated control of micronutrient malnutrition. In October 1991, the World Health Organization (WHO) and UNICEF cosponsored a follow-up meeting in Montreal to establish a policy framework for international action in eliminating micronutrient deficiencies. This meeting resulted in the identification of basic policy assumptions that should underlie national and international plans (Task Force 1992).

The challenge remained, however, to identify the specific scientific and technical opportunities for and constraints against implementing effective programs for micronutrient malnutrition control. To meet this challenge, a technical workshop was convened in November 1991 titled, "Coordinated Strategies for Controlling Micronutrient Malnutrition." The objectives of this workshop were:

- To examine the feasibility of coordinated strategies for controlling micronutrient malnutrition due to iodine, vitamin A, and iron deficiencies, and
- To identify priorities for applied research to strengthen the scientific and technical base for controlling micronutrient deficiencies.

The workshop was sponsored by the International Life Sciences Institute, a public, nonprofit foundation dedicated to research and information exchange on nutrition, food safety, environmental hazards and other health-related topics. Collaborating agencies included the Centers for Disease Control and the Program Against Micronutrient Malnutrition. The Program Against Micronutrient Malnutrition is designed to help governments achieve sustained elimination of micronutrient malnutrition through training and technical support tailored to the specific needs and capacity of each country. A steering committee of technical experts was formed to design the workshop agenda.

Workshop participants, drawn from multiple agencies and organizations from government, industry and academia, represented a wide range of disciplines and technical expertise, including nutrition, nutritional epidemiology, nutritional biochemistry, nutritional anthropology, health care delivery, agriculture, economics and food technology.

The workshop began with brief presentations on possible coordinated strategies and experiences with controlling micronutrient malnutrition in India, Thailand, Indonesia and the Philippines. These presentations were followed by three concurrent work-group sessions on information collection and dissemination, fortification and supplementation, and food-based approaches. Participants were assigned to work groups based on their areas of expertise.

The work groups, which were charged with defining the current best approaches in their area, discussed coordinated approaches to the three main micronutrient deficiencies, gaps in knowledge and priorities for future research. The workshop concluded with summary presentations by the work-group leaders and a panel discussion that further explored the potential for coordinated approaches and

recommendations for research. This paper summarizes the conclusions of each work group and presents the most important implementation issues.

INFORMATION COLLECTION AND DISSEMINATION

Scope and rationale

The work group addressing information collection and dissemination identified four ways in which information is valuable to controlling micronutrient malnutrition.

- To increase awareness of the problem for advocacy at the national and international levels,
- To garner political and financial support for control efforts from key decision makers,
- To identify populations at high risk for micronutrient malnutrition and to design effective, targeted programs, and
- To monitor and evaluate programs so that progress can be measured and further control efforts planned.

The group emphasized the importance of information dissemination. Only if decision makers have access to useful and relevant data can they make informed decisions.

Opportunities and constraints

Advocacy. When using information for advocacy, it is critical to define which decisions the effort should influence and to communicate the information effectively to those who will be making those decisions. National and international advocacy efforts can be strengthened by using the following types of information:

- The prevalence and severity of micronutrient malnutrition. Common definitions and criteria should be used so that information is compatible worldwide and comparisons can be made among populations, countries and regions. Information collected should support a compelling argument for the need to reduce the micronutrient malnutrition.
- The significance of micronutrient deficiency. This information would include the physical, developmental, social, and economic consequences of micronutrient deficiency for individuals, populations and entire countries, and the benefits of alleviating deficiencies.
- The cost-effectiveness of feasible solutions for addressing the problem.

Much of this information is available but needs to be shared more widely with the persons who can best use it, including government officials and decision makers, community leaders, health professionals such as general practitioners, and students in such training programs as medicine, nursing, nutrition and agriculture. In addition, social marketing techniques can be used to increase micronutrient consumption, as has been done by the Expanded Programme on Immunization (EPI) during the past decade. Industry can provide technical expertise, help reach populations at risk and advocate increased awareness of the problem among political officials.

Indicators. **Table 1** lists various clinical and biochemical indicators available and anticipated for defining baseline prevalence and characterizing the nature and extent of micronutrient malnutrition in a population.

Planning. Several opportunities exist for coordinated assessment of micronutrients and should be pursued. Coordinated surveys can include clinical assessment related to multiple micronutrients along with simultaneous collection of blood samples for biochemical assessment of iron, vitamin A and iodine deficiencies. Dietary assessment can also be used for vitamin A, iron and other micronutrients as well as for additional dietary factors such as energy and protein. It should be recognized, however, that dietary assessment may not always produce a precise definition of the problem.

Concentration of effort on one age group (e.g., infants or children beginning school) may also be practical, but care must be taken to ensure that other important groups (e.g., women of childbearing age) are not ignored.

Coordinated strategies should be planned in collaboration with community health workers to ensure that the strategies meet local needs and constraints. Rather than relying solely on outside expertise, these health workers or other volunteers from the local area should be trained to conduct or assist in the assessments. This training would not only provide willing and able hands for baseline surveys but would also build the area's capacity to independently conduct routine monitoring and intervention programs in the future.

A major obstacle to coordinated assessment is the lack of uniform guidelines that ensure standardized application of appropriate methodologies. Such guidelines could address common methods for establishing baseline prevalence, conducting surveys, analyzing data and using results to plan programs. Guidelines would not only reduce the need for direct technical assistance for large-scale assessment efforts but would also facilitate communication among countries, donor agencies, industry and academia.

The World Health Organization is currently initiating a coordinated data base that should include baseline data on the prevalence of micronutrient deficiencies in all countries and provide information on

TABLE 1

Indicators for surveillance of micronutrients malnutrition

Micronutrient	Indicators currently available	Indicators available in 2 to 5 y
Vitamin A	History of night blindness	Dark adaptation measurement
	Bitot's spots	Blood spot retinol
	Serum retinol	Retinol by fluorescence
	Conjunctival impression cytology	(capillary tube sample)
	Relative dose-response	
	Modified relative dose-response	
Iodine	Goiter diagnosed by palpation	
	Blood spot thyroid-stimulating hormone	
	Urinary iodine	
	Thyroid size determined by ultrasound	
Iron	Hematocrit	Blood spot ferritin
	Hemoglobin	Blood spot transferrin receptor
	Erythrocyte protoporphyrin	
	Serum ferritin	

indicators appropriate for measuring the success of control efforts. Use of these indicators worldwide would allow consistent and valid monitoring of progress toward a goal of eliminating micronutrient malnutrition by the year 2000. The data base could be kept in a clearinghouse for use by all interested individuals.

Evaluation. Many of the indicators (Table 1) are also useful for program evaluation. Process measures are also needed to determine progress toward achieving program goals. Such measures should assess knowledge, attitudes and beliefs of health workers and consumers about micronutrient deficiency and how to control it. Such information could be used to determine the association between knowledge of a deficiency and its prevalence. Individual and consumer behaviors associated with micronutrient intake should also be assessed.

National surveillance systems should be strengthened to enable continuous program monitoring and evaluation. However, the work group indicated that design of control programs need not wait until these systems are fully operational.

Country experiences

India. A nationwide survey in 1974 showed that 2% of 9 million blind persons were blind due to vitamin A deficiency. In a more recent survey, vitamin A deficiency contributed to only 0.04% of cases of total blindness. Because only survivors are examined in such surveys, this may not reveal the true picture, but the decreasing trend was corroborated by a nutrition survey among preschool-age children. However, blindness due to vitamin A deficiency still varies considerably by region.

Iron deficiency anemia is most prevalent among women of childbearing age. A recent survey revealed that more than 60% of pregnant women and preschool-age children are anemic. Cereals are the major source of iron in the Indian diet; isotopic studies have shown that iron absorption from this diet is only 3% to 5%. Hookworm disease and malaria also aggravate anemia in some areas.

A recent survey in 14 districts of different Indian states showed that the prevalence of goiter ranged from 6 to 65%. More alarming is the prevalence of endemic cretinism in all the states. Other forms of iodine deficiency disorders such as dwarfism, mental retardation and deaf-mutism, are also common in areas where iodine deficiency is endemic.

Thailand. In 1973, 37% of adults had iron deficiency anemia. In 1974 and 1975, the prevalence among pregnant women was about 30% in Bangkok and 41% to 70% in rural areas. Recent data from antenatal clinics at the provincial hospital level indicate that the prevalence has declined to about 20% in this population.

In a 1988 survey the overall prevalence of iodine deficiency was 43% in 65 districts. Within one year of implementing a control program, the goiter rate dropped to 17% in one province with a high prevalence of iodine deficiency.

Indonesia. A national survey conducted in 1978 in 23 of Indonesia's 27 provinces reported a prevalence of xerophthalmia in 15 provinces that was higher than the criteria used to define public health significance. Limited repeat surveys in five provinces from 1983 to 1986 showed that the prevalence of xerophthalmia declined significantly, from 1.16 to 0.67%. A 1991 survey in four provinces of eastern Indonesia showed that xerophthalmia is a public health problem in one province and vitamin A

deficiency (as measured by serum retinol concentrations) is a problem in all four provinces.

In the past, goiter was found in populations on almost every major island of Indonesia; prevalence was 92.5% in certain areas. A goiter prevention program was implemented in 1976, and in 1980 to 1982, a national survey of primary-school children in 26 provinces revealed that goiter was endemic in 68.3% of 666 subdistricts, and cretinism was endemic in 10% of villages. Parallel surveys conducted in 1987 and 1990 showed that both the total goiter rate and the visible goiter rate decreased significantly (from 37.2 to 27.7% and from 9.2 to 6.8%, respectively).

Current prevalence of anemia among pregnant women ranges from 39.7 to 63.4%; the national average is about 52%. The prevalence of anemia among low income female workers is 30 to 40%; among adult nonlactating and nonpregnant women who use community health centers and village health posts, the prevalence is 20 to 45%. The 1991 study in four provinces revealed that the average prevalence of anemia among pregnant women and preschool-age children was 50.1 and 49.2%, respectively.

Philippines. National nutrition surveys and smaller studies conducted since 1978 have shown that the proportion of specific population groups with deficient plasma concentrations of vitamin A ranges from 2.3 to 3.4%. The highest rates of vitamin A deficiency are in children 7 to 12 years old and pregnant women. In 1982, prevalence of xerophthalmia and Bitot's spots was 3.6%. By 1987, this rate was greatly reduced, but, in certain areas, it still hovers around 4%.

Prevalence of anemia is as high as 70% among infants and is also quite high among children age 7 to 12 years, preschool-age children, pregnant and nursing women, and the elderly. Overall, the prevalence of anemia in the country is about 37%.

For goiter, the same pattern holds true; for the country as a whole, the prevalence is only about 3.5%, but, in selected areas, especially mountainous regions, a prevalence of 66% can be found among children age 7 years or older. A procedure, developed for rapidly assessing prevalence of goiter, uses interviews with schoolchildren to determine whether any of their family members have enlarged goiters. This procedure, which roughly defines areas where goiter is endemic, can be used for program planning.

Knowledge gaps

Several gaps in knowledge, identified by the work group, can serve as the basis for defining research priorities. These research needs include the following:

- Better definition of the physiological interactions of micronutrients (e.g., how they support and interfere with each other). Where iron deficiency is common, vitamin A deficiency also seems to be more prevalent. Conversely, treatment of vitamin A deficiency often results in changes or improvement in biochemical indicators of iron. The implications of this relationship need to be defined precisely for monitoring and assessment purposes. Further studies of relationships between iodine deficiency and vitamin A are needed.
- The development of more sensitive assessment tests feasible for use in the field.
- Evaluation of the performance (e.g., sensitivity and specificity) of indicators of micronutrient status for different target groups.
- Definition of the age or sex groups that could serve as optimal target groups for multiple micronutrient assessment and surveillance.
- Guidelines for coordinated assessments of multiple micronutrients (e.g., recommendations for sample size, data management and analysis).
- The effects of various dietary interventions on dietary intake.
- Documentation of costs and benefits of individual and combined micronutrient interventions.

FORTIFICATION AND SUPPLEMENTATION

Scope and rationale

Fortification and supplementation are effective ways to increase micronutrient intake to acceptable levels. Fortification involves adding micronutrients to foods during the production process. Supplementation requires administration of micronutrients in medicinal form (e.g., tablets, capsules or injections). The fortification and supplementation work group discussed issues related to iodine, iron and vitamin A individually, as well as issues related to combined approaches.

Opportunities and constraints

Iodine. Fortification of salt with iodine has been successful, and the work group believed that such fortification should be extended to all countries where deficiency exists. Fortification levels, which depend on per capita consumption of salt and anticipated storage losses, range from 200–1000 μg/g. Potential toxicity is negligible, and the cost of salt iodinization is low. Potassium iodate is the preferred additive because it is more stable than other compounds. Storage and handling should be carefully considered because the volatile iodine is easily lost. Concerns about safety are addressed in a recent report of

the Joint FAO-WHO Expert Committee on Food Additives (JECFA) (WHO 1991).

Supplementation was considered an important short-term adjunct to a salt fortification program, which more effectively addresses the long-term problem in most countries. The main forms of iodine supplementation are oral iodized oil and iodized water. The effectiveness and safety of iodized oil programs have been amply demonstrated. From preliminary data it seems that an oral oil capsule with a dose of 480 mg of iodine will give protection for about one year. Injection can increase this protection to about three years. Both injection and oral supplementation programs have been subject to logistical problems. There are theoretical concerns in regard to iodine supplementation of pregnant women, but these are minor in areas with high prevalence of iodine deficiency.

Iron. Fortification of foods with iron is much more complex than fortification of salt with iodine because variations in dietary absorption and dietary patterns in different countries influence iron availability. In addition, because the required dosage of iron is at least 50 times more than the required dosage of iodine, taste and color changes occur in some iron-fortified foods.

The vehicles used to disseminate iron include wheat flour, cereals, sugar and salt. Some specialty foods, such as citrus drinks for children, have been tested with good results. However, these foods may not be appropriate for distribution on the open market because of variability in the amount consumed. In controlled situations such as schools, these products can be helpful.

Iron EDTA is a promising fortificant. Because it is absorbed much better than other fortificants, smaller amounts can be used, and color and taste are more acceptable. Use of iron EDTA in countries with a high prevalence of nutritional anemia seems desirable. Although EDTA is an approved food additive, iron EDTA is more expensive than other iron fortificants and has not yet been approved by any country or by the JECFA. A recommendation for use of iron EDTA from a single country would encourage other countries to use it.

Supplementation with iron is also complex because of the amount of iron needed, the dosage schedule and because of absorption and toxicity issues. Supplementation is usually made by one of several types of tablet. Absorption, which depends on the presence of and type of food in the stomach, greatly varies by individual and population. Iron excess is possible but is believed to be a limited problem in countries with a high rate of anemia. Side effects, which are common in pregnant women, the primary target group, are significant and increase with increasing dose. These side effects, including constipation and gastric distress, are minimized if the dose is taken on a full stomach, but then absorption is limited. A dose taken at night on an empty stomach may alleviate some of these problems. A new product, the gastric delivery system, may help to minimize both the side effects and absorption problems (Cook et al. 1990). Further testing of this product should be pursued.

Vitamin A. Vehicles used for vitamin A fortification include sugar, rice and some specialty foods. Vitamin A is lost when exposed to both heat and oxygen. Vitamin A fortification is also complicated by the color and slight taste it imparts to food. There is some concern about toxicity, particularly in pregnant women (IVACG 1986).

The work group was intrigued by the idea of fortifying and reprocessing broken grains of rice. This process may enable the development of a premix that would permit control of color and stability and would allow fortification with iron as well as vitamin A. Rice fortified by the process is being produced but needs extensive testing to ensure quality control, consumer acceptability and stability of the fortificant (Murphy et al. 1992).

Supplementation with oral vitamin A capsules has been tested in many countries. Dosage has been reviewed, and standard guidelines have been published by WHO and the International Vitamin A Consultative Group (IVACG) (WHO 1988). Cost is minimal, but some concerns remain about toxicity, particularly in pregnant women.

Coordinated strategies. Examples of multiple fortification opportunities identified by the work group are given in **Table 2**.

Supplementation efforts should be integrated with existing health care delivery systems whenever possible. Use of maternal and child health programs, primary health care programs and EPI activities should be explored. For example, if supplementation were linked with the EPI program, pregnant women could receive iron tablets and iodized oil with their tetanus immunization, and children could receive vitamin A supplementation as part of their routine vaccination regimen.

When planning coordinated strategies, care should be taken to address several issues, potential obstacles and constraints by doing the following:

- Involve all relevant sectors (government, industry and consumer groups) in planning and implementation. Industry is a key player in the sustained control of micronutrient malnutrition and should be supported in its free market profit-oriented approach. Industry's expertise in market development and determination should be tapped.
- Communicate with consumers to determine their perceived needs and to educate them about demanding a better product and accepting a slightly higher price for that product.
- Build an adequate infrastructure, including trained personnel and program management

TABLE 2

Possible coordinated fortification strategies

Food	Iodine	Iron	Vitamin A	Comments
Salt	X	X		Technology is now available to produce a stable formulation, but considerable testing of stability and absorption (as influenced by different dietary patterns) is required.
Rice		X	X	
Sugar		X	X	
Specialty foods, citrus drinks for schoolchildren, soft drinks, cookies, nutricubes	X	X	X	These foods are consumed in irregular amounts, which makes intake monitoring difficult. The work group advised against fortifying a wide range of products targeted at the same populations.

Countries should identify administrative structures that will suit their needs and unique situations.

- Recognize and take advantage of opportunities for fortifying new products to increase the consumption of fortified foods.
- Identify mechanisms to stimulate industry involvement, increase availability and reduce costs to consumers for fortified products. Mechanisms could include subsidies and regulatory relief to assure supplies of low cost fortificants and assist in the purchase of fortification equipment.
- Monitor and evaluate process and outcome variables. A poorly monitored program is liable to fail or be ineffective. Long-range programs should be designed, and they should incorporate evaluation that is essential to identifying progress, problems and needs. Monitoring of process measures should include the quality of the food, the level of fortification at different stages in the production and delivery system, and the tracking of micronutrient status indicators discussed by the information work group (Table 1).

Country experiences

India. The federal government in India recently liberalized the production of iodized salt to include the private sector as well as the public sector. It also made the commitment to iodize all edible salt in the country by 1992. A subsidy is provided to manufacturers to offset the cost of purchasing potassium iodate. Goiter control units have been established in all states; to monitor progress, a simple kit is used to detect the iodine in the fortified salt.

The National Anemia Control Programme has been in operation for the last two decades. Under this program, pregnant and lactating women are given tablets containing 60 mg of iron and 500 μg of folic acid, and children receive one half that dose. Each beneficiary is given one tablet per day for 100 days each year through primary health centers and maternal and child health clinics. Implementation has been very slow; a recent evaluation showed poor coverage in all states due to inadequate supplies, lack of awareness among health staff and poor compliance among women. Steps are being taken to address these problems. Initially, all nutrition interventions were isolated, vertical programs. They were subsequently integrated into other health care services, including food and nutrient supplementation, immunization, treatment of minor illness, nutrition and health education.

Iron-fortified salt, developed by the National Institute of Nutrition in Hyderabad, can provide 10 to 15 mg of iron per day, at an absorption rate of about 3% to 6%. In Calcutta, where hookworm infestation is common, the prevalence of anemia was reduced from 90 to 50–60% after introducing iron-fortified salt. Pilot attempts to produce iron-fortified salt on a commercial scale have been quite successful.

The technology for fortification of salt with both iodine and iron has been successfully developed by the National Institute of Nutrition. Laboratory studies have shown satisfactory results with respect to the stability and bioavailability of both micronutrients, and large-scale community trials are now under way.

A vitamin A distribution program, now operating in all states in the country, reaches about 30 million children between 1 and 5 years of age. Each child is given one spoonful of vitamin A syrup containing 666 $\times 10^3$ retinol equivalents (110 mg retinol palmitate) every six months. The program is implemented by auxiliary nurse midwives through primary health

centers. In areas where the program has been implemented well, the prevalence of xerophthalmia has decreased significantly. Reasons for poor coverage in other areas include inadequate supplies of vitamin A, lack of coordination between various health professionals, availability through clinics only rather than through house-to-house visits and lack of community awareness. Efforts are now being made to improve the logistics of distribution and to strengthen training and education components.

Thailand. Supplementation with iodine is accomplished using 500 μg/g of iodine in salt and two drops of iodine solution to 10 L of water in school and household reservoirs. Kits are used to test iodine levels in water. In villages, six drops of iodine solution can also be added to 750 mL of fish sauce in a bottle the size of a whisky bottle. This allows preparation to be done at the village level in an easily available container. In addition, 200-mg iodine capsules are distributed every 6 to 12 months to areas where iodine deficiency is endemic.

Thailand advocates the provision of one ferrous sulfate tablet and one multivitamin tablet daily to all pregnant women. Health officials feel that compliance is improved if only one tablet is required.

In 1976, after finding that blindness due to vitamin A deficiency occurred primarily among preschool children, Thailand established guidelines for vitamin A fortification of sweetened condensed milk. Guidelines also exist for providing foods rich in vitamin A to children of different ages, for example, breast milk for infants, rice and bananas (as a supplement) for children over four months of age, chicken liver and egg yolks for children five months old, and ivy gourd (an indigenous, green leafy vegetable) for children six months old.

Indonesia. To attain the goal that no child is born with cretinism in the year 2000, a national iodine deficiency disorders committee was established by a decree of the Ministry of Health in 1990. The national program uses three strategies: iodized oil injection as a short-term measure in areas where iodine deficiency is severe, iodized salt for human consumption as a permanent long-term strategy, and iodine added to safe drinking water as an appropriate technology in areas at high risk for deficiency.

Indonesia's intervention program for controlling nutritional anemia includes iron supplementation for pregnant women through village health posts and community health centers, nutrition education to increase the consumption of iron-rich foods, and fortification of foods with iron. The fifth in a series of five-year development plans states that, by the end of 1994, the prevalence of nutritional anemia among pregnant women should be reduced from 50 to 40%. About 80% of all pregnant women are to receive iron tablets. For the last three years, approximately 50% of all pregnant women have been given 90 to 100 tablets during their pregnancy, but the actual number of pregnant women taking iron tablets every day remains unknown. The government plans to conduct a pilot project to establish an anemia information system and to promote iron sources for pregnant women.

Xerophthalmia has been reduced significantly in some areas due to a combination of strategies: distribution of vitamin A capsules; nutrition education through village health posts; fortification of monosodium glutamate with vitamin A; and public health intervention through high immunization coverage, improved sanitation and improved health services. It is difficult to determine which strategy has had the highest impact.

Philippines. In the Philippines, the micronutrient strategy is part of an overall food and nutrition program. In areas where goiter is endemic, iodized oil capsules are mandated for children up to 14 years of age and women of childbearing age. In other areas, capsules are given only to persons with goiter. Pregnant women receive 120 mg of iron or two tablets of ferrous sulfate daily, starting from the twentieth week of pregnancy. Coverage is about 20% to 40% of all pregnant women because the program reaches only those women who attend prenatal clinics at health centers. Two tablets of iron are also given to nursing women and anemic infants and children. High dose capsules of vitamin A are provided to children with severe diarrhea, persons with moderate or severe protein energy malnutrition and persons with lower respiratory infections.

Knowledge gaps

The work group identified the need for research in the following areas:

- Effects of iron fortification and use of new fortified products on iron status.
- Feasibility of fortifying sugar with sodium iron EDTA. EDTA is not absorbed well on its own, but enhances the absorption of dietary iron. In countries where EDTA consumption levels are fairly low, sodium iron EDTA could well be approved.
- Color and stability of fortified foods.
- Absorption of micronutrients through different vehicles and under different dietary conditions.
- Safety of new products.
- Optimal number of iron tablets for pregnant women and the length of time that dosage is needed.
- Feasibility of a supplement containing both iodine and vitamin A.
- Optimal dosage for vitamin A supplements, i.e., whether small doses given frequently have a greater impact than larger, longer-lasting doses.

- Appropriate number of doses of vitamin A for children with measles. Current recommendations by WHO and UNICEF promote one dose, but recent trials are being conducted on two doses.
- Synergistic effects, in terms of disease trends and costs, of integrating micronutrient programs with other public health programs.
- Impact of water and other development programs on the problem of micronutrient malnutrition.

The work group expressed the view that it would be valuable to demonstrate the measurable benefits of fortification by conducting small pilot projects, funded by several agencies and industry, in one or two countries.

The group also support efforts by WHO and other groups to establish a micronutrient information bank that would collect and disseminate information on different countries' experiences with fortification and supplementation.

FOOD-BASED APPROACHES

Scope and rationale

Food-based approaches for the control of micronutrient malnutrition are designed to increase local production and consumption of foods that are high in specific micronutrients. The three micronutrients discussed during the workshop vary in their suitability for food-based approaches. Vitamin A, which is found in a wide variety of foods including yellow and orange fruits and vegetables, dark-green leafy vegetables, poultry liver, fish liver oils, whole milk, butter and eggs, is the most suitable. Iodine, found in marine fish, seaweed and plants from soil rich in iodine, is the least suitable. The suitability of iron for food-based strategies is moderate because foods with the best bioavailability of iron are generally expensive (e.g., meat products). Bioavailability tends to be lower in more commonly available foods (e.g., legumes).

The work group listed several advantages of food-based strategies:

- Such strategies can address multiple micronutrient problems.
- They have a community-based emphasis that promotes general community and human development.
- They are feasible in most countries but may need to be augmented by other interventions such as fortification or supplementation.
- They may result in long-term changes in consumption patterns, infant and child feeding practices and food preparation practices.
- They are cost-effective over time, and the nutrition, health and economic status of both individuals and communities benefit.

Food-based strategies are usually implemented when food sources are available but underutilized, or when food sources are unavailable or limited but can be augmented through agriculture or imports. These strategies may need to be implemented along with supplementation or fortification programs, particularly for iron and iodine.

Opportunities and constraints

Food sources available but underutilized. When food sources are available but underutilized, education in nutrition and health is the main strategy. Such education is most effective if it is designed to

- Change food consumption patterns. Educational strategies should promote increased consumption of locally available foods rich in micronutrients, such as eggs, papayas or other yellow fruits and vegetables, dark-green leafy vegetables, as well as animal products (when affordable).
- Change food preservation and preparation practices to overcome seasonal factors and guarantee a supply of micronutrient-rich foods all year long. Proper preparation would increase the bioavailability and dietary appeal of some foods, particularly to persons in greatest need (women and young children). For example, mothers can be taught how to dry certain fruits and vegetables (e.g., mangoes or green leafy vegetables), preserve them, grind them into a powder, and then add the powder to gruel for infants and toddlers.
- Link income-generating activities with food production activities (which often also generate income) when economic factors are a primary constraint on food availability. Care should be taken not to promote the production of income at the expense of proper nutrition.

Food-based approaches should identify the micronutrient problems and potential local food sources of each community. Lists of micronutrient-rich foods that can either be grown or bought locally are extremely valuable. The work group suggested that such lists be prepared by each country, with assistance from FAO, as part of intervention planning. The lists should indicate foods that must be processed before their micronutrients become available (e.g., sorghum and maize).

The work group believed that nutrition and health education would be most effective if it were

- Promoted at national, regional and local levels to develop multiple supportive constituencies.
- Designed to bring about behavioral changes among consumers so that they will select,

process, prepare and preserve foods in ways that optimize micronutrient consumption.

- Directed at policy-level officials to improve their understanding and obtain better technical and logistic support for programs.
- Integrated with other health education, agricultural and social messages being conveyed at the community level. Such coordination may increase the effectiveness of the nutrition messages and enhance their sustainability.

Messages should be tailored to the needs of unique target audiences. A range of rapid assessment procedures should be used, including both qualitative and quantitative techniques. Community participation should be solicited for these assessments, preferably through selection of small, time-limited samples rather than large groups followed over a long period of time. A variety of media (e.g., print, radio, billboards) should be used to deliver the messages, and the public health sector should teach local community health workers to develop and disseminate compatible messages.

Food sources unavailable. When food sources are unavailable or limited, food-based strategies must address the issues of food production and transportation. Unavailability may affect both urban and rural populations. Use of food coupons and promotion of kitchen gardens are two possible strategies for urban settings. Small, backyard gardens might be more appropriate for rural settings. Long-term sustainability of home gardens should take into account water availability, land availability and cost.

All too often, micronutrient-rich foods are produced at a distant site and must be transported to and marketed in the area of need. Analysis of existing infrastructures for food transport is critical to ensure local distribution of these foods without incurring unnecessary expense. Transported foods should be packaged hygienically to preserve their quality and appeal. Nutrition education, as described above, is essential to ensure consumption of the new foods.

The work group recognized the importance of agricultural policies that support the diverse diet necessary for consumers to meet micronutrient needs. The group believed that the most immediate impacts are likely to occur through agricultural and animal production at the community and household levels. In most instances, such local efforts may involve women or women's groups; hence, consideration must be given to the multiple demands on a woman's time and energy. Strategies to overcome constraints on food availability may involve not only the provision of resources, such as seed and fertilizer, but also relief from time- and energy-consuming burdens, such as hauling water. Community-development programs that reduce these burdens on women should be emphasized. Agricultural extension workers accustomed to dealing with male farmers may need to be retrained in how best to work with women.

The work group listed the following factors that should be considered when designing food-based approaches. The extent to which they represent opportunities and constraints vary considerably from country to country, depending on the sociopolitical environment in each country and the effectiveness of program planning efforts.

- The time required to change behavior may not be long if strategies are tailored to local needs and cultures.
- Distribution channels may need to be improved to ensure that the food reaches the vulnerable populations (e.g., young children).
- Communication needs to take educational status and literacy among women into account and encourage their participation in interventions to improve diet diversification.
- The status and role of women as decision makers in attaining, preparing and consuming food may need to be strengthened.
- Because food-based approaches are used in diverse situations, close cooperation is needed between local and national levels during program development so that the recommended approaches are suitable for individual communities.
- Agricultural policies may need to be altered to support dietary diversification.
- Political commitment may need to be developed at all geographic and political levels.
- Technology appropriate for the community level may need to be designed.

Country experiences

India. A pilot project was recently undertaken to evaluate the feasibility of food-based strategies. Thirty villages in three different agroclimatic regions were selected. A baseline survey was conducted to obtain information on agricultural practices, dietary habits and available vegetables and fruits with high bioavailability of micronutrients. Based on this survey, an intervention program combining nutrition education and agricultural interventions was launched. Home gardening was promoted to increase the availability of vegetables and fruits at the household level. Cultivation of perennial varieties of green, leafy vegetables was emphasized (e.g., Indian spinach, a creeper that requires little water, grows all year and is rich in β-carotene, iron and calcium).

Thailand. After a concentrated effort to improve chicken cultivation by promoting improved hygiene, vaccination and improved minimal care, the death rate for chickens dropped from 60–70% to 10–15%. This decrease resulted in an increased supply of

chicken, chicken liver and eggs. Thailand also encouraged production of nutritious foods (primarily rice, legumes and sesame), gardening, use of organic fertilizers, correction of sterilized soils, and village fisheries.

Philippines. Nutrition education in this country concentrates on promoting breastfeeding, increasing the intake of green leafy and yellow vegetables and foods rich in vitamin C, encouraging use of iodized salt in food preparation and reducing the consumption of foods containing goitrogens (e.g., cassava). Bio-intensive gardening, which was initiated as a pilot program, has been adopted nationwide. It is conducted by the Department of Agriculture, with oversight by the Department of Public Health, and involves training local villagers to produce their own micronutrient-rich foods.

Knowledge gaps

The work group recommended that research priorities focus on applied and operational questions, such as the following:

- What is the basic infrastructure required to implement food-based strategies?
- Where such a basic infrastructure does not now exist, how can it best be developed?
- How can existing infrastructures be better used?
- How can existing infrastructures for food-based strategies be augmented by supplementation and fortification strategies?

Other areas for further research and development include the following:

- Develop conceptual models, particularly for maternal and infant-feeding practices, that define micronutrient requirements for vulnerable groups and how to meet those requirements in different scenarios. These models can be accompanied by guidelines for national planners, adapting the models to local situations. Models should include information on locally available foods rich in micronutrients, the bioavailability of such foods, and preservation methods for those nutrients.
- Develop methods to increase policymakers' understanding of the cost-effectiveness of a food-based strategy, particularly the small quantities of micronutrient-rich foods actually needed and the small plots of land needed to grow adequate supplies of such foods (e.g., household-level production of vitamin A–rich foods).
- Simplify educational messages and approaches into a limited number of easy steps with specific actions, so that they can be easily tailored to a variety of populations.
- Specify the technical and logistic support required for agricultural programs designed to increase the micronutrient content of foods.
- Document food-based strategies (such as backyard vegetable gardens) that have increased micronutrient intake.
- Analyze successful food-based strategies (including those initiated by nongovernment organizations) that spread horizontally from village to village or vertically from small projects to national programs. An understanding of these processes might help in the design of other programs to control malnutrition problems. Micronutrient deficiencies that most frequently occur in pockets may be best addressed by local programs that extend to larger populations.

IMPLEMENTATION OF COORDINATED STRATEGIES

As these summaries indicate, coordinated strategies for controlling micronutrient malnutrition are technically feasible. In the area of information collection, coordinated surveys with simultaneous assessment of clinical and biochemical indicators of multiple micronutrients are feasible. Dietary assessment can also be used for vitamin A, iron and other micronutrients, as well as for additional dietary factors such as energy and protein. Furthermore, because target groups of concern (e.g., infants, children at school entry and women of childbearing age) overlap, they may prove a useful focus for examination of multiple micronutrients.

Multiple fortification is also possible using vehicles such as salt (fortified with iron and iodine), processed rice and sugar (fortified with vitamin A and iron), and citrus drinks and cookies for schoolchildren. Supplementation efforts can be integrated with existing delivery systems such as maternal and child health programs, primary health care programs and the Expanded Programme on Immunization.

Food-based strategies have also proven effective in addressing multiple micronutrient deficiencies. The best examples have been community-based programs that recognize local micronutrient problems and potential local food sources. These programs have included a nutrition and health education component designed to change food consumption patterns, to improve food preservation and preparation practices, and to link income-generating activities with food production activities.

After discussing the strengths and weaknesses of coordinated control strategies, workshop participants concluded that pilot projects are needed to test some of these strategies. The group acknowledged many unanswered questions, but stated that sufficient technical knowledge exists to support an international control effort. Technical expertise alone was deemed insufficient, however, to ensure success. A well-designed, coordinated control strategy requires strong political commitment and a supportive infrastructure.

It was acknowledged that independent expert groups, international agencies and nongovernmental organizations advocating increased effort to address a particular micronutrient (be it iodine, vitamin A, iron, etc.) must work closely together and with industry to implement coordinated strategies. Likewise, there must be clear and frequent communication among policymakers and health workers at all levels—international, national and local—to ensure commonality of purpose. It is also critical to involve industry in planning and implementing fortification and food-based control activities. Only through concerted effort in both the technical and the political arenas can coordinated control of micronutrient malnutrition be achieved.

Three specific suggestions for improving coordination among policymakers, technical experts and health professionals are summarized below.

National coordinating bodies. Strong political support is needed at the national level to facilitate technical progress in micronutrient control. Establishment of some type of national coordinating body was suggested for each country interested in initiating or strengthening its program against micronutrient malnutrition. This body should involve relevant parties in all stages of program planning, from inception through evaluation, so that they are able to advocate for improved micronutrient status. The group should include representation from technical experts in public health, social science, food technology and communications and should represent all interested sectors (e.g., industry and consumers). It is important that such coordinating bodies involve ministries of planning, interior, agriculture, education and public health, along with universities and the private sector, and be linked to a well-developed national plan of implementation.

The organizational structure of this policy-setting body would vary from country to country, depending on existing structures and philosophies. One possible structure would consist of a national nutrition committee with subcommittees for specific micronutrients. In the Philippines, such a structure was organized by the Department of Public Health. Persons at several levels are involved, including the governor and officials at the provincial, district and community levels. Indonesia has a similar structure: a committee on nutrition improvement and subcommittees on iodine deficiency disorders and family nutrition improvement. The Ministry of People's Welfare coordinates micronutrient intervention as part of an overall family nutrition improvement program, which involves several government sectors with complementary roles and active community participation. Plans consistent with the country's overall national development have been developed. These plans emphasize three goals: equitable distribution of development and its benefits, accelerated economic growth and political stability. In Thailand, food and nutrition programs are also planned in the context of broad national economic and social development goals. Preparation and implementation of these plans has increased awareness at all levels of the important link between nutrition and socioeconomic status.

Information network. The workshop participants recognized the need for close communication among all parties involved in controlling micronutrient deficiency including policymakers, government officials and staff, medical personnel, industry representatives and consumers. An effective micronutrient information network is needed to ensure the ongoing exchange of technical information that is consistent and accurate.

Technical exchange and training. Participants recommended continued interchange on new technical developments toward the elimination of micronutrient deficiencies. They suggested that mechanisms be established to ensure continuous sharing of new technical knowledge (e.g., through an information network, additional technical workshops, newsletters and manuals). Conferences on specific technical issues could be sponsored by interested agencies or organizations. Participants also identified the need for additional training and technical assistance for health professionals that will enable them to analyze problems in their own countries and develop appropriate control strategies.

ACKNOWLEDGMENT

The authors wish to express their sincere appreciation to Susan Toal for contributing her time and writing expertise to the development of this summary.

LITERATURE CITED

Cook, J. D., Carriaga, M., Kahn, S. G., Schalch, W. & Skikne, B. S. (1990) Gastric delivery system for iron supplementation. Lancet 335: 1136–1139.

DeMaeyer, E. M. (1989) Preventing and Controlling Iron Deficiency Anaemia through Primary Health Care. A Guide for Health Administrators and Programme Managers. World Health Organization, Geneva, Switzerland.

International Vitamin A Consultative Group (1986) The Safe Use of Vitamin A by Women during the Reproductive Years. The Nutrition Foundation, Washington, DC.

Murphy, P. A., Smith, B., Hauck, C. & O'Connor, K. (1992) Stabilization of vitamin A in a synthetic rice premix. J. Food Sci. 57: 437–439.

Task Force for Child Survival and Development (1992) Proceedings of Ending Hidden Hunger (A Policy Conference on Micronutrient Malnutrition). Atlanta, GA.

World Health Organization (1988) Vitamin A Supplements: A Guide to their Use in the Treatment and Prevention of Vitamin A Deficiency and Xerophthalamia. World Health Organization, Geneva, Switzerland.

World Health Organization (1991) Evaluation of Certain Food Additives and Contaminants. Thirty-Seventh Report of the Joint

103

FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 806, Geneva, Switzerland.
World Hunger Program (1989) Overcoming Hunger in the 1990's: The Bellagio Declaration. Providence, RI.

FURTHER INFORMATION

The following sample of recent work may be of interest to readers who want to further explore micronutrient malnutrition:

General

Cerqueira, M. T. & Ulson, C. M. Nutrition education in developing countries: an examination of recent successful projects. In: Beyond Child Survival: Enhancing Child Growth and Nutrition in Developing Countries (Pinstrup-Andersen, P., Pelletier, D., & Alderman, H., eds.). Cornell University Press, Ithaca, NY (in press)
Charoenkiatkul, S., Valyasevi, A. & Tontisirin, K. (1985) Dietary approaches to the prevention of vitamin A deficiency. Food Nutr. Bull. 7(3): 72–76.
International Nutrition Planners Forum (1989) Crucial Elements of Successful Community Nutrition Programs. Report of the 5th International INPF Conference. USAID Office of Nutrition, Washington, DC.
International Nutrition Planners Forum (1992) Effective Nutrition Communication for Behavior Change. Report of the 6th International INPF Conference. The Nutrition Foundation, Washington, DC.
Loevinsohn, B. P. (1990) Health education interventions in developing countries: a methodological review of published articles. Int. J. Epidemiol. 19: 788–795.
United Nations Children's Fund. (1992) 14-Point Statement following the Policy Conference on Micronutrient Malnutrition "Ending Hidden Hunger," 10–12 October 1991, Montreal. UNICEF Programme Division, New York, NY.

Iodine

Dunn, J. T. & Van der Haar, F. (1990) A Practical Guide to the Correction of Iodine Deficiency. International Council for Control of Iodine Deficiency Disorders, The Netherlands.
Hetzel, B. S., Dunn, J. T. & Stanbury, J. B., eds. (1987) The Prevention and Control of Iodine Deficiency Disorders. Elsevier, Amsterdam, The Netherlands.

Iron

Basin, S. S., Soekirman, Karyadi, D. & Scrimshaw, N. S., eds. (1979) Iron deficiency anemia and the productivity of adult males in Indonesia. Am. J. Clin. Nutr. 32: 916–925.
International Nutritional Anemia Consultative Group (1989) Guidelines for the Control of Maternal Nutritional Anemia. The Nutrition Foundation, Washington, DC.
Lozoff, B. (1991) Long-term developmental outcome of infants with iron deficiency. N. Engl. J. Med. 325: 687–694.

Vitamin A

International Vitamin A Consultative Group (1977) Guidelines for the Eradication of Vitamin A Deficiency and Xerophthalmia. The Nutrition Foundation, Washington, DC.
International Vitamin A Consultative Group (1989) Methodologies for Monitoring and Evaluating Vitamin A Deficiency Intervention Programmes. The Nutrition Foundation, Washington, DC.
International Vitamin A Consultative Group (1991) Summary of the XIV IVACG Meeting. The Nutrition Foundation, Washington, DC.
World Health Organization (1991) Vitamin A Deficiency: Time for Action. Expanded Programme on Immunization, Geneva, Switzerland.

Fortification and supplementation

Arroyave, G., Aguilar, J. R., Flores, M. & Guzman, M. A. (1979) Evaluation of Sugar Fortification with Vitamin A at the National Level. PAHO Scientific Publication 384. Pan American Health Organization, Washington, DC.
Clydesdale, F. M. & Wiemer, K. M., eds. (1985) Iron Fortification of Foods. Academic Press, Orlando, FL.
Hurrell, R. F. & Cook, J. D. (1990) Strategies for iron fortification of foods. Trends Food Sci. Technol. 1: 56–61.
International Nutritional Anemia Consultative Group (1992) Combating Iron Deficiency Anemia through Food Fortification Technology: An Action Plan. The Nutrition Foundation, Washington, DC.
Mejia, L. A. & Arroyave, G. (1982) The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala. Am. J. Clin. Nutr. 36: 87–93.

Food-based approaches

Food and Agriculture Organization (1990) Roots, Tubers, Plantains and Bananas in Human Nutrition. FAO, Rome, Italy.
Gershon, J. (1983) Alleviating vitamin A problems with home gardens. Proceedings of the 4th Asian Congress of Nutrition, Bangkok, Thailand.
Kailasapathy, K. (1988) Studies of the role of school gardens in combating malnutrition in Sri Lankan children. Ecol. Food Nutr. 21: 131–141.
McKigney, J. I. (1983) Some observations on the potential for the dietary approach to combat vitamin A deficiency. Proceedings of the 4th Asian Congress of Nutrition, Bangkok, Thailand.
Rao, B.S.N. (1991) Use of beta-carotene rich foods for combating vitamin A deficiency. Nutr. Found. India Bull. 12: 1–6.
Reynaud, J., Brun, T. & Marek, T. (1989) Impact of a women's garden project on nutrition and income in Senegal. Arid Lands, Newsletter 29, Fall/Winter.
Shrimpton, R. (1989) Vitamin A deficiency in Brazil: perspectives for food production–oriented interventions. Ecol. Food Nutr. 23: 261–71.
Susanto, D., Slamet, R. M., Sajogyo, B., Sajogyo, P., Azwar, N. R. & Karyadi, D. (1983) Dietary and household economic approaches to prevent xerophthalmia. Proceedings of the 4th Asian Congress of Nutrition, Bangkok, Thailand.
Tsou, S.C.S, Gershon, J., Simpson, K. L. & Chichester, C. O. (1983) Promoting household gardens for nutrition improvement. Proceedings of the 4th Asian Congress of Nutrition, Bangkok, Thailand.

104

THE EFFECT OF ENDURANCE TRAINING ON IRON METABOLISM

James D. Cook, M.D.

Division of Hematology, Department of Medicine, Kansas University Medical Center, Kansas City, Kansas 66160-7402, USA.

Address reprint requests to James D. Cook, M.D., Division of Hematology, Kansas University Medical Center, 3901 Rainbow, Kansas City, Kansas 66160-7402, USA.

This work was supported by NIH grant DK39246.

The effect of endurance training on hematological status has received much attention in the sports medicine literature because of concern that abnormalities may impair athletic performance. The initial emphasis was on the progressive decline in circulating hemoglobin concentration which commonly occurs in the early stages of an intense training program. This so-called sports anemia is discussed by Drs. Selby and Eichner in the present issue. Because iron deficiency is a common cause of anemia, interest in sports anemia has focussed more recently on the influence of conditioning on laboratory indices of iron status, changes that can be as dramatic as the alterations in red cell measurements. The present review is concerned with the effect of aerobic conditioning on iron metabolism. The iron status of the highly trained athlete will be reviewed and the alterations in iron balance will be discussed in relation to their possible cause. The extent to which impaired iron status influences athletic performance will be presented together with recommendations for their prevention.

EARLY EFFECTS OF ENDURANCE TRAINING

Laboratory measurements of iron status undergo significant changes within a few days of beginning an intense conditioning program. These changes occur with various types of conditioning but are particularly evident with distance running. Because of the greater vulnerability of the adolescent to iron deficiency, several studies have been performed in high school students. In eight female cross-country runners, the serum iron, transferrin saturation, and erythrocyte protoporphyrin all changed in the direction of iron deficiency

during an eight week training program but returned to baseline when the running season was over (22). These changes were associated with an 9% decline in hemoglobin concentration during the first week which then slowly reverted to preseason values with continued training. In a similar study, 12 out of 20 adolescent female runners who were not given iron supplements had no change in iron status as measured by serum ferritin whereas the remaining eight girls developed iron deficiency defined as a serum ferritin ≤20 μg/L when examined five or ten weeks after beginning a conditioning program (39). In those that developed iron deficiency, it was estimated that iron stores had fallen by 192 mg of iron representing an additional daily iron loss of 2.7 mg.

Adolescent males are less frequently iron deficient and are likewise less susceptible to the development of iron depletion with training. When examined at the beginning of a competitive season, only one of 30 male cross-country runners were iron deficient based on a serum ferritin ≤12 μg/L as compared with eight out of 20 females (48). After 11 weeks of training, three additional males and one female became iron deficient giving an overall prevalence of 17 and 45% in male and female runners, respectively. There was also a lesser decline in mean serum ferritin in males from 29 to 24 μg/L as compared with a fall from 26 to 14 μg/L in the females. Iron deficiency anemia was not observed in this study and was also uncommon in a study by Nickerson and colleagues who observed that 34% of female and 8% of male cross-country runners in the 15 to 18 year age group developed a low serum ferritin and transferrin saturation during the running season (40).

The susceptibility of distance runners to iron deficiency occurs with other types of endurance training. The effects of a particularly intense training program involving eight hours daily of isometric and isotonic physical activities was examined in 8 females and 11 males (32). Ferritin levels decreased by 50% in both sexes after four weeks of training and remained low for the remainder of the seven week training period. Significant changes were also observed in serum iron and total iron-binding capacity (TIBC) and two of the females developed iron deficiency anemia. Negative iron balance also occurs with vigorous weight training. In 12 healthy untrained males who underwent a strength-training program for eight hours weekly for six weeks, the mean serum ferritin fell from 75 to 49 µg/L reflecting a highly significant 35% decrease in iron stores although the serum iron and transferrin were not affected (52). Swimmers are less affected by intense training. In a study by Rowland and Kelleher (50), the iron status of 30 high school students was assessed at the beginning and end of a competitive swim season. Iron depletion defined as a serum ferritin ≤12 µg/L was detected in one-half of the girls at the outset but in none of the boys. The serum ferritin levels did not change significantly from the beginning to the end of the swim season leading the authors to conclude that non-anemic iron deficiency is highly prevalent in the female high school swimmer but that it is unrelated to training.

The susceptibility to iron deficiency is reduced with less rigorous aerobic training. In 24 women participating in a 35 minute exercise class on four days each week (2), a slight, but significant, decline in mean serum ferritin from 31 to 25 µg/L was observed after 13

weeks although no changes in serum iron or TIBC were observed. A modest 25% decrease in serum ferritin was reported in a group of college aged women participating in a moderate aerobic exercise training program (27) and similar changes were reported in 47 previously sedentary women (30). These studies indicate that the risk of iron deficiency is less with moderate training programs but some reduction in storage iron is common.

There is no evidence of an acute effect on iron status when conditioned athletes participate in an intense prolonged competition although a decline in circulating hemoglobin concentration is often reported. Twelve male marathon runners who had completed at least two marathon races within the previous year were evaluated during a 20 day, 320 mile road race (15). Although the pre-race hemoglobin fell from 160 g/L to 134 g/L at the end of the race, the serum iron level actually increased after 48 hours and remained elevated for the remainder of the competition. The mean hemoglobin also fell from 151 to 140 g/L in 15 professional male cyclers during a 20 day road race but there were no associated changes in serum iron, TIBC, or serum ferritin (5). In another study, the serum ferritin increased in 110 well-trained runners participating in a 20 day competition (55). Although iron balance in the highly trained athlete is not affected acutely by intense prolonged aerobic effort, there is some evidence that iron stores decline with successive seasons of training. When measurements were performed in mid-season in 28 elite Canadian athletes on two successive years, a significant fall in serum ferritin from 48 to 24 µg/L was observed in nine female synchronized swimmers and from 94 to 72

µg/L in six male speed skaters (45). In another study, a trend to decreasing serum ferritin levels in female collegiate field hockey players was observed when monitored over three successive years of play (14).

IRON STATUS OF CONDITIONED ATHLETES

The long-term effect of endurance training on iron balance is best determined by examining the iron status of highly conditioned athletes. There are numerous reports that address this topic but their interpretation is hampered by the wide variation in laboratory criteria of iron deficiency. The most reliable studies are those based on serum ferritin measurements but even with this one parameter, the cut-off level used to define iron deficiency have ranged from 12 µg/L used in most clinical reports to as high as 40 µg/L (34), a criterion that would classify most women in the reproductive age as iron deficient. The results of studies published prior to 1988 have been tabulated in a comprehensive review (37).

In a group of highly trained athletes, serum ferritin measurements were significantly lower in runners than elite rowers or professional cyclists (16). Many other studies have confirmed that iron stores are often reduced in distance runners although evidence of iron deficiency is uncommon. In one detailed study, the serum ferritin averaged only 33 µg/L in 41 trained male runners averaging 12 to 14 km daily, as compared to 73 µg/L in 36 male controls ($p<0.001$)(43). In 43 elite male runners between 20 and 46 years of age

who ran 10 to 20 km daily for many years, none lacked hemosiderin in the bone marrow or had serum ferritin values <12 µg/L although two of the men had only trace hemosiderin on bone marrow examination and serum ferritin values <20 µg/L. Many other reports indicate that iron stores are significantly lower in distance runners (8,18,26,42,62) although not all authors agree. Serum ferritin measurements in 72 elite male runners and 48 non-running controls were nearly identical with mean values of 56 and 60 µg/L, respectively and these results were little affected by excluding those taking iron supplements (1). In females, the mean serum ferritin was actually higher in runners than in controls, 26 µg/L vs 21 µg/L, respectively. When women who were not taking iron supplements were excluded, the mean serum ferritin of 15 µg/L was the same in both groups. Interestingly, one-third of the 35 male runners and two-thirds of the 37 female runners in this study were taking a daily iron supplement containing at least 18 mg iron. The authors concluded that the iron status of distance runners does not differ appreciably from the population as a whole. The same conclusion was reached from studies of the prevalence of iron deficiency in female intercollegiate athletes participating in a variety of sports (44). At the time of the initial evaluation, iron deficiency affected 31% of the athletes and overt anemia was identified in 7%. However, the prevalence was not statistically different from that observed in 66 non-athlete controls of whom 46% were iron deficient and 9% were anemic.

The balance of evidence therefore indicates that most distance runners have some reduction in iron stores as compared to sedentary controls but that iron deficient

erythropoiesis or anemia is uncommon. Iron deficiency is relatively common in those with high physiological iron requirements such as high school and collegiate female athletes but the prevalence is not convincingly higher than controls of similar age. Future studies of the prevalence of iron deficiency in the highly conditioned athlete will become increasingly difficult because of the widespread use of iron supplements.

CAUSES OF NEGATIVE IRON BALANCE WITH ENDURANCE TRAINING

There has been much discussion about factors that could explain negative iron balance with endurance training and the topic has been addressed in several recent reviews (25,37,47,65). That negative balance does occur has been demonstrated convincingly by radioiron studies in eight male elite runners with latent iron deficiency based on reduced or absent bone marrow iron stores (18). The runners were aged 24 to 32 years and had been training for at least five years. Body iron turnover was measured over several months by monitoring the decline in whole body ^{59}Fe activity with a whole body counter. The biological half-life of iron in the runners was estimated at 1000 days, appreciably shorter than values of 1300 and 2100 days in female and male controls, respectively. Since the red cell incorporation of radioiron in the runners was normal (mean 78%), it can be assumed that the accelerated loss of radioiron in these elite athletes was due to a higher rate of blood loss.

There are several reports suggesting that acute (13,26) or chronic (26,33,43,52,56) intravascular hemolysis is associated with intense exercise. Because intravascular hemolysis increases the formation of hemoglobin-haptoglobin complexes which are removed by hepatocytes, it has been suggested that the exercise-related changes in iron measurements are due to a shift in body iron stores from the reticuloendothelial compartment to the liver parenchyma resulting in a fall in serum ferritin and reduction in stainable iron on bone marrow examination (24). While this mechanism might account for some of the changes in iron status during the first few weeks of endurance training, it is unlikely that a long-term disparity would exist in the distribution of storage iron between the parenchymal and reticuloendothelial compartment.

There is convincing evidence that blood loss from the gastrointestinal tract is an important cause of negative iron balance in distance runners (35,36,40,46,62). Qualitative tests for fecal occult blood used in earlier reports have been supplanted by more precise quantitative estimates of fecal hemoglobin concentration (54). In one study, the mean fecal hemoglobin concentration was 0.99 mg/g in controls compared with 1.04 mg/g in 24 runners prior to a race and 3.96 mg/g following a race of 10 to 42 km (62). Twenty of the 24 runners had significantly increased fecal hemoglobin levels, 7 had values consistent with 3 ml blood loss and one was estimated to lose 43 ml of blood daily on two successive days. In a later study, the mean fecal hemoglobin content increased in 28 marathon runners from 1.02 to 1.48 mg/g following a race and the increase was twice as high in 13 runners who had taken analgesics before the race (46). These authors

concluded that marathon running was associated with a clinically insignificant increase in fecal hemoglobin although it could become important in those using analgesic drugs regularly. Nickerson et al. (40) studied fecal hemoglobin levels in 13 high school cross-country runners and found that out of 90 stool specimens in 20 females, 14 contained more than 4 mg/g whereas only one out of 61 specimens from 13 male cross-country runners contained over 4 mg/g. Although the increase in fecal hemoglobin before and following a competitive athletic event was not statistically significantly, the authors concluded that iron deficiency in the adolescent runner is associated with increased intestinal blood loss. The cause of the bleeding has not been identified but several theories have been forwarded. Transient gut ischemia is the most likely explanation because of the marked 80% decrease in splanchnic perfusion associated with exercise (7,62). Other explanations include erosive gastritis, trauma due to repeated impact on the bowel wall, and the common use of platelet-inhibiting analgesics by distance runners. While these various studies suggest that some increase in fecal blood loss may occur following intense prolonged activity such as a marathon race, there is less clear-cut evidence that fecal blood loss is increased during maintenance training.

The evidence for other pathways of iron loss in the conditioned athlete is not convincing. Microscopic hematuria was detected in 18% of marathon runners in the three days following a race and one specimen contained gross hematuria (58). So-called foot strike hemolysis resulting in hemoglobinemia and hemoglobinuria is commonly cited as a possible cause of accelerated iron loss (26,33,43) and a similar phenomenon has been

demonstrated with competitive swimming (56) and weight training (52). However, measurements of urinary iron loss in the conditioned athlete have shown only a modest increase. For example, Magnusson et al. reported a mean iron loss of 0.18 mg/24 hours in 11 elite runners, less than two-fold higher than values in historical controls (33). Several authors have mentioned the possibility of accelerated iron loss in sweat with intense conditioning but this was not confirmed in a meticulous study in 11 healthy men by Brune et al. (4). When iron contamination of the skin and iron loss from desquamated cells was reduced to a minimum, a very low iron loss averaging 22 μg/L sweat was reported as compared to much higher values described in previous studies. The authors concluded that variations in the amount of iron lost in sweat have a limited contribution to the variation in total body iron loss.

The modest increases in iron loss in feces, urine, or sweat could account for some reduction in storage iron in distance runners but do not adequately explain the acute changes in laboratory iron parameters during the first few weeks of an endurance training program. One explanation for the early changes is a shift of iron from stores to an expanding red cell compartment. Endurance training causes a marked increase in plasma volume but there is also an increase in total red cell mass. In one study, the total hemoglobin mass was 20% greater than in controls despite a fall in circulating hemoglobin concentration (3). An increase in muscle mass associated with some forms of conditioning such as weight training, will also increase tissue iron requirements. In many respects, the changes in iron metabolism associated with endurance training

resemble those in pregnancy: dilutional anemia with an expansion in red cell mass, a decline in iron stores that may progress to iron deficient erythropoiesis, and a prompt reversal of negative iron balance following delivery or cessation of training.

It has been suggested that impaired absorption of dietary iron contributes to the negative iron balance with endurance training. When radioiron absorption of inorganic iron (ferrous sulfate) and hemoglobin was measured by whole body counting (18), mean absorption of inorganic iron was 16.4% in eight long-distance runners as compared to 30% in eight male blood donors. The absorption of hemoglobin iron was also slightly lower in runners (13.5%) as compared to blood donors (17.8%) but in neither case was the difference statistically significant. While this report is commonly cited as demonstrating impaired absorption in the distance runner, iron absorption is highly correlated with iron stores (10) and the difference could be explained by a greater degree of iron deficiency in the blood donors.

Several authors have suggested that dietary factors contribute to the development of iron deficiency with endurance training. Because iron intake is closely linked to caloric intake, total iron intake is expected to be higher in athletes because of their greater caloric expenditure. However, the type of dietary iron ingested may be important. Because heme iron is absorbed by an independent pathway and not inhibited by dietary factors, meat consumption is a key dietary determinant of iron status (9). Snyder et al. studied female runners who were carefully matched for age, aerobic capacity, miles run per week, and

number of pregnancies and observed a significantly lower mean serum ferritin of 7 μg/L in those consuming a vegetarian diet as compared to 20 μg/L in those consuming ample quantities of red meat (60); Seiler et al reported similar findings (55). In another report, when the food intake records of iron deficient and iron replete runners were compared, total iron intake was similar but the iron deficient runners (mean serum ferritin 15 μg/L) had a significantly lower heme iron intake than iron replete athletes (mean serum ferritin 65 μg/L) (66). These studies indicate that a vegetarian diet is a significant risk factor for iron deficiency in distance runners. Other authors have suggested that the endurance athlete consumes a high fiber diet that may impair the absorption of nonheme iron. However, recent studies have shown that with a varied diet, differences in the bioavailability of nonheme iron have little effect on absorption from a complete diet when the serum ferritin is >12 μg/L (11).

THE RELATIONSHIP BETWEEN IRON STATUS AND ATHLETIC PERFORMANCE

The extent to which exercise-induced changes in iron status influence athletic performance has been debated extensively. There can be little doubt that iron deficiency severe enough to cause anemia diminishes exercise capacity and reduces VO_2 max (67). In regions of the world where iron deficiency anemia is highly prevalent, the liabilities associated with the anemic state have been clearly demonstrated. In 75 female tea estate workers in Sri Lanka who had hemoglobin values ranging from 61 to 159 g/L, dramatic differences between groups with the lowest and highest hemoglobin concentration were

observed in total treadmill time (10 vs. 18 minutes), percentage reaching the highest work load (0 vs. 100%), and post-exercise lactate levels (23). Even a modest hemoglobin reduction between 110 and 119 g/L was associated with a 20% decrease in work tolerance as compared to non-anemic controls. The relevance of these findings in relation to the conditioned athlete is difficult to assess because their decrease in hemoglobin concentration is usually due to an expanded plasma volume. In one study, running speeds were maintained despite a decline in mean hemoglobin from 160 to 134 g/L during a 20 day road race suggesting that this sports anemia does not impair athletic performance (15). Most reports indicate that true iron deficiency anemia is uncommon in the endurance athlete.

A more difficult question is whether a reduction in tissue iron can reduce performance by affecting metabolic pathways dependent on iron containing enzymes. Evidence supporting an effect of tissue iron deficiency independent of anemia was published in the classic study by Finch et al. (19), who demonstrated that severely iron deficient rats had markedly impaired treadmill running times even though their hemoglobin was increased to normal by exchange transfusion immediately prior to testing. In one group of animals, oral iron appeared to correct the defect in treadmill performance before significant increase in hemoglobin concentration occurred. However, attempts to demonstrate a similar defect of tissue iron deficiency in human subjects has not been successful. Celsing et al. (6), induced iron deficiency in nine healthy male subjects by repeated phlebotomy during a nine week period. After raising their hemoglobin concentration to normal by re-

transfusion, the mean endurance running time was 51.7 minutes as compared to 49.1 minutes in a control group, indicating no effect of tissue iron lack. No significant changes in maximal activities of several enzymes assayed in muscle biopsy specimens were seen. The reason for the disparity between findings in laboratory animals and humans is not clear but it may relate to the severity and duration of the induced iron deficiency.

One way of defining the possible limitations of iron deficiency on athletic performance has been to assess the effect of iron supplementation (5,15,21,27,31,38,41,44,49,53,56,64). In most reports, iron has not been beneficial. For example, Schoene and coworkers observed no increase in exercise performance when a group of trained female athletes with marginal iron deficiency were given two weeks of iron therapy although the blood lactate level at maximum exercise did increase significantly (53). Similarly, performance did not improve when 100 female intercollegiate athletes participating in a variety of sports were given oral iron (44). In 40 female endurance runners with mild iron deficiency, no improvement in various tests of physical work capacity occurred with eight weeks of iron supplementation despite a dramatic three-fold rise in mean serum ferritin (38) indicating that reduced iron stores, as defined by a serum ferritin <20 μg/L, do not pose a handicap to physical work capacity. When eight weeks of oral iron or placebo was given to 31 female athletes with an initial serum ferritin <25 μg/L, no improvement in VO_2 max or exercise-induced blood lactate concentration was observed despite a significant increase in hemoglobin concentration in those given iron (21).

Other workers have reported some improvement in endurance performance following iron administration. In a randomized, double-blind study in 14 non-anemic female runners with mild iron deficiency (serum ferritin <20 μg/L) full therapeutic doses of iron elevated the serum ferritin from 9 to 27 μg/L (49). Treadmill endurance time declined in all seven controls whereas six of the seven given iron improved their performance. No differences in maximal or submaximal oxygen consumption, ventilation, or heart rate were observed. Other reports indicate a marginal benefit even when the participants were more iron deficient at baseline. When the effect of daily iron supplements in 13 women was compared to 15 women given a placebo during an intensive seven week training program, a highly significant rise in hemoglobin concentration was seen in those given iron whereas two-thirds of the placebo group had serum ferritin concentrations <10 μg/L at the end of the training period (31). An increase in VO_2 max was seen at the end of the first three weeks in those given iron but no differences were observed at the conclusion of the six week trial. Thus, despite significant improvement in iron status measurements, a benefit of oral iron was demonstrated only during the early stages of the training program.

The question of whether athletic performance is improved by giving iron to those with mild iron deficiency without anemia is beset by problems in the laboratory identification of mild iron deficiency which are further magnified by the dilutional anemia which accompanies endurance training. The identification of milder forms of iron deficiency will be facilitated in future studies by measurement of the soluble truncated form of human transferrin receptor in human serum (12,20,57). The serum transferrin receptor reflects the total

mass of tissue iron and is thereby elevated with either tissue iron deficiency or increased red cell production. Controlled phlebotomy studies in normal volunteer subjects have shown that the serum receptor begins to rise at the point that iron stores are fully exhausted and continues to increase in proportion to the induced deficit in tissue iron (59). Because the serum transferrin receptor increases with enhanced erythropoiesis, a rise in the early period of endurance training may be due to an expanding red cell mass. However, in the highly conditioned athlete, the serum transferrin receptor should provide a more useful quantitative measure of latent iron deficiency than is available with existing iron measurements. The use of this measurement may be helpful in identifying a subset of distance runners with low serum ferritin who might benefit from iron replacement.

RECOMMENDATIONS

The protracted use of iron supplements by individuals who are not deficient in iron must be examined in the light of mounting concerns about the possible adverse effects of elevated body iron stores (28). The most important concern relates to hereditary hemochromatosis which is one of the most common autosomal recessive diseases in populations of Northern European descent. It is estimated that approximately 10% of Caucasian populations carry a single iron loading gene and that 0.3% to 0.5% of the US population are homozygotes (17,29). A significant but unknown proportion of these latter individuals develop irreversible damage to the heart, liver, joints, and other body tissues in midlife that can be fatal without early treatment. While extended use of iron

supplements does not cause this disease, it undoubtedly accelerates the development of clinical manifestations. Of potentially greater concern is that genetically normal segments of the population with higher iron stores may be at increased risk of common and serious disorders such as cancer (61), stroke (63), and coronary heart disease (51). If confirmed, the extended use of iron supplements without laboratory evidence of a continuing need for iron is clearly inappropriate.

The prevalence and severity of iron deficiency in those engaged in endurance training provides little support for the widespread use of iron supplements. Except in the occasional athlete who develops iron deficiency anemia, there is no apparent benefit to athletic performance of improving iron status. It is preferable to meet the modest increase in iron demands in distance runners by maintaining an adequate dietary intake of heme containing foods such as chicken, fish, and beef. Laboratory screening for iron deficiency, especially in athletes who experience a decrease in performance, is preferable to routine iron supplementation. Iron supplements should be used only if iron stores are markedly reduced as defined by a serum ferritin <20 μg/L. The one possible exception is adolescent females who are highly susceptible to iron deficiency and might benefit from a supplement of 18 mg iron daily during the competitive running season.

SUMMARY

The early weeks of intense aerobic training are associated with a moderate decrease in body iron reserves. The development of iron deficient erythropoiesis or iron deficiency anemia is uncommon except in adolescent girls who are highly susceptible to iron deficiency. The early effects of aerobic training on iron status are best explained by a mobilization of storage iron required for an expanding red cell and muscle mass. Overt iron deficiency in the highly conditioned athlete is unusual although some reduction in storage iron is a common finding. This is best explained by an accelerated blood loss from the gastrointestinal tract, particularly in those who use analgesics regularly. Most studies have failed to demonstrate any improvement in athletic performance with oral iron supplementation, especially if the baseline serum ferritin is >12 μg/L. The modest increase in iron requirements in some distance runners is best offset by an adequate intake of dietary heme iron. Because of the possible adverse effects of an excess in body iron, laboratory screening to identify iron deficiency in susceptible individuals or those who experience a deterioration in athletic performance is preferable to the extended use of iron supplements.

ACKNOWLEDGEMENTS

The author wishes to thank Leslie Kuharich for her assistance in the preparation of this manuscript.

REFERENCES

1. Balabán EP, Cox JV, Snell P, et al: The frequency of anemia and iron deficiency in the runner. Med Sci Sports Exerc 21:643-648, 1989

2. Blum SM, Sherman AR, Boileau RA: The effects of fitness-type exercise on iron status in adult women. Am J Clin Nutr 43:456-463, 1986

3. Brotherhood J, Brozovic B, Pugh LGC: Haematological status of middle- and long-distance runners. Clin Sci Mol Med 48:139-145, 1975

4. Brune M, Magnusson B, Persson H, et al: Iron losses in sweat. Am J Clin Nutr 43:438-443, 1986

5. Campanini S, Arosio P, Levi S, et al: Analysis of the iron status of professional cyclists during a 3-week road race. Haematologica 73:111-114, 1988

6. Celsing F, Blomstrand E, Werner B, et al: Effects of iron deficiency on endurance and muscle enzyme activity in man. Med Sci Sports Exerc 18:156-161, 1986

7. Clausen JP: Effect of physical training on cardiovascular adjustments to exercise in man. Physiol Rev 57:779-815, 1977

8. Colt E, Heyman B: Low ferritin levels in runners. J Sports Med Phys Fitness 24:13-17, 1984

9. Cook JD: Adaptation in iron metabolism. Am J Clin Nutr 51:301-308, 1990

10. Cook JD, Dassenko S, Skikne BS: Serum transferrin receptor as an index of iron absorption. Br J Haematol 75:603-609, 1990

11. Cook JD, Dassenko SA, Lynch SR: Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr 54:717-722, 1991

12. Cook JD, Skikne BS, Baynes RD: Serum transferrin receptor. Annu Rev Med 1993 (in press)

13. Davidson RJ, Robertson JD, Galea G, et al: Hematological changes associated with marathon running. Int J Sports Med 8:19-25, 1987

14. Diehl DM, Lohman TG, Smith SC, et al: Effects of physical training and competition on the iron status of female field hockey players. Int J Sports Med 7:264-270, 1986

15. Dressendorfer RH, Wade CE, Amsterdam EA: Development of of pseudoanemia in marathon runners during a 20-day road race. JAMA 246:1215-1218, 1981

16. Dufaux B, Hoederath A, Streitberger I, et al: Serum ferritin, transferrin, haptoglobin, and iron in middle-and long-distance runners, elite rowers, and professional racing cyclists. Int J Sports Med 2:43-46, 1981

17. Edwards CQ, Griffen LM, Goldgar D, et al: Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. N Engl J Med 318:1355-1362, 1988

18. Ehn L, Carlmark B, Hoglund S: Iron status in athletes involved in intense physical activity. Med Sci Sports Exerc 12:61-64, 1980

19. Finch CA, Miller LR, Inamdar AR, et al: Iron deficiency in the rat.^IPhysiological and biochemical studies of muscle dysfunction. J Clin Invest 58:447-453, 1976

20. Flowers CH, Skikne BS, Covell AM, et al: The clinical measurement of serum transferrin receptor. J Lab Clin Med 114:368-377, 1989

21. Fogelholm M, Jaakkola L, Lampisjarvi T: Effects of iron supplementation in female athletes with low serum ferritin concentration. Int J Sports Med 13:158-162, 1992

114

22. Frederickson LA, Puhl JL, Runyan WS: Effects of training on indices of iron status of young female cross-country runners. Med Sci Sports Exerc 15:271-276, 1983

23. Gardner GW, Edgerton BR, Senewiratne B, et al: Physical work capacity and metabolic stress in subjects with iron deficiency anemia. Am J Clin Nutr 30:910-917, 1977

24. Hallberg L, Magnusson B: The etiology of "Sports Anemia". Acta Med Scand 216:145-148, 1984

25. Haymes EM, Lamanca JJ: Iron loss in runners during exercise. Implications and recommendations. Sports Med 7:277-285, 1989

26. Hunding A, Jordal R, Paulev PE: Runner's anemia and iron deficiency. Acta Med Scand 209:315-318, 1981

27. Jensen CA, Weaver CM, Sedlock DA: Iron supplementation and iron status in exercising young women. J Nutr Biochem 2:368-373, 1991

28. Lauffer RB: Iron and Human Disease, Boca Raton, FL, CRC Press, 1992, p 1-534

29. Leggett BA, Halliday JW. Iron balance in Western societies as measured by serum ferritin, in Lauffer RB. (ed): Iron and Human Disease, Boca Raton, FL, CRC Press, 1992, p 107

30. Lyle RM, Weaver CM, Sedlock DA, et al: Iron status in exercising women: The effect of oral iron therapy vs increased consumption of muscle foods. Am J Clin Nutr 56:1049-1055, 1992

31. Magazanik A, Weinstein Y, Abarbanel J, et al: Effect of an iron supplement on body iron status and aerobic capacity of young training women. Eur J Appl Physiol 62:317-323, 1991

32. Magazanik A, Weinstein Y, Dlin RA, et al: Iron deficiency caused by 7 weeks of intensive physical exercise. Eur J Appl Physiol 57:198-202, 1988

33. Magnusson B, Hallberg L, Rossander L, et al: Iron metabolism and "sports anemia". I. A study of several iron parameters in elite runners with differences in iron status. Acta Med Scand 216:149-155, 1984

34. Matter M, Stittfall T, Graves J, et al: The effect of iron and folate therapy on maximal exercise performance in female marathon runners with iron and folate deficiency. Clin Sci 72:415-422, 1987

35. McCabe ME, Peura DA, Kadakia SC, et al: Gastrointestinal blood loss associated with running a marathon. Dig Dis Sci 31:1229-1232, 1986

36. McMann LF, Ryan MJ, Larson D, et al: Occult gastrointestinal blood loss in marathon runners. Ann Intern Med 100:846-847, 1984

37. Newhouse IJ, Clement DB: Iron status in athletes. An update. Sports Med 5:337-352, 1988

38. Newhouse IJ, Clement DB, Taunton JE, et al: The effects of prelatent/latent iron deficiency on physical work capacity. Med Sci Sports Exerc 21:263-268, 1989

39. Nickerson HJ, Holubets M, Tripp AD, et al: Decreased iron stores in high school female runners. Am J Dis Child 139:1115-1119, 1985

40. Nickerson HJ, Holubets MC, Weiler BR, et al: Causes of iron deficiency in adolescent athletes. J Pediatr 114:657-663, 1989

41. Pate RR, Maguire M, Van Wyk J: Dietary iron supplementation in women athletes. Phys Sportsmed 7:81-88, 1979

42. Plowman SA, McSwegin PC: The effects of iron supplementation on female cross country runners. J Sports Med Phys Fitness 21:407-416, 1981

43. Resina A, Gatteschi L, Giamberardino MA, et al: Comparison of RBC indices and serum iron parameters in trained runners and control subjects. Haematologica 73:449-454, 1988

44. Risser WL, Lee EJ, Poindexter HB, et al: Iron deficiency in female athletes: its prevalence and impact on performance. Med Sci Sports Exerc 20:116-121, 1988

45. Roberts D, Smith D: Serum ferritin values in elite speed and synchronized swimmers and speed skaters. J Lab Clin Med 116:661-665, 1990

46. Robertson JD, Maughan RJ, Davidson RJL: Faecal blood loss in response to exercise. Br Med J 295:303-305, 1987

47. Rowland TW: Iron deficiency in the young athlete. Pediatr Clin North Am 37:1153-1163, 1990

48. Rowland TW, Black SA, Kelleher JF: Iron deficiency in adolescent endurance athletes. J Adolesc Health Care 8:322-326, 1987

49. Rowland TW, Deisroth MB, Green GM, et al: The effect of iron therapy on the exercise capacity of nonanemic iron-deficient adolescent runners. Am J Dis Child 142:165-169, 1988

50. Rowland TW, Kelleher JF: Iron deficiency in athletes: Insights from high school swimmers. Am J Dis Child 143:197-200, 1989

51. Salonen JT, Nyyssonen K, Korpela H, et al: High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. Circulation 86:803-811, 1992

52. Schobersberger W, Tschann M, Hasibeder W, et al: Consequences of 6 weeks of strength training on red cell O2 transport and iron status. Eur J Appl Physiol 60:163-168, 1990

53. Schoene RB, Escourrou P, Robertson HT, et al: Iron repletion decreases maximal exercise lactate concentrations in female athletes with minimal iron-deficiency anemia. J Lab Clin Med 102:306-312, 1983

54. Schwartz S, Dahl J, Ellefson M, et al: The "HemoQuant" test: A specific and quantitative determination of heme (hemoglobin) in feces and other materials. Clin Chem 29:2061-2067, 1983

55. Seiler D, Nagel D, Franz H, et al: Effects of long-distance running on iron metabolism and hematological parameters. Int J Sports Med 10:357-362, 1989

56. Selby GB, Eichner ER: Endurance swimming, intravascular hemolysis, anemia, and iron depletion. New perspective on athlete's anemia. Am J Med 81:791-794, 1986

57. Shih YJ, Baynes RD, Hudson BG, et al: Serum transferrin is a truncated form of tissue receptor. J Biol Chem 265:19077-19081, 1990

58. Siegel AJ, Hennekens CH, Solomon HS, et al: Exercise-related hematuria: Findings in a group of marathon runners. JAMA 241:391-392, 1979

59. Skikne BS, Flowers CH, Cook JD: Serum transferrin receptor: A quantitative measure of tissue iron deficiency. Blood 75:1870-1876, 1990

60. Snyder AC, Dvorak LL, Roepke JB: Influence of dietary iron source on measures of iron status among female runners. Med Sci Sports Exerc 21:7-10, 1989

61. Stevens RG, Jones DY, Micozzi MS, et al: Body iron stores and the risk of cancer. N Engl J Med 319:1047-1052, 1988

62. Stewart JG, Ahlquist DA, McGill DB, et al: Gastrointestinal blood loss and anemia in runners. Ann Intern Med 100:843-845, 1984

63. Terada LS, Willingham IR, Repine JE. Iron and stroke, in Lauffer RB. (ed): Iron and Human Disease, Boca Raton, FL, CRC Press, 1992, p 313

64. Walsh R, McNaughton L: Serum iron levels in young female swimmers during pre-season phase of competition and the effects of iron supplementation upon them. J Hum Move Studies 17:229-238, 1989

65. Weaver CM, Rajaram S: Exercise and iron status. J Nutr 122:782-787, 1992

66. Weight LM, Jacobs P, Noakes TD: Dietary iron deficiency and sports anaemia. Br J Nutr 68:253-260, 1992

67. Woodson RD: Hemoglobin concentration and exercise capacity. Am Rev Respir Dis 129:S72-S75, 1984

APPENDIX B

APPENDIX B-1

DRAFT

A PROJECT PROPOSAL

A PARTNERSHIP FOR IMPROVING IRON NUTRITION IN GRENADA

THE FORTIFICATION OF WHEAT FLOUR (Using The Nutribusiness Model)

Collaborative Project With

THE GOVERNMENT OF GRENADA

the

CARIBBEAN AGRO INDUSTRIES Ltd.

and

THE CARIBBEAN FOOD AND NUTRITION INSTITUTE

Caribbean Food and Nutrition Institute
Kingston 7, Jamaica

Pan American Health Organization
Pan American Sanitary Bureau, Regional
Office of the World Health Organization

1991

136

CONTENTS

EXECUTIVE SUMMARY 1-2

I. INTRODUCTION 3-7

II. FOOD FORTIFICATION 9

II.1 Wheat Flour Fortification in the English-speaking Caribbean 10

II.2 Present Fortification Programme in Grenada 11

II.3 Change in Iron Fortification Programme in Grenada 11-13

III. OBJECTIVES OF THE STUDY 14-15

IV. METHODOLOGY 15

IV.1 Impact of Iron Fortification 15-18

V. ANALYSIS 18

V.1 Laboratory 18

V.2 Statistical Analysis 19

VI. HEMATOLOGY 19-20

VII. SURVEILLANCE 21

VIII. ECONOMIC JUSTIFICATION OF FORTIFICATION 21-22

IX. MONITORING 22-23

X. QUALITY ASSURANCE 23-24

XI. SAFETY OF FORTIFICATION 24-25

XII. TRAINING 25

REFERENCES 26-27

BUDGET 28-37

TABLE 38

FIGURE 39

SCHEDULE OF EVENTS 40-41

ANNEXES

ANNEX 1 42

ANNEX 2 43-44

ANNEX 3 45

ANNEX 4 46

138

EXECUTIVE SUMMARY

Previous studies have shown that anaemia is a public health problem in Grenada. An islandwide anaemia survey of that country was conducted in 1985-86. The results of the study showed that anaemia and iron deficiency were serious problems throughout the entire population. The prevalence of deficient plasma ferritin levels indicating no iron reserves was as high as 62% in some population groups.

Iron supplements or iron fortified foods have been found to be cost-effective in raising haemoglobin levels of iron deficient populations resulting in increased productivity.

Studies have shown that wheat flour and wheat products are the principal contributors to energy and protein among most Caribbean populations. In the case of Grenada, wheat flour is the staple food for the population and would thus be a logical choice as a vehicle for iron fortification.

In Grenada, wheat is imported and is milled locally at a central location. Baking flour which is used in the baking trade, and for home use, is fortified with iron and B-complex vitamins. Thirty-seven milligrams of iron per kilogram of baking flour is used. The counter flour which is sold for home use and mainly in rural areas in Grenada is not fortified with iron or B-complex vitamins. The ratio of sales of baking flour to counter flour is 55:45. This means that 45% of the wheat flour (counter), which is the main source of wheat flour for home consumption, is not fortified with iron or B-complex vitamins.

The purpose of this study will be to fortify all wheat flour in Grenada with a suitable type and level of iron, evaluate the impact of the fortification programme and institute an anaemia monitoring system. This will mean adding 44mg of iron per kilogram of counter flour. The type of iron added will be ferrous sulphate.

The project will use the nutribusiness strategy. This strategy promotes indigenous private sector enterprises in food processing, thus enabling these enterprises to produce higher quality foods. It is a partnership among private enterprise and governmental organizations. Nutribusiness links nutrition and the private sector to address well-defined nutritional needs of the population.

The project will last for three years and will be evaluated by sensitive hematological parameters. The objective of the study is to significantly reduce iron deficiency in the entire Grenadian population. The evaluation will be based on analyses of blood samples and questionnaire responses obtained from randomly chosen households and from antenatals attending clinics in depressed areas. Government statistics relating to health and productivity will also be used to determine trends.

This proposal seeks funding of US$56,500 to start the study.

I. INTRODUCTION

Existing data indicate that anaemia exists in all countries of the English-speaking Caribbean. It is most common in pregnant and lactating women, and in pre-school children, with country prevalences (using WHO criteria) between 27-100% and 11% and 76%, respectively.

Severe anaemia, with a haemoglobin concentration in the blood below 8 g/dl, is found in about 6% of pregnant and lactating women and 11% of pre-school children in some of the countries.

There are three major causes of anaemia in the Caribbean:

1. Poor nutrition;
2. Specific parasitic infestations; and
3. Haemoglobinopathies.

All three causes are important from a public health point of view, and all three may interact.

The major (dietary) nutritional cause of anaemia is iron deficiency, probably due to a low absorption in the intestine and an inadequate dietary intake. A dietary deficiency of folate is sometimes also responsible. Changes in food habits of some sections of the population are associated in some instances with a deficiency of vitamin B_{12}. This appears to be currently a minor problem in the English-speaking Caribbean, but it could increase.

141

Hookworm and whipworm (trichuris) are the two main parasites associated with anaemia. Infestations of hookworm are isolated in certain areas of the population. The major haemoglobinopathy causing anaemia is sickle-cell disease [1-11].

In 1972, a nutrition survey was conducted in the village of La Poterie in Grenada. Anaemia was found to be a problem in all ages studied in the survey. Thirty-eight percent of pre-school age children, 65% of school-age children, 22% of adult males, and 49% percent of non-pregnant, non-lactating females were found to be anaemic by the World Health Organization (WHO) standards. Low serum iron and folates were found in the pre-school age children and adult females [12].

Another study conducted in 1976 indicated that 51% of ante-natals were anaemic, according to suggested WHO haemoglobin standards. Eighteen point five percent of pre-school age children had haemoglobin levels below 10.5% g/dl [13]. The WHO recommended haemoglobin standard for pre-school age children is 11.0 g/dl [4].

Most recent data obtained from health clinics showed that of the 400 women assessed prenatally, 234 (58.5%) had haemoglobin levels below 11 g/dl in 1983, and in 1984, of the 479 women tested, 353 (73.7%) had low haemoglobin levels, i.e. below 11 g/dl [15].

The Government of Grenada is concerned about the poor performance of its students on the CXC Examination. Questions have been raised about the possibility

of iron deficiency contributing to the poor school performance of Grenadian school children.

In 1985, two females died of post-partum haemorrhage, which could be caused from anaemia. Because of the fear that anaemia could be a major public health problem in Grenada, the Government of Grenada requested that the Caribbean Food and Nutrition Institute (CFNI) study the problem of anaemia in Grenada and develop a programme for its control.

As a first step, a population survey of iron status was planned. The variables to be studied were demographic, social, dietary, parasitic and haematological. The blood tests were haemoglobin, hematocrit, sickling, and serum ferritin. Qualitative counts of parasites in stool samples were done by CAREC. The dietary data concentrated on frequency of consumption on iron-rich food, enhancers and inhibitors on iron absorption, and possible vehicles for iron fortification.

The sample was statistically representative, with sub-samples of pregnant and lactating women. The study was conducted in November 1985.

In summary, the findings were that anaemia was widely prevalent in all ages and gender groups ranging from a low of 14% in young men to a high of 74% in pregnant women. The next most vulnerable groups were children aged 0-5 years (with a peak of 12-24 months) and lactating women. Serum ferritin results demonstrated an almost universal lack of iron stores, pointing to iron deficiency as a significant aetiological factor. SS haemoglobinopathy was found to be present in

0.5% of the sample, and 15% had an AS genotype. Hookworm was found in 6% of the stool samples. Although other parasites were found, the load was not considered to be sufficient to be a cause of the anaemia demonstrated [16].

The qualitative pattern of the diet was satisfactory, enhancers of iron absorption were consumed often and inhibitors rarely. However, quantitative data on the diet were not collected.

In June 1986, a workshop on the Grenada Anaemia Survey was convened to discuss the results of the survey and to develop a strategy for the control of anaemia in Grenada [17].

The participants at the workshop were divided into five working groups and were requested to work out a simple, workable strategy. The groups recommended specific procedures to improve supply management, supplementation programmes, haemoglobin screening, education, and fortification. It was recommended that all flour be fortified and that the effectiveness of such a programme be evaluated by the initial blood samples and the subsequent monitoring of blood samples every six months, up to two years. [7]

After the workshop, considerable work was done on the control of anaemia in pregnant women and pre-school age children. In pre-school children regular administration of iron is difficult. In pregnant women supplementation maintains the iron status but does not correct the initial deficiency. However, to reach the entire

population other approaches must be considered. The fortification of wheat flour in Grenada should reach this objective.

Wheat flour has been fortified with iron in numerous countries around the world. Some countries have had these fortification programmes for as long as forty years. However, the fortification of wheat flour with iron has never been evaluated on a national scale.

The nutribusiness concept could be the vehicle to ensure a constructive and lasting programme. Nutribusiness links nutrition and the private sector to address well-defined nutritional needs of populations. Operationally, nutribusiness provides quality foods and food products at affordable prices through private-sector approaches. It uses environmentally regenerative production, storage, and distribution techniques and avoids pollution and inappropriate or excessive use of energy and other natural resources. Nutribusiness thus ensures optimal nutritional quality, safety, and acceptability of foods provided through commercial channels and in keeping with local cultural and environmental imperatives. The nutribusiness model addresses "well defined nutritional needs and populations". When nutritional needs are specified and target populations carefully defined, it is possible to examine how needs can be met (Annex 1).

The programme will be implemented by Caribbean Agro Industries Ltd. which is the flour mill in Grenada. Guidance and suggested levels of fortification will be provided by the Government of Grenada. The project will be evaluated by Caribbean

Agro Industries Ltd, The Government of Grenada and the Caribbean Food and Nutrition Institute (Annex 2).

Once such a programme is implemented and evaluated, it is believed that the prevalence of iron deficiency anaemia in Grenada should be reduced. It is also believed that the methodology and experience gained in Grenada would be applicable and could be used in other Caribbean countries and, possibly, in other parts of the world. A description of Grenada can be seen in Annex 3.

HOPEFUL MAJOR BREAKTHROUGHS

The following initiatives and evaluations will be attempted for the first time in this project:

First Time

1) A Government, a private sector company and an international agency will collaborate on an iron nutrition project through the Nutribusiness Approach.

First Time

2) The fortification of wheat flour with iron will be evaluated on a national scale.

First Time

3) The plasma transferrin receptor will be used to evaluate a programme on a national scale (see section VI).

First Time

4) A cost/benefit analysis will be conducted after fortifying wheat flour with iron.

II. FOOD FORTIFICATION

The fortification of foods - the addition of selected nutrients to foods in order to improve their nutritive value - is a commonly used strategy for improving or maintaining the nutritional status of a population by increasing the daily intake of specific nutrients. The technology for adding nutrients to foods is well developed and has been used in many countries for many years. Primary examples are the addition of iodine to salt, vitamin A to fats and sugar, and vitamin C to juices. The most widely used food fortification technology is for the addition of B-vitamins and iron to cereal foods such as flour, bread, meals, and pasta. The fortification of wheat flour with vitamins and iron is presently being used in more than 20 developed and developing countries. Also, in at least 12 countries that do not produce their own flour, the imported flour is required to be fortified with vitamins and minerals. The fortification of foods has been shown to be a simple, effective and relatively inexpensive way of introducing nutrients, such as bioavailable iron, into the daily diet.

The technology for the fortification of cereal food, such as wheat flour with iron, involves two major components:

1. The installation and operation of the equipment for adding the nutrient;
   a. in reasonable amounts
   b. on a fairly regular basis
2. Consumption by those in the lower economic levels, the ones most in need of the nutrients. Since cereal foods are the primary staple food for so many

of the world's population, especially in developing countries, they are the most frequently selected vehicles for fortification.

II.1 Wheat Flour Fortification in the English-speaking Caribbean

Wheat flour and wheat products are the principal foodstuffs consumed in the Caribbean, except Guyana. As can be seen in Figure 1, this wheat and wheat flour is imported from West Germany, the United States, Canada and Puerto Rico. Some of the pre-ground wheat flour imported into the Caribbean is fortified with vitamins and iron.

Most wheat flour imported into the Caribbean and ground locally is fortified with vitamins and iron except in Suriname. These wheat products are fortified at the following levels:

1.	Thiamine	-	4.4	-	5.5 mg/kg
2.	Riboflavin	-	2.6	-	3.3 mg/kg
3.	Niacin	-	35.0	-	46.0 mg/kg
4.	Iron			- 28.0	36.0 mg/kg
5.	Calcium	-	1.1	-	1.4 mg/kg

Fortification with calcium is optional and is usually not done.

The iron added in both pre-ground as well as locally ground wheat is in the form of either ferrous sulphate or reduced iron. Ferrous sulphate has a higher bioavailability than does reduced iron of a large particle size. The reduced iron size used is 44 mu in size. Studies have shown that this large particle size reduced iron is

absorbed to a limited size. Since wheat provides 17 and 24% of available energy and 18 to 30% of protein for those Caribbean countries from which information is available, this produce needs special mention in relation to fortification with iron [18].

II.2 Present Fortification Programme in Grenada

In the case of Grenada, wheat flour is the staple food of the population. The amount of wheat flour produced and available on a per capita basis can be seen in Table 1. This is the logical choice as a vehicle for an iron fortification programme.

In Grenada, presently 99% of wheat flour used is milled locally at the central location. Both baking flour and counter flour are produced. Baking flour, which is used in the baking trade and some home use, is fortified with iron, thiamine, riboflavin and niacin. The iron is added at 37.0 mg/kg of baking flour and is in the form of ferrous sulphate. Forty-five percent of the flour is counter flour. This means that 45% of the wheat flour (counter flour) which is the main source of wheat flour for home consumption and probably the flour purchased by the lower socio-economic group, is not fortified with iron or B-complex vitamins.

Both baking flour and counter flour are sold in 100 lb. bags to bakeries and supermarkets. The supermarkets usually divide both wheat flours in 2, 8, 10 or 15 lb. bags. A two-pound bag of baking flour costs EC$1.66, and a two-pound bag of counter flour sells for EC$1.56. Baking flour is also sold in five-pound labelled packets.

II.3 Change in Iron Fortification Programme in Grenada

As part of an iron fortification programme of wheat flour in Grenada, specifications will be established and plans developed with the Grenadian Caribbean Agro

Industries Limited (The flour mill), to ensure that the form of iron added to all wheat flour produced in the country be ferrous sulphate.

The wheat flour component of the fortification programme can be viewed both as:

1. An upgrade of the nutritional value: the change in iron form used in a food already part of an intervention; and
2. As a new nutrition intervention involving the fortification of counter flour with ferrous sulphate or electrolytic reduced iron and B-vitamins.

This intervention will make iron fortified flour available to people who have not had access to fortified flour.

About 55% of the wheat flour used in the country is baking flour and is produced at the Caribbean Agro Industries Limited in St. George's, Grenada. The type of flour currently produced is used in commercial bakeries to produce breads of all types and is available for some home use. The flour is fortified with B-vitamins and iron by use of a specially prepared premix which is added to the flour using typical food fortification technology. This premix contains enzymes and oxidation materials in addition to the vitamins and iron. These ingredients are not usually included in standard premixes. The form of iron used in the premix is ferrous sulphate and is added at the level of 37 mg/kg of baking flour.

Although precise costs are not available at this time, preliminary calculations suggest a slight increase in cost for adding the premix to the counter flour. The producer has agreed to absorb the increase in the cost as part of his effort for improving the nutritional value of the flour.

The remaining 45% of the flour used in Grenada is "counter flour". This flour traditionally is used by the majority of the population for general home use and probably used by the lower socio-economic group, since it is less expensive than the baking flour. Available information indicates that this flour is not fortified with any nutrients, including iron, so that the general population which consumes counter flour have not been recipients of any added iron in their daily diet through the use of counter flour. The counter flour should be fortified with B-vitamins and iron. Ferrous sulphate could be considered as the iron source of choice. A premix for this use will be much less complicated, ingredient-wise, than that used for the bread flour. It is expected that this premix also will undergo a long holding time in the mill before use, but no problems are anticipated with the use of ferrous sulphate (which is the standard for comparison) which is available from producers of premises. The cost to fortify wheat flour with B-vitamins and iron (ferrous sulphate) to current U.S. standards is about US$0.90 per tonne. The iron level for this fortification would be 44 mg/kg of flour.

In review, the iron source contained in the premix, used to fortify the wheat flour produced in the country, should be ferrous sulphate. The required iron level in all wheat flours should be determined regardless of iron source used.

A programme for the iron fortification of counter flour will involve the addition of iron such as ferrous sulphate in the mill at the time of production. The iron will be blended into a premix for ease and accuracy of addition to the meal. A level of 44 mg per kilogram of counter flour is suggested. Ferrous sulphate will be used.

The project will be self-sustaining in that Caribbean Agro Industries Ltd. has agreed to continue the fortification of wheat flour with B-complex vitamins and iron after the initial project has been completed.

III. OBJECTIVES OF THE STUDY

The general aim of the project is to significantly reduce iron deficiency in the entire Grenadian population through wheat flour fortification. To this end we will need to have all appropriate monitoring devices in place so that the benefits of the improved fortification can be observed. Such benefits may relate not only to health effects but also to worker productivity and academic achievement. Measures in the latter areas will of necessity be crude but their importance is such that they cannot be overlooked. Additionally, it will be necessary to look more closely at the high risk group: antenatals living in depressed areas.

Specific Objectives

1. To evaluate the short term impact of the change in fortification on iron status, general health and productivity of the entire population by:
   a) A survey of households which are a representative of the entire population.
      1) Hematology
      2) Food Consumption patterns
      3) Socio-economic parameters
   b) Analysis of Government statistics.
      1) Health Parameters
      2) Productivity
      3) Educational achievement

2. To evaluate the impact of the change in fortification on the detailed hematology of pregnant women attending clinics in depressed areas.
   1) Hematology
   2) Socio economic parameters
3. To improve the country wide surveillance system for monitoring anaemia and its effects. This system will have the following features:
   a) It will identify appropriate indices of productivity that are routinely part of Government statistics.
   b) It will identify appropriate health indicators that are or should be a part of Government statistics.
   c) It will ensure that appropriate observations are taken in the hospitals and clinics and transmitted to the health authorities.
   d) It will include a sub-system for the quality control of wheat flour through random sampling in food retail outlets activities and bakeries.

IV. METHODOLOGY

IV.1 Impact of Iron Fortification

The evaluation of the impact of the change of iron fortification of wheat flour derives from several sources. The first is the improved iron status of a random sample of households and after the institution of the change in fortification. The second relates to the measurement of maternal mortality and morbidity. The third

relates to productivity and academic attainment data collected by the establishment of an improved surveillance system. These changes will be evaluated for three years and some of the parameters will be continually measured and evaluated after the initial three year period of the project.

Household Survey

The population of Grenada is approximately 97,495 persons. A 1.02 percent sample will be 1,000 persons. If we assume approximately four persons per family, 250 families will be chosen for the study. However, to allow for families moving during the study 300 families will be chosen from a random sample of households chosen by enumeration districts (ED's) from the entire population. A single well-structured pre-tested questionnaire will be administered to the same households at the start of the project. The questionnaire will be administered by trained interviewers. It will focus on age, sex and employment status of household members and purchasing and consumption patterns.

The questionnaire will contain questions regarding the length of time flour is held at home before consumption. This would be important to ascertain whether the fortified flour is used within the designated shelf life. Qualitative food consumption data will be collected on each of the households. This will be done by asking questions on which food items are eaten rarely, once or twice weekly, three to seven times per week, or more than once per day. This should include some measurement of meat, fish, poultry, legumes, coffee, tea and citrus fruits and or other vitamin C rich foods. A larder survey may be conducted on a sub-sample of households.

A finger prick blood sample will be taken and the haemoglobin, plasma ferritin, and the transferrin receptor levels would be determined from each family member except pre-school age children under six months of age.

The same questionnaire including information on food consumption and finger prick blood samples will be collected at the eighteenth and thirty-six month on the same households.

Pregnant Women

The Government of Grenada has recently completed a study identifying Economically Depressed Communities in Grenada. A sample of 200 pregnant women that are attending antenatal clinics will be chosen from these depressed communities (See Annex 4). Only those pregnant women who enter the clinics at 16-20 weeks of pregnancy will be studied. Any woman with sickle cell disease will be excluded from the study. A questionnaire will be administered which will include name, age, and gestational period.

A blood sample will be taken and a hemoglobin, plasma ferritin and plasma transferrin receptor level determined. The blood samples will be taken at the beginning of the study and will be repeated at 18 and 36 months on ante-natals attending the same ante-natal clinics.

Other Parameters

The hospital system consists of main multidisciplinary hospital (General Hospital) in the capital (240 beds), a smaller general hospital in a rural area (40 beds) and a general hospital in Carriacou (32 beds).

Information will be collected on a yearly basis on mortality due to anaemia. Also in each of the three hospitals the number of patients admitted with a diagnosis

of anaemia will be recorded. The cost of treating these anaemic patients particularly patients admitted who are pregnant, will be recorded. This will include the cost of maintaining the bed, blood transfusions, etc. This information will be collected on a yearly basis.

Information will also be collected on a yearly basis to include passes in the CXC examination, low weight per age of pre-school age children, infant mortality rate, banana production, cocoa production, nutmeg production and gross national product.

V. ANALYSIS

V.1 Laboratory

The blood samples will be analysed at the Clinical Laboratory in Grenada and the Iron Nutrition Monitoring Laboratory at CFNI, Jamaica. The Department of Haematology, Kansas Medical Centre, Kansas City, U.S.A., will serve as a reference laboratory. A finger prick sample of blood will be collected from each person at the start. The sample will be taken in a plastic disposable blood collection device by using a disposable spring loader lancet. Twenty micro-litres of blood will be taken by Hemocue micro-cuvette and the micro-cuvette put into the Hemocue Photometer. The haemoglobin will be determined by the Hemocue method [20]. The whole blood sample will be kept cold and sent to the Central Hospital Laboratory, where it will be spun at 2,000 r.p.m. for 10 minutes. The plasma will be removed and frozen. The frozen plasma samples will be hand-carried to CFNI, Jamaica.

The plasma ferritin and the transferrin receptor level samples will be determined by the ELISA [21-22].

V.2 Statistical Analysis

1. Simple graphical analyses of trends in various Government statistics for productivity, educational attainment and health. These will include analysis of retrospective data where possible.
2. Simple graphical analysis of trends in various haematological measures as applied to the various sub population groups:

   0-5 years, 6-4 (M), 6-14 (F), 15-44 (M),

   45 and over (M), 45 and over (F), antenatals.

   This will be supplemented by ANOVAs to compare measures before and after the change in fortification.
3. An appropriate technique for statistical analysis of quality control data (flour) is yet to be worked out.
4. Data on flour consumption will be related to haematological parameters using correlational techniques.

VI. HEMATOLOGY

Estimating the severity of mild iron deficiency assumes greater importance in population studies. Earlier surveys relied on single cut-off levels of laboratory parameters to distinguish normal from iron-deficient segments of a population. In the evaluation of the Health and Nutrition Examination Survey (HANES II) in the United States, combinations of measurements were used to define prevalence of impaired

iron status or iron deficiency anaemia. (23) Another recent approach is to use algorithms to estimate body iron quantitatively in each sampled individual. (24) Because of the close correspondence between the serum ferritin and body iron stores, this calculation is reliable in otherwise healthy individuals if residual iron stores are present. At the other extreme in patients with overt anaemia, the deficit in body iron can be estimated quantitatively from the decrease in circulating haemoglobin concentration. Between these limits, estimating mild deficits in functional iron is more difficult. In previous report, combinations of the serum ferritin, transferrin saturation, and erythrocyte protoporphyrin were used to estimate body iron between the occurrence of storage depletion and the development of anaemia (24).

A recent study has shown that the serum reception level is a reliable index of early tissue iron deficiency. Of all the measurements examined, the serum receptor was the most reliable quantitative guide to a deficiency in the functional iron compartment. Therefore, the iron status of a population could be fully assessed by using serum ferritin as a measure of iron status, serum receptor as a measure of mild tissue iron deficiency, and haemoglobin concentration as a measure of advanced iron deficiency (25).

This could be a major methodological breakthrough. This will be the first time that the receptor level has been used on a national scale. Hopefully such a sensitive indicator as the receptor level could be shown to reduce the time necessary to evaluate an iron nutrition project.

The haemoglobin, ferritin and receptor levels can be estimated on a finger prick sample of blood which will greatly facilitate the conduct of the study. Also all these measurements will be conducted in Grenada and Jamaica and will be the first time that these two sensitive hematological indicators will be used in developing countries.

VII. SURVEILLANCE

A surveillance system will be designed and left in place after the fortification of counter flour has been evaluated. The present surveillance system consists of estimating the haemoglobin level on pregnant women. This system will continue. From 1985-1988 hemoglobin levels were collected on pre-school age children but was discontinued because of a lack of hemoglobinometers. Data will continually be collected on maternal mortality and morbidity, low birth weight infants, weight per age on infants and pre-school age children, etc. Quality control will be conducted on both counter and baking flour by Caribbean Agro Industries and the Produce Chemist Laboratory. (See
Section X. Quality Assurance).

VIII. ECONOMIC JUSTIFICATION OF FORTIFICATION

The end results in food fortification programmes are the improvement in iron status of the target population, increased productivity and reduced adverse consequences arising from poor iron status. Food fortification is justified when these positive attributes could be traced to the programme.

Approximately 10% of iron contained in wheat flour is absorbed. Thus 10% of the known level of iron added per unit wheat flour consumed is expected to enhance the iron status which in turn would enhance work output. The literature suggests worker output of between one and two percent is associated with a one percent

increase in the Hb status. Among populations with severe iron deficiency anaemia, supplementation has resulted in increases in Hb status of 25 percent and over (26). Under such conditions, worker productivity could increase 25-50%.

A series of economically unquantified adverse consequences are associated with poor iron status. These include maternal mortality and morbidity, poor growth and development, impaired immune system, reduced learning capacity and lassitude.

At the rate of 44 mg per kg of iron added to the wheat flour and an average per capita consumption of 0.15 kg flour per person per day, 0.66 mg or iron is expected to be absorbed. This represents 66% of the iron retention requirement of adult males and post-menopausal females. Since approximately 55% of the flour consumed is fortified at 37 mg/kg with iron whose bio-availability is suspect, an additional contribution of less than 33% is anticipated from the change. This additional contribution from a single fortified food source could have a significant impact on the haemoglobin status and thus on worker productivity. The cost of the supplementation estimated at $0.90 per tonne is negligible while the benefits could be great. Levin reports studies in which the benefit cost ratios of iron fortification were in the order of 5-8 in Indonesia, 30-47 in Kenya and 49-79 in Mexico (26). The fortification of wheat flour in Grenada is intuitively justifiable. This study will test the economic justification and other benefits of the proposed changes.

10 %

IX. MONITORING

The importance of establishing the efficiency of an iron fortification programme cannot be over-emphasized. It is seldom possible to measure the impact of a fortification programme once it is implemented, and this is particularly true with respect to iron fortification. In contrast to fortification with nutrients such as niacin, thiamine, or riboflavin, where the objective is to prevent the occurrence of isolated deficiencies, it is feasible to alter body iron stores of an entire population through dietary manipulation.

The customary approach to monitoring efficiency is to conduct a pilot study, but there are several drawbacks to this. During a preliminary trial, the supply of a fortified food is usually ensured whereas, this is not necessarily the case when implemented on a national scale. Another important limitation of preliminary iron trial is that because of the inhibiting effect of the diet on iron absorption, changes in iron status in a population produced by iron fortification occur very slowly, often only after several years. Pilot studies, therefore, delay the implementation of a fortification programme and are very costly because of the long duration of the trial.

It is therefore planned to start the study on a national scale and carefully monitor the population by using very sensitive haematological indices.

X. QUALITY ASSURANCE

During stage one and throughout an iron fortification programme, quality assurance activities must be carried out to verify the iron content of the fortified

flours. Sampling and testing for iron content and sorting out effect should be carried out routinely at the mills during the production of the products. The in-plant quality testing should be the responsibility of and carried out by the companies, since they have the laboratory facilities and good technical personnel with which to do this. This will be conducted by Caribbean Agro Industries Ltd.

Samples of products should be drawn periodically from the market shelf by designated government representatives and analyzed for iron content. This is needed to verify both the presence of added iron and in the proper amount. These samples will be analyzed at the Produce Chemist Laboratory, St. George's, Grenada. This level of quality assurance activity is necessary to ensure that there is a given level of iron intake each day based on expected consumption. A successful intervention can be accomplished only when the iron is present in the food on a continuing basis in the correct amount. Anything less than this could lead to unsatisfactory results due to lack of proper control rather than a failure of the intervention itself.

XI. SAFETY OF FORTIFICATION

The possible adverse effect of adding large amounts of iron to the diet in certain segments of a population has been discussed from time to time and deserves comment. In reviewing studies in which possible adverse effects of iron have been reported, it is important to define the route of iron administration. When large doses

of Imferon (iron dextran) were given by deep subcutaneous injection to new born infants, an increased frequency or fatal septicemia was noted, but it was not clear, whether this was due to iron or due to the intramuscular injection. There is some published evidence that the frequency of malaria may increase when full doses of therapeutic oral iron are administered to a susceptible population.

On the other hand, it must be stressed that no adverse effects have yet been demonstrated when conventional levels of iron fortification have been implemented in a population. Homozygotes for the iron loading gene, i.e. **idiopathic hemochromatosis**, have been demonstrated to occur in 1:300-500 American whites but this genetic disorder has never been clearly identified in a black individual. Countries such as Sweden are continuing to fortify the diet at a level that provides approximately 40% of dietary iron intake without demonstrable adverse effects. Therefore, there seems to be little basis for concerns for a fortification programme that will double the intake of dietary iron even in iron replete individuals. Nevertheless, because of the lack of information on the maximal consumption of wheat flour by older children and adults, it is desirable to establish consumption patterns of this food vehicle throughout the population.

XII. TRAINING

Preliminary discussions have taken place for training members of the Grenadian Food and Nutrition Council. This training will probably consist of the Systems

Analyst, U.W.I., Mona, Jamaica, in developing a training course for the computer analyst and members of the council attending training courses in the U.S.A. on Training for Project Managers. The CFNI Public Health Nutritionist will train all Public Health Nurses on the use of the Hemocue for determining the haemoglobin level and the latest techniques for taking blood samples.

REFERENCES

1. Simmons, W.K. Nutritional Anaemia in Jamaica. West Ind Med J 28:199.

2. Simmons, W.K., Sinha, D.P. Anaemia in the Cayman Islands: Its Prevalence and Control. CFNI-J-24-80, May 1980.

3. Simmons, W.K. et al. A Survey of the Anaemia Status of Pre-school Age Children and Pregnant and Lactating Women in Jamaica. Am J Clin Nutr 1982, 35:319.

4. Simmons, W.K., Gurney, J.M. Nutritional Anaemia in the English-speaking Caribbean Suriname. Am J Clin Nutr 1982; 35:327.

5. Simmons, W.K. Anaemia in the Caribbean: Its Prevalence and Causes. CFNI-J-10-83, February 1983.

6. Serjeant G.R. The Clinical Features of Sickle Cell Disease. North Holland Publishing Co., Amsterdam, 1974.

7. Simmons, W.K. et al. Anaemia in Ante-natals in the Turks & Caicos Islands. W.I. Med J 1981; 36:210.

8. Simmons, W.K. The Control of Anaemia in the English-speaking Caribbean. CFNI-J-34-83, 1983.

9. Simmons, W.K. Programmes for the Prevention of Anaemia in Jamaica. West Ind Med J 1984; 33:92-96.

10. Bramble, D. and Simmons, W.K. Anaemia in Ante-Natals in Montserrat. West Ind Med J 1984; 33:92-96.

11. Simmons, W.K. Nutritional Anaemia in Ante-natals in the English-speaking Caribbean. CFNI-J-22-85.

12. Caribbean Food and Nutrition Institute, La Poterie, Nutrition Survey, Grenada Survey, Grenada, W.I. CFNI, May 1972.

13. Gueri, M. Nutrition Status of Young Children in Grenada. Caribbean Food and Nutrition Institute, 1976.

14. World Health Organization. Nutritional Anaemia. Tech Ref Ser No. 503, WHO, Geneva, 1972.

15. Ministry of Health, Grenada, Personal Communication.

16. Caribbean Food and Nutrition Institute. Anaemia of Iron Status of the Grenadian Population. Mimeo document. CFNI-J-17-86. CFNI, Kingston, Jamaica, 1986.

17. Caribbean Food and Nutrition Institute. A Strategy for the Control of Anaemia in Grenada. Mimeo document CFNI-J-19-86, CFNI, Kingston, Jamaica, 1986.

18. Gurney, J.M. Nutritional Considerations Concerning the Staple Foods of the English-speaking Caribbean. Ecol Food and Nutr 1975; 4:171.

19. Caribbean Food and Nutrition Institute. Recommended Dietary Allowances for the Caribbean. CFNI, 1979.

20. Bridges N, Parvin R.M. and Van Assendelft, O.W. Evaluation of a New System for Haemoglobin Measurement. Reprinted from American Clinical Products Review, April 1987.

21. Flowers, C.H., Kuizon, M., Beard, J.L., Skikne, B.S., Covell, A.M. and Cook, J.D. Am J Hematol 1986; 23:141-151.

22. Flowers, C.H., Skikne, B.S., Covell, A.M. and Cook, J.D. The Clinical Measurement of Serum Transferrin Receptor. J Lab Clin Med 1989; 114:368-377.

23. Dallman, P.R., Yip R. Johnson C. Prevalence and Causes of Anaemia in the United States, 1976 to 1980. Am J Clin Nutr 39:437, 1984.

24. Cook, J.D., Skikne B.S., Lynch, S.R., Reusser, M.E. Estimates of Iron Sufficiency in the US Population. Blood 68:726, 1986.

25. Skikne, B.S., Flowers, C.H., and Cook, J.D. Serum Transferrin Receptor: A Quantitative Measure of Tissue Iron Deficiency. Blood 1950, 75: 1870-1876.

26. Levin Henry M. Benefit-cost analysis of anaemia interventions and social marketing. Stanford University, 1986.

APPENDIX B-2

ADHERENCE TO IRON SUPPLEMENTATION DURING PREGNANCY: DETERMINANTS AND EFFECT ON HEMATOLOGIC VARIABLES.

A research proposal.

Eva-Charlotte Ekström

Nutrition Officer

World Health Organization

September 1991

Tanzania Food and Nutrition Center
PO Box 977
Dar es Salaam

List of contents:

I. Background

II. Conceptual model

- A. Theoretical basis
- B. Focus of study

III. Objectives

IV. Previous studies

V. Relevance of study

VI. Magnitude of the problem and study area

- A. Nutritional anemia during pregnancy in Tanzania
- B. Study site

VII. Study design

- A. Introduction
- B. Phase I
  1. Specific objectives
  2. Coverage of pregnant women by MCH clinic
  3. Base-line information on Hb distribution
  4. Health beliefs and developement of questionnaire
     - (i) objectives and study population
     - (ii) methods
- C. Phase II.
  1. Specific objectives
  2. Methodology
  3. Measures of adherence and hematology
- D. Phase III.
  1. Specific objectives
  2. Study population and sample size
  3. Treatment groups
  4. Data collection frame
  5. Assessment of cost-effectiveness

VIII. Analysis

- A. Hypothesis testing
- B. Sociologic part

IX. Ethical considerations

X. Collaborating Institutions

XI. Budget

XII. References

I. BACKGROUND

Iron deficiency anemia among pregnant women has for a long time been recognized as a major public health problem. This is particularly true for developing countries where anemia makes a large contribution to pregnancy complications and maternal mortality (ACC/SCN 1991, WHO 1979, Fleming et al 1986).

It is quite clear from a number of studies that supplementation of elemental iron during pregnancy is a possible means to prevent a fall in hemoglobin levels and decrease the prevalence of anemia (Simmons 1990, Charoenlarp et al 1988, Fleming et al 1986) So far, most efforts to solve the anemia problem has focused on identifying appropriate dose, iron compound and formulation to ensure a positive change in the blood parameters. However, when attempts have been made to apply these clinical findings in public health programmes results have been less successful. Apart from logistic problems with distribution of the drug, a major reason for this is suggested to be non-compliance, or preferably non-adherence, to the iron regimen on part of the pregnant woman (WHO 1990).

Although adherence is a prerequisite for a successful iron supplementation programme limited research has been done on the determinants and the magnitude of the impact the constraining factors have. Therefore, this study will focus on the adherence aspect of iron supplementation, and in particular on impact of side-effects. However, measurements of hematologic outcome will also be included to assess if a variation in adherence is of importance for anemia prevalence.

II. CONCEPTUAL MODEL

A. Theoretical basis

The importance of using theoretical models in studying health behavior, like adherence to prescribed medication has been stressed (Christensen 1978, Leventhal 1985). Application of a theoretical model assists the researcher in understanding how predictive variables of adherence may interact and alter their respective impact on adherence. Use of models further facilitate comparisons to be made between studies in different settings.

Although there have been attempts to combine the numerous models that describe health related behavior (Cumming et al 1980) still the most frequently used model, in particular in studies of adherence, is the "Health Belief Model" (HBM). The model is a psychosocial model and as such it is limited to accounting for as much of the variance in the individuals' behavior as can be explained by their attitudes and beliefs (Janz and Becker 1984). It was originally developed by Rosenstock 1966 and further modified by Becker and Maiman 1975, Becker et al 1979, and Rosenstock et al 1988). The basic variables remain the same and the theory argues that whether a person will comply to

recommended health behavior depends on the individual's: 1) perceived susceptibility of the disease or condition, 2) the perceived severity of the condition if contracted, 3) perceived benefits of the treatment recommended and 4) perceived difficulties or barriers to the recommended behavior.

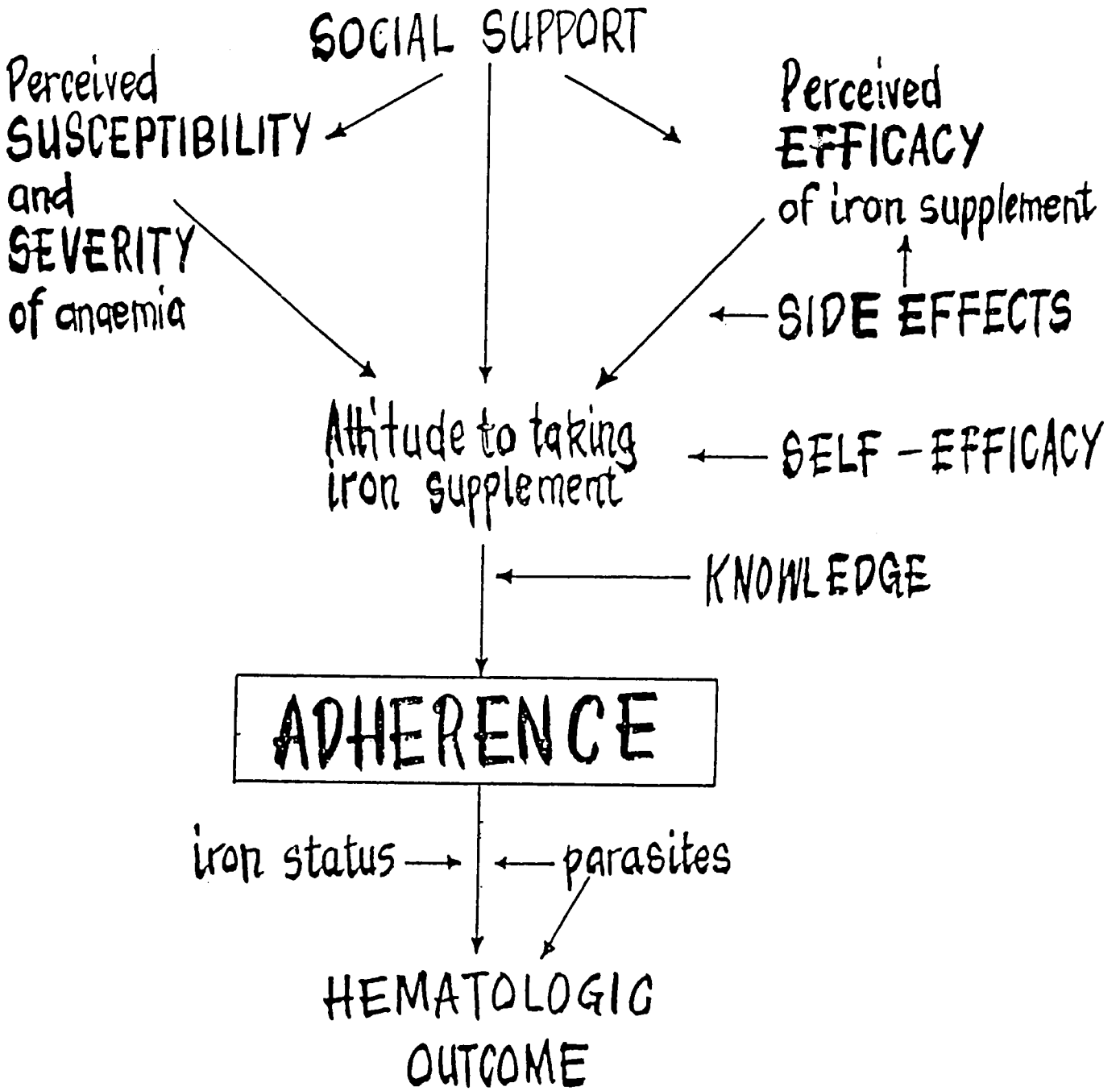
The conceptual model (fig 1.) for this study has incorporated these attitude and belief variables of the HBM mentioned above but also included two other variables, self-efficacy and social support. Self-efficacy is the individual's assessment of her ability to follow the recommended behavour, in this case take the iron supplement. Although these variables fit conceptually in the HBM they have not generally been elaborated (Janz and Becker 1984). However, more recent studies of health related behavior tend to discuss and include these aspects more in depth (Becker 1985, Kelly et al 1991 and Rosenstock et al 1988)

The patient-doctor/provider interaction is another aspect discussed in health behavior studies (DiNicola and DiMatteo 1984, Hall et al 1988, Hulka 1979). In the present study, only one health center is to be included and the provider of the iron supplement will be the same for all subjects. Thus little variation in the interaction is expected, in particular since essential parts of the interaction, the information component, will be standardized. Because of this, only one aspect of the interaction, client's understanding of the regimen, will be included in the study.

B. Focus of study:

The focus of the intervention part of the study is the impact of side-effects on adherence. As a means to create a variation in the prevalence of side-effects, but also for evaluative purposes a new type of iron preparation will be compared with conventional iron supplements. The new iron supplement contain ordinary ferrous sulphate but combined in a matrix of hydrocolloid which in contact with gastric juices dissolve and form a mix which will float on top of the stomach content. The matrix is slowly disintegrated delivering the iron comp[illegible] over an extended period of time to the upper part of the [illegible]. The gastric delivery system (GDS) has the advantages of [illegible]ing less side-effects at the same time as it has a better absorption than both the conventional iron preparations and the so called slow-release types (Cook et al 1990, Simmons 1990).

Figure 1 Conceptual framework



III. OBJECTIVES

The overall objectives for the study are:

- Study patterns of adherence to iron supplementation and factors that determine it, focusing on importance of side-effects.

- Make a cost-effectiveness analysis of the usefulness of GDS iron supplement as a means to increase adherence and improve hematologic outcome.

IV. PREVIOUS STUDIES

Limited reliable information exists on levels of adherence to iron supplementation. This is probably partly due to difficulties in measuring adherence. The most applied techniques have been, pill count, recall by user, and biologic marker. All these techniques have their shortcomings. Pill count method may have an effect on adherence, pills may be kept in other containers, and pills may be dumped before counting episode. Recall by user have a tendency to be an overestimate of adherence and biologic marker is a costly method which also pose the problem of individual variation in the biologic measures even under conditions of perfect adherence. Neither does spot check measures represent long-term adherence (Becker and Rosenstock 1984, Cramer et al 1990, Feinstein 1990, Rudd et al 1989).

A new technique is now available consisting of a small microprocessor imbedded in an ordinary pill bottl : cap which will record each time the pill bottle is opened (Cramer et al 1989, Rudd et al 1990). Use of this equipment may overcome many of the previous measurement problems (except cost!), and it is now possible to monitor adherence in a better way.

One of the major reasson suggested for poor adherence is the gastro-intestinal side-effects of the treatment (Afifi et al 1966, and DeMayer 1989). The prevalence of side-effects depends on dose, type of iron compound, dosage scheme, if it is taken with meal or not, and it also has a geographical variation (Bonnar et al 1969, Charoenlarp et al 1988, Cook et al 1990, Hallberg et al 1966, Hallberg and Sölvell 1966, Sjöstedt et al 1977). However, if present, side-effects will decrease if supplementation is continued and after about a week they will be significant reduced (Charoenlarp et al 1988, Cook et al 1990). suggesting that if side-effects have an impact on adherence it is of most importance in early stages of supplementation.

Limited information on side-effects' impact on adherence can be extracted from literature. In an iron supplementation trial in northeastern Thailand where the side-effects were high the subjects were persuaded by field personnel to continue taking the supplements (Charoenlarp et al 1988) and the high adherence observed may thus not be indicative of what would have happened outside the trial. In the same study, an unsupervised group receiving 240 mg elemental iron was compared to a supervised one.

Poor results in the unsupervised group could possibly be due to side-effects and decreased adherence as suggested by the authors (Charoenlarp et al 1988). In a recent iron supplementation trial in Jamaica where the side-effect prevalence, (depending on symptom) varied between 4-27 percent (Simmons 1990) adherence to supplement is also likely to have been affected by close supervision of field personnel. Furthermore, the measurements of adherence was reported as being unreliable (Simmons 1990).

Two studies have reported a relation between level of adherence and hemoglobin level at term of pregnancy. In a supplementation trial in Jerusalem self-reported level of adherence was divided into three groups; "good" "not so good" and "poor compliance". Although there was no significant difference in hemoglobin and hematocrit level in third trimester between the three groups there was a slight trend of better hematologic values for the "good" group followed by the "not so good" (Gofin et al 1989).

Another study in England, the subjects were divided into two groups depending on a hemoglobin level above or under 12 g/dl at term of pregnancy. Throughout pregnancy a number of stool samples had been taken and tested for iron as a measure of adherence to supplementation. The low hemoglobin level group had significantly lower number of positive tests, 25 percent compared to 86 percent in the high hemoglobin level group (Bonnar et al 1966).

Even though there are no conclusive results on the negative impact of side-effect on adherence, efforts have been made to develop iron preparations with less frequent side-effects. The so called "slow-release" preparations where the iron compound is released gradually on its passage through the intestine tried to meet this demand. Apart from being very expensive the "slow-release" preparations have had a reputation of poor absorption (Cook et al 1990).

The new gastric delivery system (GDS) preparation where the iron compound is released during an extended time period in the stomach does not have the problem of low absorption. On the contrary it has shown a 3-4-fold higher absorption compared to conventional iron preparations (Cook et al 1990). Since the ingredients of the formulation are not expensive it may be that the cost of GDS capsules could be competitive with conventional iron tablets (Simmons 1990).

Women's health beliefs may also affect adherence. A number of specific beliefs have been reported to have a negative impact. These include form of preparation of a medication (tablets, liquid, injections) where one of these can be regarded as more effective in general, or not compatible with the pregnant condition. Frequently it is mentioned that color of the iron supplement may play an important role. In case anemia is perceived as a weakness of the blood it may be favorable to have red iron tablet. Taste of the iron supplement may also play a role (WHO 1990, Galloway 1990).

A common worry among pregnant women is that they will have big babies which will cause difficult deliveries. If the iron tablets is believed to make the fetus big and strong which has been reported it is likely to have a negative effect on use of the supplement (WHO 1990).

V. RELEVANCE OF STUDY

In the overall efforts to reduce the high maternal mortality in developing countries one of the recognized strategies is to prevent iron deficiency anemia. It is established that iron supplementation during pregnancy has a positive effect on hemoglobin level, - if the supplement is taken as prescribed.

Although logistic problems of distribution of the iron supplement have sometimes been recognized as the major problem for adherence (ACC/SCN 1991) it is not necessarily the case that the supplement would be taken as prescribed if available. To ensure, once defaulting distribution systems are working, that the iron supplement provided will be effective it is essential to get a better understanding on adherence and its determinants.

Due to difficulties in measuring adherence limited reliable information is available on adherence level itself or on the magnitude of the impact of the factors that determine it. This is also true for the possible negative influence which side-effects may have.

As cultural factors play an important role in adherence it may be limitations for the extent to which generalizations can be made, even so it is still felt as important to investigate if reducing side-effects can be a possible strategy to improve adherence in a more limited setting. With the now available measurement technique it is possible to get valid information on adherence and in the case side-effects are a problem, the recently developed GDS formulation may be provide some remedy. A cost-effectiveness analysis will provide basis for decisions to substitute conventional iron preparations for GDS ones.

Regarding the study component of health beliefs and attitudes the application of a theoretical base in the conceptual framework will provide a more comprehensive picture of the determinants of adherence where the variables will have an explanatory capacity. Many studies do lack that quality and merely produce lists of descriptive variables that statistically do or don't correlate with adherence.

One could argue that even if the variables have an explanatory capacity the information is not useful unless it can be included in programme activities to improve adherence. Although there are no studies on adherence to iron supplementation that have used a theoretical base which can show this usefulness, the HBM has successfully been used for identifying intervention points for inducing behavioral change in other contexts (Becker et al 1979, Jones et al 1988).

181

VI. MAGNITUDE OF THE PROBLEM AND STUDY AREA

A. Nutritional anemia during pregnancy in Tanzania

Anemia during pregnancy is a major health problem in Tanzania. It is one of the most common reason for antenatal hospital admission in Dar es Salaam region and it is also a leading factors for maternal mortality (Kavishe 1982). A study at Muhimbili Medical Centre showed that in early 1988, 26 of 71 (37%) maternal deaths could be attributed to anemia (Kaisi 1988). A large study of anemia during pregnancy was conducted in 1977. A total of 1317 women were evaluated when they came for their first antenatal clinic visit. The mean hemoglobin level was 9.3 gm% and none of the women had a hemoglobin level above the recommended cut-off of 11 gm% (Mwanukuzi E and Nhonoli AM 1972).

The major causes of anemia during pregnancy are malaria and iron deficiency. However there is limited information on their relative contribution to anemia. It is depending on malaria prevalence and thus on region and season. A recent study in Zanzibar (TFNC 1991) showed a prevalence of 41.2 of anemia in women using WHO cut-off of <12 g/dl for non-pregnant and <11g/dl for pregnant women. The same study demonstrated that 29.2 of the women were iron deficient as defined by a free erythrocyte protoporphyrin level above 70 microgram/dl of red blood cells. Anemia and iron deficiency showed a statistical significant correlation while malaria (prevalence 8.1 %) did not in this particular study. Although there is a need of more comprehensive information on anemia in Tanzania it is beyond all doubts that it is a major problem that need to be tended to.

B. Study site.

The study is proposed for Ilula villages in Iringa region about 45 km from Iringa town. The villages lie on the main road Dar es Salaam to the Zambian boarder. The census for the villages in 1978 was 5657. Health care is provided by the units, one Roman Catholic dispensary and a Lutheran dispensary and this study will have the Lutheran dispensary as a basis. From previous studies in the area it is reported that antenatal coverage is high but with a big variation in gestational age at register (mean 23, range 9-35 weeks) and number of visits to the clinic (mean 6.3 and range 0-12) (Möller et al 1989).

Ilula villages are comparable with other rural villages in Tanzania although they probably are somewhat more developed due to the closeness of the road. Still there is not electricity.

Although no quantitative information on anemia is available from Ilula anemia is assessed by the medical assistant at the MCH to be very common (Ballart 1991). In the previous study in the area anemia was noted as the most common pregnancy complication (Kavishe et al 1987).

VII. STUDY DESIGN

A. Introduction

The study is divided into three phases. The first phase includes an assessment of the coverage of the antenatal clinic in Ilula, base-line information on hemoglobin distribution near term of pregnancy and a sociologic study on beliefs of health and illness during pregnancy as part of preparation of draft questionnaires.

The second phase is a pretest of the developed questionnaire as well as a test of the intervention components and the methodology for their assessment, in particular the measurement of adherence. The last phase is the actual study where all the proposed factors affecting adherence will be measured as well as adherence itself and hematologic outcome.

B. Phase I. October - November 1991

1. specific objectives

The specific objectives for phase 1 are:

- To assess coverage of pregnant women by the antenatal clinic in Ilula.
- To assess hemoglobin distribution near term of pregnancy in an unsupplemented population
- To describe common health beliefs related to pregnancy.
- To develop draft questionnaires.

2. Coverage of pregnant women by the MCH clinic.

In the villages to be included in the main study all household where a child has been born the last two months will be visited. The household to be visited will be identified by ten-cell leaders who knows the household composition and whether a child has been born in his ten-cell the last three months. At the visits information on mother's attendance at MCH clinic, such as number of visits, gestational age at registration and reason for participation or non-participation will be collected. The aim is to find out how large proportion of the pregnancies in the area are covered by the MCH clinic. It is expected that approximately 100 mother-newborn pairs will be interviewed.

A subgroup of this study population will be used for the study of health beliefs related to pregnancy.

3. Base-line information on hemoglobin distribution near term of pregnancy in an unsupplemented group.

According to present information there has not been any iron supplement available in Ilula health center for a long time. Before starting the distribution of iron supplement a base-line assessment of hemoglobin levels near term of pregnancy is going to be conducted. The possibility of using this information as a quasi control group in comparisons of hematologic outcome of iron

supplementation is going to be investigated. In order to do so background data on the subjects like socio-economic status, reproductive history, recalled morbidity and present malaria will be collected. Hemoglobin will be assessed by finger-prick and use of hemocue hemoglobinometer. Blood samples will be spotted on filter paper for later assessment of ferritin and erythroprotoporhyrin (see also phase III hematology).

Collection of information for two months is expected to provide a sample of approximately 100.

4. Description of common health belief during pregnancy and development of questionnaires.

(i) Objectives and study population

The study population for this section will be a subgroup of the subjects for the coverage study which are mothers with new born babies. Interviews will also be made with health personnel as well as traditional birth attendants.

The objectives of the study are

a) to describe general health beliefs and practices common during pregnancy.

b) identify a local concept of anemia which overlaps with modern medicine's definition.

This concept will be used in developing a cultural acceptable message related to the benefits of iron supplementation and in the intervention phase of the study for assessment of the perceived susceptibility and severity to anemia as well as other occasion when referring to anemia.

c) to create a list of illnesses and symptoms common during pregnancy.

For the main study, the most frequent side-effects of iron supplement will be included in the list which will act as an instrument to measure side-effects. The list will also be used for assessing symptoms of anemia which could trigger adherence to supplement.

d) investigate if there is a concept of preventive care which include medication of some sort.

If existing, parallels could be drawn with this concept in explanations of the function of iron supplement.

e) develop draft questionnaires

(ii) Methods

The specific methods for, in a structured way, collect

information of qualitative nature (like health beliefs) have been described by Bernard 1988 and Weller & Romney 1988 among others. The sequence or rather circular way of applying them has been proposed by Winch 1991.

The methods to be applied are a) identification of domain of interest, b) free listing c) charts d) pile sorts e) paired comparisons, and indicator questions, which are common methods applied in social science.

A brief description of these methods;

Identification of a domain is defining the area of interest in which the questions should focus on. For this research it is ilnesses or problems during pregnancy. Free listing is developement of a list of the most common problems and chart is a way to structure the cause, treatment and symptoms of each of the illnesses. Through pile sorting it is possible to find out which illnesses are related to each other and paired comparisons can be used as a tool for ranking how important or serious different illnesses are perceived to be. Indicator questions or statements are commonly used in assessment of attitudes.

A base-line questionnaire for assessment of household characteristics, including household composition, socio-economic status, and major means of living will be produced as well as one for the pregnant woman's reproductive history and obstetric information. A crucial variable in the study is gestational age. A previous study in the area showed that using last menstrual period gave a valid assessment of duration of pregnancy (Möller 1989). Even so fundus height will also be measured as a complementary method.

C. Phase II. Pilot test of questionnaires and outcome measures. December 1991-February 1992.

1. Specific objectives.

The specific objectives of phase 2 are;

- Pre-test of questionnaires.
- Pre-test of methodologies and procedures for assessing adherence and hematologic outcome.

2. Methodology.

Phase 2 involves a testing of all variables to be measured in the main study. For a period of three months data will be collected according to the schedule set for the main study. This involves a pretest of the questionnaires developed in phase 1 for the assessment of a) household characteristics; b) variables on health beliefs and attitudes in conceptual model and c) test of methods for assessing adherence and hematologic outcome.

Apart from testing the actual assessment instrument for the various variables this phase is a practical preparation for management of data collection in the main study.

3. Measures of adherence and hematologic outcome

(i) Hematology

Because of difficulties in handling venous blood samples under the existing field condition the hematologic measurements selected have been limited to those which can be done with capillary blood and do not need to be centrifuged or stored in a freezer. Capillary blood will be collected by finger prick and all samples will be taken by the same person. The measurements are: hemoglobin, free erythrocyte protoporphyrin (FEP), and sickle cell test.

Hemoglobin level will be assessed by Hemocue system which use disposable microcuvettes. The technique has been compared with standard laboratory hemoglobin methods and good reliability and accuracy has been found (Bridges et al 1987). The method is particular convenient for field conditions as it does not require any sample or reagent preparation and can be run by use of a battery.

FEP will be assessed by a micro-flourometric method (Orfanos et al 1977). The method needs a small amount of blood 2-3 drops which can be spotted on a filter paper and stored protected from sunlight for analysis later in the laboratory.

Sickle cell will assessed by microscopy.

At Kansas university a method is under development for assessment of ferritin in whole blood spotted on a filter paper. Samples will be collected in this study for the possibility of later assessment of ferritin.

Blood slides for assessment of malaria will be prepared.

(ii) Adherence

This study intend to use a new equipment for measuring adherence to medical treatment "Medication Event Monitor System" MEMS (Cramer et al 1989). It is a small microprocessor imbedded in a pill bottle cap. The microprocessor register each time and date the pill bottle is opened. Information about 1000 medication events can be stored for later retrieval by use of the computer software developed together with the microprocessor. This equipment allow detailed information about adherence to be collected without the knowledge from the pregnant women which decrease the risk of altering the adherence behavior due to the study intervention. The assumption is that every time the pill bottle is opened the tablet is actually taken.

The microprocessor method of measuring adherence will be used during the first month of supplementation where there is a need

of accurate information on a daily basis. As the possibility exist that the woman will open the pill bottle regularly and through away the tablet this method will be validated by testing for iron in stool samples (Afifi et al 1966).

For the follow-up of adherence assessment (week 4, 8 and 12 weeks) the method using stool samples will be used.

D. Phase III. Intervention study, March 1992- March 1993.

1. Specific objectives

The specific objectives for the intervention part of the study are the following;

- To assess level of initial adherence to iron supplements.
- Study patterns of adherence and its determinants.
- Test if use of gastric delivery system (GDS) iron preparation with reported lower frequency of side-effects has an improved effect on adherence compared to conventional preparations.
- Compare cost-effectiveness of conventional and GDS iron preparation on adherence.
- Compare effectiveness in improving hematological parameters related to anemia (in particular hemoglobin) between conventional and GDS iron preparations.
- Compare cost-effectiveness of conventional and GDS iron preparations on hemoglobin level.
- Test the explanatory capacity of the components in the health belief model on variation in adherence.

2. Study population and sample size

Of all women registering for MCH clinic at Ilula dispensary women with a gestational age less than 27 weeks will be offered to participate in the study and their consent to do so will be sought. Based on the health center's routine women with hemoglobin less than 8.5 g/dl will be excluded from the study and referred to Iringa hospital. Women with, or developing pregnancy complications such as: requiring blood transfusion; emergency obstetric intervention; eclampsia; or chronic diseases will also be excluded from the study.

The following calculations were made to estimate the number of women it may be possible to enroll per month. Based on information from the health center approximately 50 children are born at the clinic per month. If 80 percent of these have participated in MCH clinic before delivery there will be 40 children per month. Of these, assume 75 percent have participated before week 27 in pregnancy which lead to 30 pregnant women per month who can be offered to participate. If 80 percent accept to participate about 25 women per month can be entered in the study.

The proposed sample size is 250 women which will require a data collection of 13 months. A sample size of this magnitude will enable a difference in adherence proportion between the two iron

preparation of 20 percent to be detected and a difference in hemoglobin level of 0.35 g/dl to be identified. A difference of less than 0.5 g/dl is probably of little biologic importance (INACG 1984).

3. Treatment groups

Initially all women that agree to participate in the study will be treated for hookworm infestation with a single dose of Albendazole 400 mg.

The women will randomly be assigned to receive either conventional or GDS iron preparation. The conventional ferrous sulphate preparation will provide a dose of 120 mg elemental iron as recommended by WHO 1989. The GDS ferrous sulphate has shown to have a 3-4-fold higher absorption rate than ferrous sulphate elixir (Cook et al 1990), and a dose of 50 mg elemental iron in form of GDS capsules has proven, in an anemic (8-11 g/dl of hemoglobin) population, to give the same or better hematologic response than 100 mg elemental iron of conventional type (Simmons 1990, ACC/SCN 1991). The elemental iron dose for GDS preparation will thus be 50 mg per day.

The dose of conventional ferrous sulphate can be taken once a day or as two administrations of 60 mg each. To reduce the frequency of side-effects it is generally recommended to take the dose in two administrations which will be applied in this study. The GDS iron supplement is to be taken once a day with a meal which in this study will be the evening meal.

Since the positive effects of iron supplement is established in an anemic population it is not ethical to include a placebo or control group without iron in the present study.

However, designing a study without a placebo group create some problems. One of these is difficulties in evaluating the side-effects as there are a number of placebo side-effects. Neither is it possible to assess the absolute effect of iron supplementation.

However if, which is likely, there will be a non-compliant group in the study it may to certain extent be possible to use this group as a quasi-experimental control group. In interpretation of the results considerations have of course to be made to the fact that the group is a self selected one and not randomly assigned. Still the use of this group as a control group may be acceptable, in particular if it is possible to statistically show that it, regarding essential variables, is comparable with the rest of the study population. Suggestions for when it may be appropriate to use non-adherer as a control group is outlined in the analysis section. Furthermore, the base-line information of distribution of hemoglobin near term of pregnancy may be used as another quasi-control group.

4. Data collection frame

For each of the recruited subjects data will be collected at registration (week 0 of supplementation) and at week 2, 4, 8, and 12 of supplementation. The information that will be collected at these occasions are:

Registration:
- base-line information
- illnesses
- determinants of adherence
- hematology (incl malaria)

Week 2 of supplementation:
- illness and side-effects by 2 weeks recall
- adherence by microprocessor

Week 4 of supplementation:
- illness and side-effects by 2-weeks recall
- adherence by microprocessor and stool sample
- determinants of adherence, follow-up

Week 8 of supplementation:
- illness and side-effects by 4-weeks recall
- adherence by stool sample

Week 12 of supplementation:
- illness and side-effects by 4-week recall
- adherence by stool sample
- hematology (incl malaria)
- determinants of adherence, follow-up

Provision of iron supplements will be made until end of pregnancy.

5. Assessment of cost-effectiveness of iron supplementation.

To calculate cost the following information will be collected:

- cost of the two different iron supplement per pregnancy.
- cost for health personnel
- cost for transport
- cost for facilities

The first cost, the iron supplement, will differ between the two types of formulation. The cost difference will be used for calculation of marginal cost of using the GDS supplement instead of the conventional preparation.

The cost for the next three ingredients in the supplementation programme will not differ between the iron supplements. For later calculations of total programme cost the assumption will be that a basic health delivery system already exists and that the iron supplement will be one of several drugs delivered to the center. The cost factors for the mentioned items will thus be calculated

as shares of the total budget for the health centers cost for the mentioned ingredients. The share which will be attributed to the iron supplementation will be based on an assessment of how large proportion of the health center's time is used for information about, and distribution of iron supplement.

The effectiveness part of the calculation will have two variables. The first is a measure of the process of the supplementation programme, an output measure, which in this case will be number of adherers. The second variable is an outcome measure, per definition a biologic variable, in this case it will be hematologic outcome.

VIII. ANALYSIS

A. Hypothesis testing;

The intervention part of the study is the use of GDS iron preparation. A number of hypotheses regarding characteristics of the GDS supplement will be tested whether they are true or not. The analyses will be done using EPI-INFO and SPSS statistical packages which include statistical methods such as, t-tests, analysis of variance and multiple regrression analysis.

Hypothesis 1. GDS iron preparation produce less side-effects compared to conventional iron preparation.

Hypothesis 2. Use of GDS iron preparation will lead to better adherence to regimen.

Hypothesis 3. Use of GDS iron preparation will maintain or improve hematologic status better than conventional iron supplement.

Hypothesis 4. Side-effects of iron supplementation have a negative effect on adherence.

Hypothesis 5. A better adherence will result in better hematologic outcome.

Hypothesis 6. GDS iron supplement is more cost-effective in terms of adherence than conventional iron supplement.

Hypothesis 7. GDS iron supplement is more cost-effective in terms of hematologic outcome.

B. Analysis of the sociologic part.

The health belief model:
The components in the health belief model will be assessed on their respective importance for the variation observed in adherence by pathway analysis.

The health beliefs contribution to adherence patterns will be investigated by assessing the overall explanatory capacity of the model.

The general questions will be compiled into a descriptive section.

19

IX. ETHICAL CONSIDERATIONS

The subjects in the proposed research project are women in Ilula villages that, at start of study, within the last three months delivered a baby (phase 1; antenatal coverage study) and pregnant women attending antenatal clinic at the health center in Ilula (phase I; hemoglobin base-line study, phase II; pilot test of methodology and phase III; intervention study)

The subjects for the antenatal coverage study will be identified by approaching the community leaders, in particular ten-cell-leaders, who know each household and its composition within his/her administrative unit. Initial contact will be at their respective house. Subject for the other part studies will be approached when the visit antenatal the clinic.

At the first encounter with the subject candidate, before a request is made about participation in the study, they will be informed about the research project, its aims and what a participation would involve on their part. Since the population to large extent is illiterate written consent is not feasible and oral consent has to be taken. The subjects will be informed that if they choice to participate and later change their mind they can withdraw any time from the study without any penalties or consequences for their continuous participation and use of the clinic's service.

If the ongoing research for some reasons has to terminate and debriefing can't be done within the project's time frame the village leaders will be asked to inform the subjects.

The intervention part of the study is the test of two iron supplement which are randomly assigned to the women. Both preparations have the same active compound, ferrous sulphate. The dose of elemental iron is in the conventional formulation 120 mg as recommended by WHO. The GDS iron preparation has a 3-4-fold higher absorption rates (Cook et al 1991) and have resulted in the same or better hematologic response, in an anemic population, when the dose has been 50 mg of elemental iron (Simmons 1990). A dose of 50 mg of elemental iron will be used for the GDS iron preparation. Due to ethical reason this study will not have any placebo or control group.

In the intervention part of the study the data collection include taking two finger prick blood samples. Finger prick sample is already before the initiation of the study routinely taken in the clinic at registration but an additional sample is needed after 12 weeks of iron supplementation. Finger prick blood samples as such does not involve any risks for the patient. However, HIV virus is prevalent in Tanzania and strict precautions has to be taken to avoid transmission of the virus. The steps that will be taken are the following; only one person will take and handle all the blood samples in the study. This person will, even if previously trained, receive further training in safety measures regarding handling blood samples possibly contaminated with HIV virus. Training will be done by a medical doctor at the

laboratory department of TFNC who has previously trained persons in blood sampling techniques. Protective gears for the person taking the blood samples will be made available as well as equipment for handling contaminated supplies and for its destruction. Finger prick samples will be taken by use of disposable supplies only.

The data collection and interviews will be carried out by field workers living in the area who are familiar with local customs and fluent in kiswahili.

The data collected will be confidential. The subjects privacy will be ensured by limiting the number of persons who have access to the raw data. In analysis and presentation of the results all subjects will be codified and no names will be revealed.

The study is funded by the Tanzanian government through loans from the World Bank, by World Health Organization, and by Scandinavian Africa Institute, Uppsala, Sweden.

X. COLLABORATING INSTITUTIONS

The project is part of the TFNC/WHO research collaboration in Nutrition. Personnel from TFNC will mainly be drawn from the Anemia programme and from WHO the nutrition officer is participating.

In Ilula villages, personnel from the Health Center will be part of the collaboration.

XI. BUDGET

In Tanzania shilling if not otherwise stated.

Phase 1. Preparatory phase October - November 1991.

Transport:	164.000	
Per Diem & Salaries:	272.000	
Stationaries:	11.000	
Instrument & Eqiupment:	79.000	
total	526.000	≈(2300 US$)

Phase 2. Pilot test questionnare, December- February 1992.

Transport:	306.000	
Per diem & salaries:	310.000	
Stationaries:	11.000	
Instrument & Equipment:	89.000	
total	716.000	≈(3100 US$)

Phase 3. Intervention study, March - March 1993.

Transport:	1176.000	
Per diem & Salaries	828.000	
Stationaries:	45.000	
Instrument & Equipment:	347.000	
total	2396.000	≈(10400 US$)

Phase 4, Analysis and dissemination of results, April -July, 1993.

Analysis:	86.000	
Seminar in Ilula:	278.000	
total	366.000	≈(1600 US$)

Lease of instruments for measure of adherence and purchase of hemoglobinometer.

Adherence instrument:	36.480 SEK	
Hemoglobinometer:	2.500 SEK	
total	38.980 SEK	≈(6300 US$)

Grand total 23.700 US$

The project is funded ~~through~~ by the Tanzania Government through loan from the World Bank (4000 US$), WHO/AFRO (5000 US$), WHO/Head quarters (applied for 12700 US$) and Scandinavian Africa Institute (2000 US$) applied for.

XII. REFERENCES

ACC/SCN. Report of the joint ACC/SCN workshop on iron deficiency control (Dublin, 6-8 June. ACC/SCN News, Geneva 1991.

Afifi AM, Banwell GS, Bennison RJ, Boothby K, Griffiths PD et al. Simple test for ingested iron in hospital and domiciliary practice. Brit Med J 1966;1:1021-1022

Babbie E. The practice of social research. Wadsworth publishing company, Belmont CA, 1989.

Ballart 1991. Personal communication after her field visit to Ilula village.

Becker MH and Maiman LA. sociobehavioral determinants of compliance with health and medical care recommendations. Medical Care 1975;13:10.

Becker MH, Maiman LA, Kirscht J, Haefner DP, Drachman RH and Taylor DW. Patient perceptions and compliance: Recent studies of the health belief model. In: Compliance in health care. Eds Haynes, RB, Taylor DW and Sackett DL. The Johns Hopkins University Press, Baltimore 1979.

Bernard HR. Research methods in cultural anthropology. Sage Publications, The International Publishers, Newbury Park, 1988.

Bonnar J, Goldberg A and Smith J. Do pregnant women take their iron? The Lancet 1969;(March 1):457-58

Bridges N, Parvin M and van Assendelft O. Evaluation of a new system for hemoglobin measurement. Am Clin Prod Rev, 1987;6:22-25.

Charoenlarp P, Dhanamitta S, Kaewvichit R, Silprasert A, Suwanaradd C, Na-Nakorn S, et al. A WHO collaborative study on iron supplementation in Burma and Thailand. Am J Clin Nutr 1988;47:280-97.

Christensen DB. Drug-taking compliance: A review and synthesis. Health Service research, summer 1978, 171-187.

Cook JD, Carriaga M, Kahn SG, Schalch W and Skikne B. Gastric delivery system for iron supplementation. The Lancet, 1990;335:1136-39.

Cramer J, Mattson R, Prevey M, Scheyer R and Quellette V. How often is medication taken as prescribed? A novel assessment technique. JAMA 1989;261:3273-3277.

Cramer JA, Scheyer RD and Mattson RH. Compliance declines between clinic visits. Arch Intern Med 1990;150:1509-10.

Cummings KM, Becker MH and Maile M. Bringing the models together: An empirical approach to combining variables used to explain

health actions. J Behavioral Med, 1980;3(2):123-145.

DeMaeyer EM. Preventing and controlling iron deficiency anemia through primary health care: A guide for health administrators and programme managers. Geneva: World Health Organization 1989.

DiNicola DD and DiMatteo MR. Practitioners, patients, and compliance with medical regimens: A social psychologic perspective. In: handbook of psychology and health. Vol IV, Social psychological aspects of health. Eds Baum A, Taylor SE and Singer JE, Lawrence Erlbaum associates, publishers, Hillsdale, New Jersey 1984.

Feinstein AR. On white-coat effects and the electronic monitoring of compliance. Arch Intern Med 1990;150:1377-78.

Fishbein M and Ajzen I. Attitudes and opinions. Ann Rev Psychol 1972;23:487.

Fishbein M and Ajzen I. Belief, attitude, intention and behaviour: An introduction to theory and research. Reading, MA: Addison-Wesley Publishing Co., 1975.

Fleming AF, Ghatoura GBS, Harrison KA, Briggs ND and Dunn DT. The prevention of anemia in pregnancy in primigravidae in the guinea savanna of Nigeria. Ann Trop Med Parasit 1986; 80(2):211-233.

Galloway R. Determinants of medical compliance. Literature review done as a consultant to the World Bank, June 1990.

Gofin R, Adler B and Palti H. Effectiveness of iron supplementation compared to iron treatment during pregnancy. Public Health 1989;103:139-45.

Hall JA, Roter DL and Katz NR. Meta-analysis of correlates of provider behavior in medical encounters. Medical Care 1988;26(7):657.

Hallberg L and Sölvell L. Succinic acid as absorption promotor in iron tablets. Acta Medica Scandinavia, 1966(supp no 459:3):23-35.

Hallberg L, Ryttinger L and Sölvell L. Side-effects of oral iron therapy. Acta Medica Scandinavia 1966 (supp no 459:3):3-10.

Haynes RB. Introduction. In: Compliance in health care. Eds Haynes RB, Sackett DL and Taylor DW. Johns Hopkins University Press, Baltimore MD, 1979.

Hulka BS. Patient-clinician interaction and compliance. In: Compliance and health care. Eds Haynes RB, Taylor DW and Sackett DL. The Johns Hopkins university press, Baltimore 1979.

INACG. The design and analysis of iron supplementation trials. A report of the International anemia consultative group.THe Nutrition foundation, Washington 1984

196

Janz NK and Becker MH. The health belief model: A decade later. Health Education Quarterly, 1984;11(1):1-47.

Jones SL, Jones PK and Katz J. health belief model intervention to increase compliance with emergency department patients. Medical care, 1988;26(12):1172-84.

Kaisi. Report on maternal mortality at Muhimbili Medical Center, Dar es Salaam, for the first quarter of 1988. Unpublished.

Kavishe, FP. Nutritional anemia in Tanzania: A review. Tanzania Food and Nutrition Center Report no 738, 1982.

Kavishe FP, Ballart A, Maletnlema TN, Gebre-Medhin m, Ljungqvist B, Möller B and Lushino O. Maternal and child mortality and morbidity in Ilula village, tanzania. TFNC report no 1080, August 1987.

Kelly RB, Zyzanski SJ and Alemagno SA. Prediction of motivation and behavior change following health promotion: Role of health beliefs, social support and self-efficacy. Soc Sci Med 1991;32(3):311-320.

Langlie JK. Social networks, health beliefs, and preventive health behaviour. J of Health and Social Behavior, 1977;18(September):244-260.

Leventhal H. The role of theory in the study of adherence to treatment and Doctor-Patient interactions. Medical Care, 1985;23(5):556-563.

Mwanukuzi E and Nhonoli AM. Anemia in expectant mothers. East African Medical J 1972 (49) 2:101-107.

Möller B. The outcome of pregnancy and antenatal acre in rural Tanzania. Acta Univ Upsaliensis, comprehensive summaries of uppsala dissertations from the faculty of medicine 177. Uppsala 1988.

Möller B, Lushino O, Meirik O, Gebre-Medhin M and Lindmark G. A study of antenatal care at village level in rural Tanzania. Int J Gynecol. Obstet, 1989, 30:123-131.

O'Reilly. Methodological issues in social support and social network research. Soc Sci Med 1988;26(8):863-873.

Orfanos A, Murphey W and Guthrie R. A simple flourometric assay of protoporphyrin in erythrocytes (EPP) as a screening test for lead poisoning. J Lab Clin Med; 89(3):659-665.

Rosenstock IM. Why people use health service. Millbank Mem Fund Q 1966 ;44:94

Rosenstock IM, Strecher VJ and Becker MH. Social learning theory and the health belief model. Health Education Quarterly, 1988;15(2):1175-183.

197

Rudd P, Byyny RL, Zachary V, LoVerde ME, Titus C, Mitchell WD and Marshall G. The natural history of medication compliance in a drug trial: Limitations of pill counts. Clin Pharmacol Ther 1989;46:169-76.
Rudd P, Ahmed S, Zachary V, Barton C and Bonduelle D. Improved compliance measures: Applications in an ambulatory hypertensive drug trial. Clin Pharmacol Ther 1990;48:676-85.

Simmons WK. Evaluation of a novel delayed-release formulation for iron supplementation in pregnancy. Report no 5, Maternal Nutrition and Health Care programme research report series. International Center for Research on Women. 1990.

Sims L. Towards an understanding of attitude assessment in nutrition research. J Am Diet Assoc, 1981;(78):460-466.

Sjöstedt JE, Manner P, Nummi S and Ekenved G. Oral iron prophylaxis during pregnancy - A comparative study on different dosage regimens. Acta Obstet Gynecol Scand 1977;60(supp):3-9.

Tanzania Food and Nutrition Center. Anemia survey in women and children in Zanzibar. Draft report 1991.

Weller SC and Romney AK. Systematic data collection. Qualitative Research Methods Series no 10. Sage Publications, The International Publishers, Newbury Park 1988.

Winch P. Personal communication.

Winch P. Qualitative methods in community-based research. BAckground paper prepared for a meeting on Participatory Operational Research, 19 June 1991, UNICEF area office for Tanzania and Seychelles, Dar es Salaam, Tanzania.

World Health Organization. The prevalence of nutritional anemia in women in developing countries. Geneva: WHO 1979.

World Health Organization. Iron supplementation during pregnancy: Why aren't women complying? A review of available literature. WHO/MCH/90.5. Geneva: World Health Organization 1990.

APPENDIX B-3

APPENDIX B-3a

FINAL PROJECT REPORT

Title : Gastric delivery system iron trial on pregnant and non pregnant women.

Location : Surabaya - Indonesia

Duration : November 1992 - June 1993

In Country Budget:

Central Office : DR Soetomo Hospital

Key Personel : DR.Dr. Poedji Rochjati
Principal Investigator

Research : Dr. Marsianto
Project Coordinator

Service Delivery : Mrs. Sridati - Midwife
Mrs. Cholilah - Midwife
Mrs. Farida - Midwife

BACKGROUND :

It is presently estimated that over a billion people in the world are anemic and that the major cause of this anemia is a deficiency in the supply of dietary iron relative to the iron needs of a population. The population segment most vulnerable to this nutrional iron deficiency is pregnant women. The global prevalence of nutritional anemia during pregnancy exceeds 50 % and although this figure is much higher in Third World countries, no region or society is exempt. Recent studies have highlighted the significant of iron deficiency anemia during pregnancy. Mild anemia is associated with an increase in the rate of premature delivery and low birth weight whereas more severe is estimated to account for up to 20% of maternal deaths during parturition. In light of the dismal success in reducing the global prevalence of anemia in this highly vulnerable segment of the population, there is a need to develop novel methods to improve the iron status of pregnant women.

For the past several decades. the provision of oral iron supplements to pregnant women has been the primary method to decrease the prevalence of anemia. A commonly cited reason for the ineffectiveness of iron supplementation in developing countries is the reluctance of women to adhere to a regimen that is ofte associated with gastrointestinal side effects. One way of circumventing this problem is to supply iron in a slow release form while retaining it in the stomach proximal to the iron

absorbing region of the small intestine, a formulation referred to as a gastric delivery system (GDS). Recent studies indicate that this preparation not only eliminates the side effect of oral iron but also results in a more efficient assimilation of the nutrient. The present investigation was undertaken to evaluate the hematological efficacy of GDS in a field setting.

Maternal mortality in Indonesia in high (M.M.R = 250-718/100,000 LB). As in most developing countries, the major causes of maternal mortality are hemorrhage, sepsis and toxemia. Maternal anemia is recognized risk factor for maternal death due to hemorrhage. In one study in Indonesia, the maternal mortality ratio for anemic women was substantially higher (700/100,000) than for non-anemic women (190/100,000). In a study noted in World Health Statistics 1982, pregnant women in Indonesia had the lowest average hemoglobin levels among women in 5 countries in the region. The National Household Health Surevey of 1985/86 showed a prevalence rate of over 73 % of anemia in women of childbearing ages.

There has not been sufficient research to document the etiology of the problem of anemia in Indonesia; however, poor absorption of dietary iron due to rice-based diets appears to be an inportant contributing factor. In other countries in the region, supplementation of dietary iron with iron sulfate has achieved reductions of anemia of fifty percent. However, there appears to be many obstacles to widespread acceptance of iron sulfate suplementation as a public health strategy for reduction of the prevalence in all areas, the awareness of the need for and benefit from these iron tablets among women, and the problems of continued compliance where the tablets are available. Many women who have access to the tablets simply do not want to take them because of the perception that the side-effects (nausea, constipation, etc) outweigh the potential benefits of taking them.

Recently, a new formulation and delivery system for iron has been tested, and in trials in Jamaica, appears to significantly lower the rate of adverse effects while still reducing anemia. If true, this method of iron delivery would significantly decrease problems with compliance. This new delivery system is called the Gastric Delivery System for Iron (GDS Iron).

GOLD AND OBJECTION :

Is the GDS Iron pill as effective in anemic pregnant women at raising hemoglobin levels as the standard Ferrous Sulfate tablets, and is it better tolerated ?

Is compliance less of a problem with the GDS Iron tablet when compared to a standard ferrous sulfate formulation and a placebo that contains no iron ?

.MOV

These questions will be answered by two separate trials in different study populations. The studies will be done in Surabaya, Indonesia by the research group at RS Dr Sutomo Hospital. Additional support will be provided by experts from the University of Kansas. Logistic support and technical assistance will also be provided by the Mother Care Project through the USAID/Jakarta TAACS Adviser.

RESEARCH METHOD AND MATERIAL :

TRIAL 1

Summary :

The first trial will take place in a group of anemic pregnant women and will involve three groups of women. The first group will be given the GDS Iron pill for a period of 90 days during the last three months of their pregnancy. The last two groups will be given the GDS-1420 and GDS 6925, therapy for the same time period during the last 3 months of their pregnancy.

After obtaining informed consent, the women in the three groups will be randomly allocated to each group following screening to determine that they meet the criteria for gestational age and anemia. All groups will be treated identically, seen every 30 days and given pills in packets of 30 for a total of 90 days (4 visits prior to birth. They will have hemoglobin, ferritin and transferrin levels measured at there points during the pregnancy. The analysis will compare biochemical parameters with reports of compliance and side effects in the two groups to determine if the GDS Iron formulation is as efficacious as ferrous sulfate, and if it has a higher compliance rate.

Sample size and study length :

Three groups of pregnant women with hemoglobin between 8.0 and 10.9 g/dl will be enrolled, with total 300 women. The women will be recruited from 2 prenatal care clinics (unit I and unit II). Each clinic sees an average of 20 women per day, and estimates are that each week 25 anemic pregnant women will be enrolled per clinic, for a total of 50 women per week. All the women will be enrolled by the and of the 6th week of the study. Allowing 90 days for the duration of the pregnancy, and 30 days for the required follow-up period, two weeks for data entry and cleaning, and four weeks for data analysis and report writing, the study will be completed by the end of the 7th month.

Enrollment :

Pregnant women aged 15-45 attending the prenatal clinics will be enrolled. The goals of the study will be explained to them, and they will be asked to participate in a screening process to determine their eligibility. They will have a fingerstick hemoglobin determination done, and if anemic (8.0-10.9 gm/dl) will be asked to participate in the study. Following receipt of written informed consent, two vacuatainers of blood will be taken, baseline social, demographic, nutritional, family planning and previous pregnancy/birth date will be taken. Women screened and found to be anemic but not choosing to enter the study will be told that they are anemic and counseled regarding taking iron.

Data gathering :

Fallowing the first clinic visit where baseline data will be gathered, the women will be seen at intervals of 30 days. A social worker will interview the women and record answers to questions concerning compliance and side effects. Following the interview, women will be given a 30 day supply ofpills and asked to return in 30 days. Women in the study will have transportation paid for when returning to the clinic for visits. Women who do not return for the clinic visits will be visited by the social workers at home if possible. At the third and fourth visit blood will be taken for, transferrin receptor and ferritin. Weight and MUAC will be taken also. The hemoglobin and ferritin will be determined at the last visit.
Frosen samples will be sent to the University of Kansas for determination of transferrin receptor. Data will be entered in a database using Epi-Info by a data entry person.

Data Analysis

The data will be analyzed first with descriptive statistics and then by stratified analysis. Following this, logistic regressing will be done if the stratified analysis is not sufficiently powerfull. Socio-demographic variables (age, religion, education); acuupation, number of persons in househould, number of children); reports of compliance and side effect (diarrehea, nausea, loss of appetite, heartburn, abdominal cramps, constipation, color of stool, MUAC, hemoglobin, ferritin, transferrin receptor) and others (abbreviated nutritional history, menstrual history, etc) will be determined for the three groups to see if there are significant differences between the GDS Iron group, the GDS 1420 group and the GDS 6925.

-104

Ethical considerations :

The study does expose any of the women ,fetuses or babies to health risks. Rather, it provides the pregnant women with a major health benefit as it identifies them as being anemic during their pregnancy (which they would not have known) and offers therapy for this. Any women who are anemic at the end of the study (non-responders) will be given a supply of iron sulfate and counseled regarding its appropriate use. The major risks in the study are those resulting from the drawing of blood for biochemical tests. These risks are minimal.

Trial 2

Summary

The second trial will take place in anemic non-pregnant women and will involve three groups of non-pregnant, anemic women. The first group will be given the GDS Iron pill for a period of 60 days. The second group will be given GDS 3163 therapy in an identical appearing capsule for the same time period. The third group will receive an identical appearing GDS 4041. The study will be double blinded with neither the women nor the reaseachers knowing who is in which group. After obtaining informed consent, the women in the three groups will be randomly allocated to each group following screening to determine that they meet the criteria for anemia. All three groups will be treated identically, seen every 30 days (3 visits including final). They will have, ferritin and transferrin levels measured at the two poinst and haemoglobil measurement at the first and last visit and the analysis will compare biochemical parameter with report of compliance and side effects in the three groups. This data will be used to draw conclusions as to whether the GDS Iron pill is significantly better (less side effects, higher degree of compliance, decreases rates of anemia) than the others.

Sample size and study length :
Three groups of non-pregnant women with hemoglobins between 8.0 and 10.9 g/dl will be enrolled with 100 women in each group. The women will be enrolled from two sub-health centres from this village.
Each subcentre sees approximately 50 patient per day and it is estimated that each week 25 women meeting the criteria will be enrolled, for a total of 50 women for the two clinics. Thus, the three groups of 100 women will require 6 weeks to enroll.
Allowing 60 days for the duration of the study, 2 weeks for data entry/cleaning, four weeks for analysis and report writing, it will require 5 months to finish the study.

Enrollment :

Mentruating women aged 15-45 who lives in Mojo village who are not pregnant or breats-feeding will be enrolled.
The goals of the study will be explained to them, and they will be asked to participate in a screening process to determine their eligibility. They will have a fingerstick hemoglobin determined done, and if anemic (8.0 - 10.9 g,/dl) will be asked to participate in teh study. Following receipt of written informed consent, two vacutainers of blood will be taken, baseline social, demography, nutritional, family planning nad previous pregnancy/ birth data will be taken. Women screened and found to be anemic but not choosing to enter the study will be told that they are anemic and counseled regarding taking iron.

Data gathering

Following the first visit where baseline data will be gathered, the women will be seen at intervals of 30 days. A social worker will interview the women and record answers to qoeustions concerning compliance and side effects. Women in the study will have transportation paid for when returning to the for visits. Women who do not return for the clinic visits will be visited by the social workers at home if possible. At the second and third follow-up visits (30 days and 60 days) blood will be taken for, transferrin receptor. Weight and MUAC will be taken at each visit also. The hemoglobin and ferritin will be determined at last visit again.
Frozen samples will be sent to the University of Kansas for determination of transferrrin receptor. Data will be entered in a dabase using epi-info by a data entry person.

Analysis :
The data will be analyzed first with descriptive statistics and then by stratified analysis. Following this, logistic regression will be done if the stratified analysis is not sufficiently powerful. Socio-denographic variables (age-religion, education accupation, number of persons in houshold, number of children, etc.); report of compliance and side effect (diarreheam nausea, loss of appetite, heartburn, abdominal cramps, constipation, color of stool, menstrual history,etc); biochemical and physical parameters (height, weight, MUAC, hemoglobin, ferritin, transferrin receptor) and others (abbreviated nutritional history, menstrual history, history of other drugs/medicines,etc) will be determined for the three groups to see if there are significant differences berween the GDS Iron group, and the other groups

104

Ethical considerations :

The study does not expose any of the women to health risks. In reality, it provides them with a major health service, as it identifies them as being anemic (which they would not have known) and offers therapy for this. Any women who are anemic at the end of the study (placebo group, non-responders)will be given a supply of iron sulfate and counseled regarding its appropriate use. Women whose niochemical parameters indicate that the anemia is probably not the result of iron deficiency will be counseled regarding seeking the appropriate treatment. The major risks in the study those resulting from the drawing of blood for biochemical test. These risks are minimal.

Personnel and Logistics :
Both studies will be done by the Research Group at Dr.Sutomo Hospital. The studies will be done concurrently, but with staggered starting poinst to avoid logistic and personnel problems. The Research Group will have a study Coordinator, and investigators. The studies will share ancillary personel who are two social workers (one for each clinic), two blood drawing technicians (one for each clinic), and a data entry/secretary/computer operator.

A computer with hard disk, monitor and printer will be purchased for data entry and analysis. The specialized software for data input and analysis will be provided. Two Hemocue machines with equepment such as vacutaners/bleeding equepment, laboratory supplies, computer paper and diskettes will be purchased locally with money provided from the budged. The budget will provide funds for transport of the women to the clinic appointments, and for the social workers to visit women who have missed appointments. Funds will also be provided for training, for communications and mailing and for publication of the final report.

Samples will be taken in duplicate (two vacutainers).
Both will be frozen and periodically collected by the AID TAACS Adviser and sent to the University pof Kansas for determination of transferrin receptor.

Data analysis will be done by the principal investigators with the help of the AID TAACS Adviser.

201

APPENDIX B-3b

GASTRIC DELIVERY SYSTEM IRON TRIAL

Introduction:

Maternal mortality in Indonesia is high (MMRatio= 250-718/100,000 LB). As in most developing countries, the major causes of maternal mortality are hemorrhage, sepsis and toxemia. Maternal anemia is a recognized risk factor for maternal death due to hemorrhage. In one study in Indonesia, the maternal mortality ratio for anemic women was substantially higher (700/100,000) than for non-anemic women (190/100,000). In a study noted in World Health Statistics 1982, pregnant women in Indonesia had the lowest average hemoglobin levels among women in 5 countries in the region. The National Household Health Survey of 1985/86 showed a prevalence rate of over 73% of anemia in women of childbearing ages.

There has not been sufficient research to document the etiology of the problem of anemia in Indonesia; however, poor absorption of dietary iron due to rice-based diets appears to be an important contributing factor. In other countries in the region, supplementation of dietary iron with iron sulfate has achieved reductions of anemia of fifty percent. However, there appears to be many obstacles to widespread acceptance of iron sulfate supplementation as a public health strategy for reduction of the prevalence of anemia. Among the problems are the lack of availability in all areas, the awareness of the need for and benefit from these iron tablets among women, and the problems of continued compliance where the tablets are available. Many women who have access to the tablets simply do not want to take them because of the perception that the side-effects (nausea, constipation, etc) outweigh the potential benefits of taking them.

Recently, a new formulation and delivery system for iron has been tested, and in trials in Jamaica, appears to significantly lower the rate of adverse effects while still reducing anemia. If true, this method of iron delivery would significantly decrease problems with compliance. This new delivery system is called the Gastric Delivery System for Iron (GDS Iron). This study proposes to examine two questions:

> Is the GDS Iron pill as effective in anemic pregnant women at raising hemoglobin levels as the standard ferrous sulfate tablets, and is it better tolerated?
>
> Is compliance less of a problem in non-pregnant women with the GDS Iron tablet when compared to a standard ferrous sulfate formulation and a placebo that contains no iron?

These questions will be answered by two separate trials in different study populations. The studies will be done in

Surabaya, Indonesia by the research group at RS Dr Sutomo Hospital. Additional laboratory support will be provided by experts from the University of Kansas. Logistic support and technical assistance will also be provided by the MotherCare Project through the USAID/Jakarta TAACS Adviser.

TRIAL 1

Summary:

The first trial will take place in a group of anemic pregnant women and will involve two groups of women. The first group will be given the GDS Iron pill for a period of 150 days during the last four months of their pregnancy and the first month postpartum. The second group will be given the standard ferrous sulfate therapy for the same time period during the last 4 months of their pregnancy and the first month postpartum.

After obtaining informed consent, the women in the two groups will be randomly allocated to each group following screening to determine that they meet the criteria for gestational age and anemia. Both groups will be treated identically, seen every 30 days and given pills in packets of 30 pills for a total of 150 days (5 visits prior to birth, at birth and a final time 30 days following birth). The women will have hemoglobin, ferritin and transferrin levels measured at three points during the pregnancy (entry, 60 days and at birth) and at 30 days postpartum. The analysis will compare biochemical parameters with reports of compliance and side effects in the two groups to determine if the GDS Iron formulation is as efficacious as ferrous sulfate, and if it has a higher compliance rate.

Sample size and study length:

Two groups of pregnant women with hemoglobins between 8.0 and 10.9 g/dl will be enrolled, with 150 women in each group. The women will be recruited from 2 prenatal care clinics. Each clinic sees an average of 10 women per day, and estimates are that each week 25 anemic pregnant women will be enrolled per clinic, for a total of 50 women per week. All the women will be enrolled by the end of the 6th week of the study. Allowing 120 days for the duration of the pregnancy, and 30 days for the required follow-up period, two weeks for data entry and cleaning, and four weeks for data analysis and report writing, the study will be completed by the end of the 7th month.

Enrollment:

Pregnant women aged 15-45 attending the prenatal clinics will be enrolled. The goals of the study will be explained to them, and they will be asked to participate in a screening process to determine their eligibility. They will have a fingerstick hemoglobin determination done by hemocue, and if anemic (8.0-10.9 gm/dl) will be asked to participate in the study. Following

receipt of written informed consent, two vacutainers of blood will be taken, baseline social, demographic, nutritional, family planning and previous pregnancy/birth data will be taken. Women screened and found to be anemic but not choosing to enter the study will be told that they are anemic and counseled regarding taking iron.

Data gathering:

Following the first clinic visit where baseline data and the first blood sample will be gathered, the women will be seen at intervals of 30 days. A social worker will interview the women and record answers to questions concerning compliance and side effects. Following the interview, women will be given a 30 day supply of pills and asked to return in 30 days. Women in the study will have transportation paid for when returning to the clinic for visits. Women who do not return for the clinic visits will be visited by the social workers at home if possible. At the second return visit, at birth, and at 30 days postpartum blood will be taken for hemoglobin, transferrin receptor and ferritin. Height, weight and MUAC will be taken also. Blood will be obtained in a pediatric vacutainer to avoid the impression of large volumes of blood being taken, which would decrease participation. The sample will be checked with the hemocue for hemoglobin and then frozen. Periodically, the TAACS Adviser will pick up samples and ship them frozen to Kansas for determination of ferritin and transferrin receptor. Five percent of samples will also have hemoglobin checked for quality control. Data will be entered in a database using Epi-Info by a data entry person.

Data Analysis:

The data will be analyzed first with descriptive statistics and then by stratified analysis. Following this, logistic regression will be done if the stratified analysis is not sufficiently powerful. Socio-demographic variables (age, religion, education, occupation, number of persons in household, number of children); reports of compliance and side effects (diarrhea, nausea, loss of appetite, heartburn, abdominal cramps, constipation, color of stool); biochemical and physical parameters (height, weight, MUAC, hemoglobin, ferritin, transferrin receptor) and others (abbreviated nutritional history, menstrual history, etc.) will be determined for the two groups to see if there are significant differences between the GDS Iron group and the ferrous sulfate group.

Ethical considerations:

The study does not expose any of the women, fetuses or babies to health risks. Rather, it provides the pregnant women with a major health benefit as it identifies them as being anemic during their pregnancy (which they would not have known) and offers therapy for this. Any women who are anemic at the end of the

study (non-responders) will be given a supply of iron sulfate and counseled regarding its appropriate use. Women whose biochemical parameters indicate that the anemia is probably not the result of iron deficiency will be counseled regarding seeking the appropriate treatment. The major risks in the study are those resulting from the drawing of blood for biochemical tests. These risks are minimal.

Study schematic:

Subjects: Anemic pregnant women
Treatment: double blinded GDS-Fe (150)/Ferrous sulfate (150)
Outcome measures: Hgb, Transferrin, ferritin, wt/MUAC, GI sxs

```
Scrng
0          30         60         90         120/Birth 30
|---------|---------|---------|---------|---------|
1          2          3          4          5          6

<----------Therapy for both groups (blinded)------->

Bldg                  Bldg                  Bldg      Bldg
```

Laboratory:
Dr Sutomo: Hgb, height, weight, MUAC
U Kansas: Ferritin, transferrin receptor, 5% samples Hgb.

TRIAL 2

Summary:

The second trial will take place in anemic non-pregnant women and will involve three groups of non-pregnant, anemic women. The first group will be given the GDS Iron pill for a period of 90 days. The second group will be given ferrous sulfate therapy in an identical appearing capsule for the same time period. The third group will receive an identical appearing placebo containing biologically inert ingredients for the same time period. The study will be double blinded with neither the women nor the researchers knowing who is in which group. After obtaining informed consent, the women in the three groups will be randomly allocated to each group following screening to determine that they meet the criteria for anemia. All three groups will be treated identically, seen every 30 days and given pills in packets of 30 pills for a total of 90 days (3 visits including final). They will have hemoglobin, ferritin and transferrin levels measured at three points (baseline, 60 and 90 days) and the analysis will compare biochemical parameters with reports of compliance and side effects in the three groups. This data will be used to draw conclusions as to whether the GDS Iron pill is significantly better (less side effects, higher degree of compliance, decreases rates of anemia) than the standard ferrous sulfate pill or the placebo.

Sample size and study length:

Three groups of non-pregnant women with hemoglobins between 8.0 and 10.9 g/dl will be enrolled, with 100 women in each group. The women will be enrolled from two busy family planning clinics. Each clinic sees approximately 50 patients per day and it is estimated that each week 25 women meeting the criteria will be enrolled, for a total of 50 women for the two clinics. Thus, the three groups of 100 women will require 6 weeks to enroll. Allowing 90 days for the duration of the study, 2 weeks for data entry/cleaning, four weeks for analysis and report writing, it will require 6 months to finish the study.

Enrollment:

Menstruating women aged 15-45 attending the family planning clinics who are not pregnant or breast-feeding will be enrolled. The goals of the study will be explained to them, and they will be asked to participate in a screening process to determine their eligibility. They will have a fingerstick hemoglobin determination done, and if anemic (8.0-10.9 gm/dl) will be asked to participate in the study. Following receipt of written informed consent, two vacutainers of blood will be taken, baseline social, demographic, nutritional, family planning and previous pregnancy/birth data will be taken. Women screened and found to be anemic but not choosing to enter the study will be told that they are anemic and counseled regarding taking iron.

Data gathering:

Following the first clinic visit where baseline data will be gathered, the women will be seen at intervals of 30 days. A social worker will interview the women and record answers to questions concerning compliance and side effects. Women in the study will have transportation paid for when returning to the clinic for visits. Women who do not return for the clinic visits will be visited by the social workers at home if possible. At the third and fourth visits (60 days and 90 days) blood will be taken for hemoglobin, transferrin receptor and ferritin. Weight and MUAC will be taken at each visit also. The hemoglobin and ferritin will be determined at Dr Sutomo Hospital. Frozen samples will be sent to the University of Kansas for determination of transferrin receptor. Five percent of samples will also have hemoglobin and ferritin checked for quality control. Data will be entered in a database using Epi-Info by a data entry person.

Analysis:

The data will be analyzed first with descriptive statistics and then by stratified analysis. Following this, logistic regression will be done if the stratified analysis is not sufficiently powerful. Socio-demographic variables (age, religion, education, occupation, number of persons in household, number of children,

etc.); reports of compliance and side effects (diarrhea, nausea, loss of appetite, heartburn, abdominal cramps, constipation, color of stool, menstrual history, etc.); biochemical and physical parameters (height, weight, MUAC, hemoglobin, ferritin, transferrin receptor) and others (abbreviated nutritional history, menstrual history, history of other drugs/medicines, etc.) will be determined for the three groups to see if there are significant differences between the GDS Iron group, the ferrous sulfate group and the placebo group.

The analysis will be done in Surabaya by the principle investigator and co-investigators with the assistance of the TAACS Adviser.

Ethical considerations:

The study does not expose any of the women to health risks. In reality, it provides them with a major health service, as it identifies them as being anemic (which they would not have known) and offers therapy for this. Any women who are anemic at the end of the study (placebo group, non-responders) will be given a supply of iron sulfate and counseled regarding its appropriate use. Women whose biochemical parameters indicate that the anemia is probably not the result of iron deficiency will be counseled regarding seeking the appropriate treatment. The major risks in the study those resulting from the drawing of blood for biochemical tests. These risks are minimal.

Study schematic:

Subjects: 300 anemic menstruating women:

GDS	100 women
FeSO4	100 women
Placebo	100 women

Treatment: double blinded GDS-Fe/Ferrous sulfate/placebo
Outcome measures: Hgb, Transferrin, ferritin, wt/MUAC, GI sxs

```
Scrng
0          30         60         90
|---------|---------|---------|
1          2          3          4

<--Blinded therapy X 3 groups->

Bldg                  Bldg       Bldg
```

Laboratory:
Dr Sutomo: Hgb, weight, MUAC
U Kansas: Ferritin, transferrin receptor, 5% samples Hgb

Personnel and Logistics:

Both studies will be done by the Research Group at RS Dr Sutomo Hospital. The studies will be done concurrently, but with staggered starting points to avoid logistic and personnel problems. The Research Group will have a Study Coordinator, and each study will have a Principal Investigator and Co-investigators. Where possible, the studies will share ancillary personnel.

A computer with hard disk, monitor and printer will be purchased for each study for data entry and analysis. The specialized software for data input and analysis will be provided by the TAACS Adviser, as well as assistance in using it. Four Hemocue machines with sufficient cuvets for all samples will be purchased. Other equipment such as vacutainers/bleeding equipment, laboratory supplies, computer paper and diskettes will be purchased with money provided from the budget. The budget will provide funds for transport of the women to the clinic appointments, and for the social workers to visit women who have missed appointments. Funds will also be provided for training, for communications and mailing and for publication of the final report.

Samples will be taken in a pediatric vacutainer to avoid the impression of large volumes of blood being taken which would decrease participation. The hemocue machine will be used to determine hemoglobin. Following this, it will be frozen and periodically collected by the TAACS Adviser and sent to the University of Kansas for determination of ferritin and transferrin receptor. Five percent of those sent to Kansas will have hemoglobin values checked for quality control purposes.

Data analysis will be done in Surabaya by the principal investigator and co-investigators with the help of the TAACS Adviser.

APPENDIX B-3c

GASTRIC DELIVERY SYSTEM (GDS) PROJECT

SURABAYA, INDONESIA

JULY 9-20, 1993

CAROL FLOWERS

217

TABLE OF CONTENTS

		Page
ACKNOWLEDGEMENTS		1
I.	EXECUTIVE SUMMARY	2
II.	PURPOSE OF VISIT	4
III.	BACKGROUND	5
IV.	TRIP ACTIVITIES	7
V.	RESULTS/CONCLUSIONS	12
VI.	RECOMMENDATIONS	14

ACKNOWLEDGEMENTS

I would like to express my sincerest thanks for the gracious assistance of the following persons, without whose help this trip would not have been the successful venture that it was: Dr. Samuel G Kahn, Dr. James D. Cook, Dr. Poedji Rochjati, Dr. Marsianto, Dr. Mary Hansell, Dr. Kenneth Farr and Ratna Kurniawata.

I. EXECUTIVE SUMMARY

The first shipment of samples from the gastric delivery system (GDS) project in Surabaya arrived at the University of Kansas Medical Center in poor condition. Therefore, Dr. Samuel Kahn of the Office of Nutrition at the US Agency for International Development (USAID), Washington, DC, and Dr. James Cook of the University of Kansas Medical Center, requested that Carol Flowers travel to Indonesia to observe, first-hand, the collection and processing of samples for storage and also the preparation of these samples for shipment to the U.S.A. Dr. Kenneth Farr of the USAID Office in Jakarta, Indonesia gave authorization for this visit.

The goal of this consultancy was to visit the GDS project, meet with the principal investigator and the project coordinator and discuss the collection and preparation of the samples for storage and for shipment. In addition, aims were to visit the clinics to observe the methods of collections, processing and storage of samples. In the event this process could be improved this would be discussed with the principal investigator and a list of recommendations left in country.

In addition, the consultant would provide instruction and assistance in the preparation and packaging of the remaining samples and duplicates of samples from the original failed shipment, for transport to the University of Kansas.

A. Key Findings

1. The first shipment of samples to Kansas arrived thawed with no dry ice evident. The labels were not suitable for freezing and had come loose on

many tubes. Marking of the tubes had been done with non-permanent ink. When the labels became wet, on thawing, the ink ran, making many of the numbers unreadable. Values for the transferrin receptor measurements were lower than expected in samples from iron-deficient women, indicating there had been some deterioration of the samples.

2. Blood samples were not collected into fresh, clean and sterile vacutainer tubes. The tubes used for processing were not adequately washed to prevent possible cross-contamination or bacterial contamination of the blood if they were at ambient temperature for any length of time.
3. The samples were stored and shipped in glass tubes which risk breakage from repeated freezing and thawing or rough handling during shipment.
4. The freezer in which the samples were stored malfunctioned over one weekend. The samples thawed before they could be transferred to another freezer.
5. Finger-stick methods employed during the study often necessitated milking of the finger to obtain blood for hemoglobin (Hgb) readings, introducing the possibility of artificially low values.

B. Key Recommendations

1. Shipments of samples should be made more often during the term of the project with enough dry ice to make the entire journey. Adequate instructions should be given for proper preparation and packing to persons involved in this process.

2. Adequate supplies should be provided for proper collection, processing and storing of samples.
3. Incorporation of laboratory or technical personnel into the planning stage of the project would be crucial.
4. Adequate supervision at the outset of the project would catch potential difficulties before they become major problems.

II. PURPOSE OF VISIT

The University of Kansas is providing laboratory support for the Indonesian GDS project. Unfortunately, the first shipment of samples arrived in very poor condition and consequently may not be fully used. Because there appears to be a problem in the correct preparation and shipment of the samples, both R&D/H/Office of Nutrition and the University of Kansas felt the latter should send one of their staff to oversee the process of sample collection and shipment preparations in order to ensure arrival of viable samples.

Under this scope of work, Carol Flowers would meet with Dr. Poedji, the principal investigator, to discuss the process for collection and preparation of the samples for shipment. Methods would include first-hand observation and evaluation of the practices used by persons involved in the GDS project for the collection, processing, and storage of blood samples, visits to the clinics and health center where the actual blood collections were done, discussions with key personnel to assess any potential areas where problems may have occurred, instruction and assistance with preparation and packaging of the remaining samples and

duplicates of the first failed shipment, and return with viable samples to the University of Kansas Medical Center for laboratory analysis.

III. BACKGROUND

It is presently estimated that over a billion people in the world are anemic and that the major cause of this anemia is a deficiency in the supply of dietary iron relative to the iron needs of the population. The population segment most vulnerable to this nutritional iron deficiency is pregnant women. The global prevalence of nutritional anemia during pregnancy exceeds 50% and although this figure is much higher in Third World countries, no region is exempt. Recent studies have highlighted the significance of iron deficiency anemia during pregnancy. Mild anemia is associated with an increase in the rate of premature delivery and low birth weight, whereas more severe anemia is estimated to account for up to 20% of maternal deaths during parturition. In the light of the dismal success in reducing the global prevalence of anemia in this highly vulnerable segment of the population, there is a need to develop novel methods to improve the iron status of pregnant women.

For the past several decades the provision of oral iron supplements to pregnant women has been the primary method to decrease the prevalence of anemia. A commonly cited reason for the ineffectiveness of iron supplementation in the developing countries is the reluctance of women to adhere to a regimen that is often associated with gastrointestinal side effects. One way of circumventing this problem is to supply iron in a slow release form while retaining it in the stomach,

proximal to the iron absorbing region of the small intestine, a formulation referred to as GDS. Recent studies indicate that this preparation not only eliminates the side effects of oral iron but also results in a more efficient assimilation of the nutrient.

There has not been sufficient research to document the etiology of the problem of anemia in Indonesia; however, poor absorption of dietary iron from rice-based diets appears to be a contributing factor. Recently, the new delivery system GDS has been tested in field studies in Jamaica and it appears to significantly lower the adverse side effects while still reducing anemia. This would then decrease problems with compliance. Therefore the present study was undertaken in two separate trials in Surabaya, Indonesia. The first trial would assess the ability of the GDS formulation to reduce anemia in pregnant women while reducing side effects in comparison with the standard ferrous sulfate tablet. The second trial would assess compliance in groups of anemic non-pregnant non-lactating women.

These studies would be done at the Dr. Soetomo Hospital by the research group there and the laboratory analysis of samples collected during the study would be done at the University of Kansas Medical Center.

It was expected that, because of the low Hgb values, the measured transferrin receptor levels would range from normal levels to those elevated due to increased erythropoiesis in iron deficiency anemia. However, study samples which arrived in Kansas for evaluation had unusually low receptor levels indicating deterioration of the samples. This was most likely due to increased temperature during shipment as they had arrived with no dry ice and completely thawed. Because of the poor condition of the samples when they arrived it was suggested that an attempt be

made to determine the cause and salvage the rest of the study by transporting, by hand, the remaining samples and the duplicates of those already sent.

IV. TRIP ACTIVITIES

On Friday July 9, I left Kansas City for Indonesia carrying a large cooler with which to transport frozen plasma samples to KU from Surabaya. Phone calls to airline agents and officials had assured us there would be no problem transporting the samples on board the airplanes, provided the proper letters of verification of the non-hazardous nature of the shipment were in hand. These letters were supplied by the Department of Health and Human Services, Centers of Disease Control in Atlanta, Georgia and the USAID office in Jakarta, Indonesia.

On Sunday, July 11, I arrived in Jakarta, Indonesia and on Monday met with Mission officials at USAID in Jakarta for briefing. I met with Mary Jo Hansell and Ratna Kurniawata to discuss the condition of the samples as they had arrived in Kansas City. We discussed my scope of work and the concerns that the project was salvageable. I listed for them the things I needed to accomplish in Surabaya to make it so. We also discussed Dr. Kahn's hopes to obtain individual Hgb values for each blood sample collected, and Dr. Cook's desire to obtain a copy of the revised protocol as it was finally administered. Ratna gave me the letter for Customs Officials to aid in return of the samples to the USA. I met Dr. Kenneth Farr when he delivered this letter to Ratna.

On Tuesday, July 13, I flew to Surabaya where I was met by Dr. Marsianto. We discussed the layout of the Dr. Soetomo Hospital and the logistics of sample

collection, processing, and finally transportation for freezing and storage. He described the distances from clinics and health centers to the central storage area and the time taken for each day's activities. Dr. Marsianto indicated that samples collected from the two pregnancy clinics at the hospital were frozen immediately after collection and separation of the plasma, however because the power supply to the clinic area was unreliable they were transferred and stored in a freezer located next to the operating room (OR). This electricity was more dependable, however, the freezer did malfunction over one weekend and the thawed samples had to be transferred to another on Monday. The samples from the Mojo Health Center were kept at the center until the day's collection was complete then carried back to the hospital where they were processed in the delivery room, which is one of the few areas open 24 hours a day. The rest of the hospital closes at 1:00pm. Ambient temperature during these collections was 25-30° C.

On Wednesday, I met with Dr. Poedji to discuss the problems with the original shipment and my schedule of activities while at Dr. Soetomo hospital. She gave some background information of the medical situation in Indonesia. There are four types of hospitals in the country, types A,B,C and D. There are only two type A hospitals in Indonesia, one in Jakarta and Dr. Soetomo in Surabaya. These have all areas of specialization and sub-specialization. The Dr. Soetomo hospital has 1500 beds in the inpatient area and each of the outpatient clinics sees between 50 and 200 patients a day. The hospital closes at 1:00pm Monday - Thursday and Saturday, but closes at 11:00 am on Friday as most of the staff are Moslem and this is their time of worship. Type B hospitals employ only doctors in five or six

areas of specialization and type C only three or four. Type D are the Health Centers located in each provincy.

I was introduced to, and chatted briefly with, the hospital director, Professor Karjadi Wirjoatmodjo. We then walked to the Obstetrics and Gynecology Department where I met with the head of the department, Professor R. Prajitno Prabowd. From here we visited the Pregnancy Clinic I where the first part of Trial I took place. Here I watched while the nurse screened a patient for Hgb with a finger-stick and HemoCue reading. The Hgb was in the target range of 8.0-10.9, therefore 3 cc of blood was drawn via venous puncture using a sterile syringe with needle. The blood was transferred to a vial containing powdered EDTA and mixed. It was then transferred to another tube for centrifugation and separation of the plasma from red cells. The plasma was pipetted to a small vacutainer tube using a glass transfer pipet, sealed with a stopper and immediately frozen in a small freezer located in the clinic. At the end of the day, these tubes were carried to the central freezer located in the OR. After each sample was drawn the vial, tube, and pipet were washed with soap and water or just water and inverted to dry for the next day's collections.

Then we visited the Pregnancy Clinic II where the second half of Trial I was conducted. The women seen in this clinic were from families of government workers, with a higher standard of living than those in Clinic I. The process was essentially identical to that in Clinic I except the distance to the central freezer was longer.

The methods employed at both clinics for obtaining blood from finger-sticks encourages milking of the fingertip and can sometimes give artificially low Hgb

221

measurements. I was concerned that the vials, tubes and pipets were reused again and again without proper washing procedures. This can introduce cross-contamination or bacterial contamination if the blood is at ambient temperatures for extended periods of time.

Dr. Marsianto indicated that there is an inherent problem, in Indonesia, with the study design. There is a prevailing fear or reluctance to have blood drawn from venipuncture. Many subjects were lost because of the number of blood draws involved in completion of the study.

On Thursday, I met the midwives involved in Trial II with non-pregnant anemic women at the Mojo Health Center. I met with the director of the health center briefly and,because there were no patients there,we went to do home visits for the follow-up blood collection on some of the study subjects. We only found one of these subjects at home. The blood was drawn from finger-stick for Hgb and then from venipuncture with a 3 cc syringe. The blood was transferred, as before, to the vial containing EDTA. However,after mixing, it was carried in the vial during the rest of the trip until we reached the hospital. We walked to the area's community center where blood was drawn from one of the nurses for an additional fresh sample. From here we walked back to the Delivery Room, where the centrifuge is located, for separation of the plasma. The processing was the same from this point as I have described for the other clinics. However, if there were more patients in one day than available centrifuge tubes, those already used were rinsed, shaken dry, and reused for subsequent samples. Residual water in these tubes would slightly dilute subsequent samples. The Delivery Room staff were in the midst of several deliveries while we were working there.

In the afternoon I met the laboratory personnel who would be assisting in the preparation and packaging of samples for transport to the USA. A schedule of activities was set up as special arrangements had to be made since we would be working in the afternoons, after regular work schedules had finished. We spent the rest of the day sorting and remarking samples with permanent markers.

Thursday and Friday were spent at the OR freezer sorting and remarking samples. This would not have been necessary if the markings had been in permanent ink and proper storage racks had been supplied initially so that duplicate samples could have been separated at the time of collection.

On Saturday, the tubes were packed 10-15 per group, wrapped with a rubber band and packed into individual Ziploc bags, each bag was secured with an additional rubber band. This method of packing was used to ensure adequate cushioning for the tubes in the least possible space. The samples were returned to the freezer as soon as each package was complete. On Monday morning, all samples would be packed in the cooler with 30 lbs of dry ice.

I met with Dr. Poedji and discussed my findings and recommendations with her, returned to the hotel where I drafted these recommendations, and had them typed for her.

Monday, after final packing, we travelled to the airport where we encountered difficulties with the Singapore Air agents. They insisted that an excess baggage ticket be purchased if the cooler was to travel with me on the plane. I had no difficulties at the Singapore Airport or on the NorthWest Airlines flights. However, I had problems again with the agents for American Airlines in Chicago. It is my belief that a phone call from an embassy official to the airport personnel for each

229

of the airlines would have completely eliminated all of the difficulties that I encountered. Apparently telephone calls to the airlines alone were not sufficient.

V. RESULTS/CONCLUSIONS

The trip to Indonesia allowed me to make observations of the actual blood collections and processing at the various clinic sites. I was able to view actual procedures done by the personnel involved in the two research trials. This enabled me to determine several potential problem areas. There may be several factors that have acted in concert to affect the measured protein values. These would have a cumulative effect on the plasma samples.

1. The most significant factor may be the actual shipment of plasma to Kansas. These arrived thawed with no dry ice evident. It is not known how long they were in this condition. There was either not enough dry ice to make the entire journey or it was not replenished in Singapore as had been previously arranged.
2. During the actual collection of samples, the heat of the Surabaya climate seems only a possible factor in those samples collected at the Mojo Health Center. These would have been at ambient temperature for 2-4 hours, either at the center or on home visits, until they could be processed at Soetomo hospital. The plasma may have warmed while being prepared for shipment, however, either of these conditions would only contribute to small losses.
3. Some of the earlier samples from the studies thawed when the OR freezer malfunctioned over a weekend. These were transferred to another freezer on

Monday, however it is not known how long they were thawed or how warm they got.

4. A factor in the low receptor values may also be the lack of adequate supplies for the blood collection, processing and storage. The blood was collected into vials containing EDTA prepared by the laboratory personnel. It was mixed and transferred to centrifuge tubes, centrifuged and then transferred to clean 3 ml glass tubes with a glass transfer pipet. This glassware was either rinsed or washed with soap or detergent and allowed to air dry. Blood proteins stick tenaciously to glass surfaces and with inadequate washing procedures these would carry over to the next sample. Any residual soap would also carry over. Bacterial contamination is a possibility if the blood samples are kept at ambient temperatures for extended periods of time. Any of these factor could affect measured proteins values.
5. If there were more samples in a day's collections than there were available centrifuge tubes, these were rinsed, shaken dry and reused for subsequent samples. Any residual water left clinging to the surfaces would dilute the next sample.
6. Adequate supplies were not provided for proper storage of the samples.
7. The finger-stick methods employed by the nurses introduced the possibility of artificially low Hgb values. These were only measured on the first and last blood draws.
8. Mike Linnan is the only person with the keys to the capsule codes as administered in the final revised protocol.

9. Analysis of a representative sampling of duplicate tubes of plasma returned with me to KU indicates the transferrin receptor values average 30% higher than those measured on the same samples from the May shipment. This would point to the shipping process as the major factor in the deterioration of the samples.

I believe that inadequate attention was given to technical details for completion of this project. A few more dollars spent on permanent markers and proper storage containers would have eliminated many problems. More careful attention to the process of packaging and shipment of samples plus an adequate supply of dry ice would have prevented deterioration of samples.

VI. RECOMMENDATIONS

It is my feeling that if a future project hopes for the best possible results there are several points that need careful consideration.

1. More frequent shipments should be made to the USA with careful attention given to proper packaging procedures and an adequate supply of dry ice inside the foam container to make the entire journey to the US without replenishment.
2. Care should be given that samples are handled the shortest amount of time and the least number of times.
   a. Individual blood collection tubes containing EDTA should be provided.
   b. Storage tubes should be provided that are appropriate for freezing and shipping. These should be polypropylene microtubes with snap-caps or

screw-caps. These should have frosted "write-on" surfaces. Humidity in Indonesia makes adhesive labels unusable.

c. Storage racks or containers which take little space would facilitate separation of duplicates as they are collected rather than trying to accomplish this when preparing samples for shipping.

3. Markers that have permanent ink are imperative.
4. Disposable transfer pipets for transferring plasma to the freezing tubes would be an ideal solution, however, lacking this, a detailed protocol of stringent glassware washing procedures would be essential.
5. Input from laboratory personnel at the planning stages of the project would be extremely helpful.
6. Close supervision by USAID/John Snow, Inc. (JSI) personnel at the outset of the project might help spot potentials problems early.
7. Contact of an embassy official with airport personnel of authoritative position would avert difficulty in transportation of samples to the USA.
8. If samples are carried by USAID or JSI personnel, I would recommend carrying bungee cords for tying down the container on board the aircraft.
9. More frequent shipments of samples would eliminate the potential for losses of major portions of any study.

NOTE: Federal Express does have service out of Surabaya. This would probably make more frequent shipments easier.

APPENDIX B-4

APPENDIX B-4a

JOINT MICRONUTRIENT MISSION
IRON PERSPECTIVE

I. Executive Summary

A. Assessment

1. The National Nutrition Survey conducted at 5 years intervals in the Philippines in more than 3000 households provides an adequate data base to monitor the prevalence of nutritional anemia.
2. It is strongly recommended that serum ferritin determinations be performed in a high proportion of sampled individuals to establish the true prevalence of iron deficiency anemia (IDA) rather than the prevalence of all types of anemia. This is especially important in adult males and individuals over 50 years of age who often have anemia that is not IDA. Serum ferritin measurements are less important in high risk groups for IDA such as pregnant women and infants under the age of 12 months.
3. The extent to which malaria infection is contributing to the prevalence of anemia in population studies should be determined.
4. Studies are needed to establish appropriate hemoglobin cut-off levels for anemia in the Filipino population and specifically whether WHO criteria are valid in the Philippines.
5. Selected field studies are needed to determine the contribution of parasitic infection to IDA and the benefit of controlling related infections.
6. Given the large variety of methods currently used to measure hemoglobin levels, efforts at standardization are important. Purchase of a limited number of portable hand-held digital instruments such as the HEMOCUE system would be helpful for periodic calibration of measurements in local health clinics and laboratories.

B. Supplementation

1. Efforts to provide iron supplements to **all** pregnant women and low birth weight infants without prior hemoglobin determinations should be strengthened because the iron needs of these groups can only be met by medicinal iron. These programs should be revised to expand coverage and increase efficacy.If there is going to be a significant delay in the introduction of iron-fortified weaning foods, a case could be made for universal iron supplementation of all infants between 6 and 12 months of age.
2. On the other hand, it is unlikely that supplementation programs which require detection of anemia by a hemoglobin determination before providing supplements (screening-based intervention) are cost-effective. The investment in procuring laboratory equipment, training of personnel, and maintaining laboratory supplies would be better invested in the development of fortification programs to *prevent rather than treat* IDA.
3. Because many children with moderate or severe protein-energy malnutrition do not suffer from IDA initially, it may not be advisable to target iron

supplements to them. Provision of iron-enriched food supplements should meet the relatively modest increase in their iron requirements.

4. Because prior studies by FNRI personnel have shown that many commercial preparations of medicinal iron are very poorly absorbed, strict pharmaceutical guidelines should be established and implemented. The use of UNICEF tablets should be strongly encouraged because of high bioavailability and low cost.

*5. Studies to improve compliance and efficacy of iron supplementation programs in pregnancy should be developed once supply problems in regional health clinics are solved. It is possible that innovations such as one tablet taken daily at bedtime and encouragement to continue supplements for a few days despite minor side effect will increase effectiveness.

*6. The efficacy and cost effectiveness of newer iron delivery systems which eliminate gastrointestinal side effects such as the gastric delivery system (GDS) should be examined in small pilot studies.

*7. Consideration should be given to the use of simple inexpensive screening methods to monitor compliance rates in pilot efficacy studies of supplementation. Examination of tissue paper fecal samples using a "spot" test for iron could be an effective way of checking for recent ingestion of iron tablets.

C. Fortification

1. Efforts to develop iron-enriched foods should be given the highest priority for the control of IDA. Fortification of a staple food such as rice or wheat and the development of inexpensive weaning foods are of key importance.

2. No more than one iron-fortified staple food consumed by adults should be introduced at a time. The effect of one fortified food on the iron status of the population should be fully assessed before introducing a second fortified product.

3. The preliminary studies of iron-enriched **rice** ("FVR" rice) are promising but additional work is required to establish the feasibility and effectiveness of using this vehicle when extended to a production level. This includes studies of availability of the fortificant (radioisotopic measurements in humans), losses of the added iron during preparation of the rice in a home setting, evaluation of untoward color or taste reactions when consumed with a normal diet, etc. The establishment and support of a task force for rice enrichment is strongly encouraged.

4. Further studies are needed to evaluate the potential for fortifying **wheat** flour in view of the expanding consumption of wheat-based foods (20% of cereal consumption in Metro Manila). The strong appeal of this vehicle is that there are only 12 milling facilities in the country (all in Manila) and that the technology for fortifying wheat with iron is fully established and immediately available.

5. There is an urgent need for iron-fortified **weaning foods** to cover the iron needs of infants. Attention should initially be focussed on Insomix, a low-

cost weaning food which is prepared at the household level. An iron-enriched rice premix (packets) could be used.

D. Dietary Modification

*1. Further studies on the effectiveness of dietary modification such as promoting the intake of iron-rich foods and foods high in ascorbic acid are needed before promoting widely this approach as a means of controlling IDA. Increasing the consumption of meat, fish and poultry will be effective but it is unlikely that the diet can be altered sufficiently in low income segments of the population to influence iron balance.

* Denotes proposals that will require preliminary work to establish feasibility before implementation

II. Prevalence and Causes of Iron Deficiency Anemia

There is abundant evidence available from reports obtained prior to, and during, the JMM that there is a exceptionally high prevalence of iron deficiency anemia (IDA) as judged by isolated hemoglobin or hematocrit measurements. The most recent information is the Food and Nutrition Institute (FNRI) 1987 National Nutrition Survey which showed a prevalence rate for anemia of 70% in 6 month to 11 month old infants and a rate of approximately 40% in children aged 1-12 years. The prevalence of anemia in adult women and men was 39 and 21% respectively while the rates in pregnant and lactating women were 45 and 51% respectively.The overall prevalence for all ages and sexes was 37.2% These prevalence figures are among the highest of any country from which anemia survey data is available.

The prevalence rates for anemia in the Philippines have not only been stable for many years but appear to be increasing in most age and sex categories. When the 1987 figures are compared with the same FNRI national survey performed in 1982, the prevalence of anemia in the under-one-year olds was only 51% in 1982 as compared with 70% five years later. In general, the 1982 figures were about 10 percentage points lower than in 1987; the composite prevalence rate was 27% in 1982 and 37% in 1987.This trend of increasing anemia prevalence is also evident when compared to surveys in mid-1970's, at least in certain age and sex categories. In one such survey conducted in 2351 individuals (Marzan AM. Phil J Nutr, 1976:14-24), the prevalence rate in adult men was only 7% as compared with 21% in 1987. The prevalence of anemia in pregnant women, however,has remained stable over this period at about 45%. While there may be other factors contributing to the apparent increase in the prevalence of IDA such as sampling design or differences in laboratory methodology, there seems to be little doubt that iron deficiency remains a serious public health problem in the Philippines and that measures to reduce IDA during the past decade and a half have not been effective.

The increasing prevalence of anemia in the Philippines raises the question of whether causes of anemia other than iron deficiency may be contributing significantly. In recent

national surveys, there has been no attempt to obtain more specific laboratory indices of IDA such as serum ferritin levels although these assays may be performed in some age and sex categories in the present national survey that will be concluded later this year. There is no reason to suspect that the major cause of anemia is not iron deficiency in high risk segments of the population such as infants and pregnant women. In isolated studies, iron deficiency has actually been confirmed in the latter group. In a recent study in 88 healthy primigravidas, the mean serum ferritin in first trimester was 22.6 ng/ml but by the time of delivery, over 80% of women had ferritin levels below 12 ng/ml, the widely accepted cut-off level for iron deficiency. Virtually all of the anemic women had serum ferritin levels in the diagnostic range for iron deficiency. (Perlas et al., SE Asian J Trop Med Public Health 1992;23:691-697).

The reliability of isolated hemoglobin levels as an index of IDA does become a significant question in lower risk segments of the population. For example, it is very doubtful that the primary cause of anemia in individuals over 60 years of age in the 1987 survey (prevalence, 47%) is IDA and it also unlikely that the 20% rate of anemia in adult men is due to iron deficiency.Two factors could explained these anemia figures. The first is that the normal hemoglobin level in Filipinos is lower than in the predominantly Caucasian populations on which the current WHO cut-off levels for anemia are based. It is now evident, for example, that the mean hemoglobin in African-Americans is an average of 0.8 gm/100 ml lower than whites resulting in a correspondingly high prevalence of anemia in blacks. The other possible explanation for the high anemia rates in certain segments of the Filipino population is disorders other than iron lack such as chronic infection, inflammatory disease or protein deficiency. Chronic disease states rather than IDA accounts for most of the anemia seen in elderly subjects and are the probable explanation for the high prevalence of anemia in the national survey. This fact has already prompted workers at the FNRI to perform serum ferritin levels in a significant proportion of the elderly subjects in the current national survey and it strongly advised that this assay be performed in as many of the low or intermediate risk segments of the population (e.g. adult men and women) as possible . These measurements will provide far more reliable indices of IDA and will avoid investing large amounts of funds to alleviate anemia due to causes other than iron deficiency. This of great importance because the objective of the iron portion of the micronutrient initiative is to reduce the prevalence of IDA, not anemia.

There have been few, if any, recent studies in the Philippines to identify specific causes of iron deficiency or explain the increasing prevalence rates. It is, in fact, very difficult to do this assessment because the etiology of iron deficiency a given population is usually obscure. As in all populations, the predominant cause is an imbalance between dietary iron supply and accelerated iron needs due to growth and pregnancy.Data are available on the average iron intake of different segments of the population in the Philippines but these are of limited value because the bioavailability of iron is far more important than total dietary iron intake. Apart from meat,poultry and fish consumption which are of unquestioned importance by virtue of the highly available heme iron they contain, the key dietary determinants of iron status are poorly understood. Nevertheless, it is likely that the increasing prevalence IDA in the Philippines is related to a reduced quality of the diet because of economic constraints. There are virtually no iron-fortified foods available to

segments of the population in greatest need. For example, the very high rate of anemia in infancy is clearly due to the lack of available iron-fortified weaning foods and formulas that have virtually eliminated IDA in infancy in most industrialized countries. Iron supplementation for anemic infants is being considered at the present time but has not be implemented in any significant portion of the county. One important potential cause of iron deficiency which is of uncertain significance in the Philippines is parasitic infection. Hookworm, the only parasite with an undisputed association with iron deficiency, appears to be relatively uncommon. It is doubtful that other parasites such as tapeworm are important causes of iron deficiency. It is possible that the iron deficiency program would benefit by field studies to determine the major correlates of parasitic infections with IDA.

There have been several studies of iron supplementation in pregnant Filipino women and because this is an important component of the iron program in the Philippines, the results of one of the larger trials, performed nearly a decade ago, will be reviewed here.[1] In a preliminary study, the bioavailability of iron from a ferrous sulfate tablet obtained from UNICEF was compared with ferrous sulfate obtained commercially in the Philippines and with a preparation marketed in the USA. Each of the iron preparations was given to fasting subjects and the rise in plasma iron was measured. The volunteer subjects included 101 healthy, young, non-pregnant women who were evaluated using a cross-over design in which the absorption from a solution of chemical grade ferrous sulfate was compared with one of the commercial preparations. The relative bioavailability of the UNICEF tablet was 71% and 80% when measured in separate studies whereas the US preparation was 98% available. In contrast, the bioavailability of two commercial preparations obtained in the Philippines was 9.1 and 10.4% . When based on the cost of bioavailable iron, the two locally manufactured tablets were more expensive than the cost of the imported tablet by a factor of two.

This study was followed by a large field study of iron supplementation in pregnant mothers in which compliance and efficacy of several iron preparations were assessed. The study was conducted in 112 barangays (villages) in 9 municipalities of Bulacan, a province approximately 45 km northeast of Manila. Pregnant women between week 18 and 24 of gestation and with hemoglobin above 8 g% were entered into the trial. The key comparisons were as follows: UNICEF - 60 mg iron daily without supervision, UNICEF - 120 mg iron daily without supervision, UNICEF - 60 mg iron daily with supervision, and UNICEF - 120 mg iron daily with supervision. Thus, two dose levels (one or two tablets daily) were compared using both supervised and unsupervised administration. Follow-up examinations of hemoglobin and serum ferritin were obtained at intervals up to 100 days. A total of 1661 pregnant mothers were surveyed but only 30% or 502 qualified and of these, there was a high drop-out rate of 10% after 30 days, 20% at 60, and 30% at 100 days.

---

[1]This and other studies are summarized in a monograph obtained at FNRI entitled "Coordinated Research Program for the Control of Nutritional Anemia in the Philippines (1980-1982). This will be referred to subsequently as the CRPCNA monograph.

The hemoglobin response indicated that IDA was the primary cause of the high prevalence of anemia in these pregnant women. In contrast to the decline of approximately 1 g/100 ml in hemoglobin in the placebo group, the hemoglobin increased in all women given some form of iron. The most dramatic response occurred in women given a single UNICEF tablet with supervision (hemoglobin 12.3 g/100 ml as compared to a baseline of 10.2 g/100 ml). In the unsupervised group, a slightly higher hemoglobin response was obtained as compared with one tablet daily but the response differences were not dramatic. It should be noted that the most common cause for the high drop-out rate was the side effects of iron.

III. Current Perspectives and Priorities

A. Government Perspective

From the comments of Secretary Flavier, Undersecretary Tan, and other government officials, there is a good deal of uncertainty about the optimal program in the Philippines to control IDA and during our initial interaction with government officials, they expressed a need for "help" in developing an appropriate program. At the same time, they did not seem to be disinterested in the anemia problem but instead seemed committed to including IDA in their micronutrient program. Thus, from the government perspective, the initiatives for IDA are in their infancy and there is awareness of need to bolster this component of their micronutrient initiative.

B. Community Leaders, Industry, and NGO Representatives

There is a similar degree of unfamiliarity with what needs to be done to control IDA by these potential contributors to the micronutrient initiative. In our site visits, many community leaders were unaware of iron deficiency as a nutritional problem although there was knowledge that anemia is a nutritional concern. There has been far more emphasis on the importance of Vitamin A and iodine deficiency within the country and less awareness of what can, and should, be done about IDA. Industry has invested no special effort in iron fortification of food products but in our discussions at the NFA, this interest is developing because of the publicity given to FVR rice. Likewise, NGO representatives have been focussed more on efforts to alleviate iodine and Vitamin A deficiency although most are aware of the anemia problem within the country. There is a lack of support for the control of IDA among NGO representatives and a major question is what agency will undertake this initiative as their primary objective. Whereas the interest in Vitamin A deficiency is well represented by HKI and iodine deficiency is spearheaded by UNICEF, no agency has committed their resources to the control of IDA. Although we do not have the opportunity to discuss this problem with the WHO representatives within the Philippines, interest in the control of IDA by WHO was commented upon indirectly on different occasions. *It would helpful if Linda Keiss and Dr. Horwitz could add their comments here about their session with the WHO representatives.*

C. International Donors Operating in the Philippines

See above comment. Except for a possible interest by WHO in the control of IDA, no other international donors have an expressed interest in this aspect of micronutrient deficiency.

D. USAID Mission Perspective

No specific comments in relation to IDA are required here.

IV. Assessment of Infrastructure Related to Micronutrient Malnutrition

a. Service Facilities

Because very little attention has been paid to the control of IDA in the past, there was little evidence of activity relating to the detection or treatment of iron deficiency at the health clinic level. Iron tablets have been provided to pregnant women for many years but supplies at the few local health clinics that were visited were low or nonexistent. Supplies have been curtailed in recent months as a result of the process of devolution although most health workers indicated that iron tablets were available in the past in most health clinics. It was difficult to get a clear picture of the extent of compliance of pregnant women with iron supplementation. Side effects from iron do seem to be a significant problem and in at least one health clinic, the comment was made that women prefer not to use the inexpensive tablet provided by UNICEF but rather to purchase their own iron supplements at local pharmacies. The possible reasons are discussed in Section V. There does seem to be a general awareness of what anemia is and what its side effects are but there was little evidence that this was a priority in any of the clinics that were visited.

B. Laboratory Capabilities and Personnel Training

Except for the provision of iron supplements to pregnant women, supplementation programs in the Philippines are based on a prior hemoglobin measurement demonstrating the presence of anemia. There is no systematic hemoglobin screening procedure, however, and hemoglobins are usually performed only when the individual presents with illness. There appeared to be a wide range of approaches to the detection of anemia. In one hospital laboratory, the microhematocrit was used, whereas in most other settings, some form of hemoglobin determination is made. The technology ranges from the crude Sahli hemoglobinometer to more accurate spectrophotometric determinations. It appears that the facilities for performing a hemoglobin determination are not present in most health clinics. Individuals suspected of being anemic are sent to a local laboratory where they are required to pay for a hemoglobin measurement. They may or may not then return to the health clinic to obtain iron supplements.

The laboratory capabilities and personnel training in reference to IDA is therefore variable and inconsistent.

C. Food Processing and Quality Control Capabilities

There are no foods that are currently produced or distributed which are fortified with iron. The efforts relating to the fortification of rice, wheat, or weaning foods are discussed in other sections. We did not have an opportunity to interact with a pharmaceutical manufacturer but, as mentioned in other sections of the report, there seems to be little or no regulation of iron medication. Because a significant proportion of pregnant women apparently obtain their iron supplements at local pharmacies, some attention to the quality of iron supplements available within the country would be rewarding.

V. Past and Present Micronutrient Control Programs

The primary control strategy for IDA during the past two decades has been the provision of iron supplements to all pregnant women beginning in the second trimester or 20th week of pregnancy. Two iron tablets daily, each containing 60 mg of elemental iron, are recommended. In addition, iron supplements are provided to those infants, lactating mothers, and preschool children who have been found to be anemic by laboratory measurement either at routine screening at health clinics (infants are usually screened at 6 and 12 months of age) or on the basis of suspicious symptoms or signs.

The effectiveness of these strategies is difficult to assess on the basis of available evidence. Efficacy can only be ascertained from the limited contact with health care workers during the present JMM field visits. It is likely that the iron supplementation program for pregnant women has had some impact because the prevalence rate for anemia has remained stable and is roughly similar to that which has been reported in other developing countries. Apparently, the lack of supply of tablets has not been a major problem until recent months when the availability has been sharply curtained in the process of devolution. None of the clinics that were visited had more than a few days supply of tablets for one individual. As in most supplementation programs, poor compliance due to gastrointestinal side effects appears to be a significant problem, causing refusal of the supplement in one-third to one-half of women. Interestingly, many women prefer "chocolate" coated iron supplements that are available commercially to the tablets provided by UNICEF. This preference is probably explained by the marked difference in the availability of the iron in the two preparations. When assessed by the rise in serum iron levels in fasting subjects given iron, it was shown that the availability of commercially available supplements in the Philippines was less than 10% of that absorbed from an iron formulation marketed in the USA; the UNICEF tablet had a relative availability of over 70%(see CRPCNA monograph). It seems likely that the coated iron tablet obtained locally is preferred because the iron is relatively unavailable for absorption in the gastrointestinal tract and therefore does not produce the common side effects of nausea, vomiting and epigastric pain.

242

It is even less likely that supplementation of infants or children found to be anemic in local health clinics is having a significant impact. One problem is the availability of hemoglobin or hematocrit determinations. While such determinations are immediately available in some clinics, patients must be referred to a local laboratory in others at a cost that many patients consider too high to have the measurement performed. The routine screening of infants less then one year of age is probably more effective but this also depends on laboratory equipment and the training, skill, and motivation of the workers in the health clinic. Moreover, the definition of anemia varies from clinic to clinic; some workers believe that hemoglobin levels above 8-9 gm/100 ml do not constitute a significant anemia problem. Mothers also complain of the staining of the teeth and mouth from the liquid iron preparation. It is highly unlikely that screening based interventions in infants and children are having any significant influence on the prevalence of IDA in the Philippines.

As an outgrowth of the of the National Nutritional Council (NNC) meeting held during the JMM visit in which an integrated micronutrient program was discussed , a framework plan for the control of IDA in the Philippines was proposed. A detailed review of strengths and weaknesses of these strategies might therefore be useful.The proposal will focus on iron supplementation rather than fortification.Iron tablets will be provided to pregnant women (cost, where M = 1 million pesos, 218M),lactating women (72M) and school children (5M). Iron drops will be given to infants (218M) and low birth weight (LBW) infants (78M). Iron syrup will be given to preschoolers (356M). The projected total cost of these components is about 1 billion pesos. There are several additional components of the IDA plan including the provision of iron to all school children suffering from moderate or severe protein energy malnutrition (PEM), promotion of gardening, provision of small livestock, revision of assessments methods and development of iron fortified foods; most of these are listed under research and development (see appendix).

The major reservations about these expanded supplementation efforts relates to their cost effectiveness. It is likely that the overall effectiveness of iron supplements in pregnancy could be increased by reducing the number of tablets from two to one daily, emphasizing the need to persist with the supplements despite gastrointestinal side effects (because these often subside after a few days), improving compliance by taking the tablet at one time of day etc. The latter may be especially beneficial if the tablet is taken at bedtime rather than with food which impedes absorption.Also, troublesome side effects may not occur before the woman falls asleep. Given the high cost of the pregnancy program, some field studies to assess efficacy will indicate ways in which the program can be altered to improve efficacy. In recent studies, iron determinations on small fecal specimens have provided an excellent compliance guideline; the strong reaction of fecal material to a chromogenic reagent for iron that is typically seen with the recent intake of medicinal iron could probably be detected reliably by performing a spot test on a fecal sample smeared on toilet tissue.

Whereas there is a rationale for supplying iron to pregnant women, it is unlikely that widespread supplementation of infants and preschoolers will be effective. This has not been used in other countries to any extent except in LBW infants who require much larger amounts of iron than can be provided in iron-fortified foods. It will be important to assess the effectiveness of this approach in pilot studies before extending the program to the

national level.The need for additional iron in children with PEM should also be evaluated; the anemia in these children is not usually due to iron lack, at least until their weight begins to increase with supplemental feedings.

In summary, the are a large number of constraints with a screening based intervention program employing medicinal iron.The indicators for screening are highly unreliable whether based on symptoms such as weakness, fatigue and shortness of breath or on signs such as conjunctival or facial pallor. As a result, a large majority of iron deficient individuals will go undetected. The cost of the laboratory examination will often preclude measurements in the lower socioeconomic segments. Many of the screening methods are highly unreliable and even reliable methods will not perform adequately with unskilled technologists. Medicinal iron has several problems that invite poor compliance such gastrointestinal side effects, staining of the teeth with liquid preparations to say nothing of the cost which deter many individuals who cannot afford to purchase the supplement. Sustaining medicinal iron over any significant period of time is another important limitation. Few, if any,iron intervention programs that require a laboratory measurement on a individual basis have been shown to justify the investment.

VI. Recommended Single Micronutrient Strategies

The limitations of supplementation programs in which medicinal iron is given to individuals identified as anemic by laboratory testing are outlined in the previous sections. In this section, the advantages of food-based strategies to reduce IDA will be discussed and emphasized as the most cost-effective long-term method. The efforts to introduce iron-fortified food products are now well underway by Dr. Florentino and colleagues at FNRI.The following is a discussion of this program and the potential for its success in the micronutrient program for IDA control in the Philippines.

Although several food vehicles were discussed during our evaluation, only a very limited number warrant detailed consideration for iron fortification. The prime candidates are rice, wheat flour and cereal-based weaning foods. Other possibilities are fish paste which is consumed primarily by the lower income groups, and salt which is much discussed as a possible vehicle for double fortification with iron and iodine. While the latter has appeal in the context of a coordinated micronutrient program,there is no clear evidence from investigators in India, who have explored this approach for the past many years, that the technological problems have been fully solved. The other, more remote, possibility for double fortification is the combined addition of iron and iodine to the water supply of elementary school children but there are only preliminary reports of iron-fortified water and none of double micronutrient addition.

Because rice is consumed extensively throughout the country by virtually all segments of the population, this vehicle offers major advantages. The technology of rice fortification is based on studies conducted almost a decade ago at FNRI in which a surface-coating method was developed using ferrous sulfate as the iron source and a coating material prepared from ethyl cellulose-methyl cellulose-chloroform-isopropyl alcohol. A premix of coated rice is prepared by spraying or roller mixing followed by air-drying. When the dried grain is diluted 1:200 with uncoated rice, 6 mg elemental iron/100 g would be

consumed after accounting for losses during washing and cooking. This exceptionally high level of fortification would provide roughly an additional 18 mg iron/day in adult consuming 300 g rice daily or as much as two-third of the total dietary iron intake daily. Because the maximum level of iron fortification in any country at the present time is 40% of total dietary iron intake (Sweden), it would be prudent to reduce the planned level of rice fortification to approximately 2 mg iron/100 g rice.Needless to say, the impact of introducing this fortified food will have to monitored in the iron-replete segments of the population (serum ferritin) as well as the effect on IDA prevalence.

The are several important technical questions that need to be addressed as this technology is expanded from a laboratory scale to a production level. One important question is the bioavailability of the added ferrous sulfate. In prior studies with the surface-coating method for fortification of rice with iron and other nutrients, the coat has not fully dissolved in the gastrointestinal tract resulting in markedly reduced absorption levels. Radioisotopic iron absorption studies in humans were performed several years ago in the Philippines using the proposed technology but the isotopic tag was added to the food rather than the ferrous sulfate used as the fortificant. Curiously, iron absorption increased as the level of fortification iron was increased rather than declined as usually occurs when the iron content of a meal is increased. These prior studies would not detect impaired release of the added ferrous sulfate. Additional radioisotopic studies in humans are highly desirable before embarking on a large scale fortification program with rice. Another obvious concern is the loss of added iron during washing and cooking of the rice when the premix is prepared in a large production facility.Acceptance by the consumer of rice that initially contains a few (fortified) grains that are whiter and later darker than the uncoated rice grains, is another possible reason for rejection of the product by consumers.

It is planned to initially fortify rice provided by the National Food Authority (NFA), a low cost commodity which supplies less than 10% of rice consumed in the country.This is regarded as an initial step before extending coverage to commercial rice mills.There is little doubt that the most important issue related to iron fortification is the large number of milling facilities in the Philippines, currently estimated at more than 3500. Because of the modest increase in the cost of fortified rice if not subsidized by the government, there may be no incentive for millers to add the premix or for consumers to buy the fortified rice. Thus, the degree of expansion of rice fortification beyond NFA rice is unpredictable. Even the enthusiasm for rice fortification by the NFA is measured, in part because of concern that they will not be able to sell the fortified product.

The other main potential vehicle for iron fortification of a staple food is wheat flour. Whereas the consumption of wheat-based foods such a bread products and noodles was believed in the past to be limited to higher income segments, the intake has increased in recent years to cover a much broader socioeconomic segment. In urban areas such as Metro Manila, it is estimated to constitute about 20% of cereal intake, the remaining 80% being rice. In rural areas, however, the intake of wheat-based products is much lower. The strong appeal of wheat fortification with iron is that the technology is highly developed from decades of experience in North America and Europe. The cost of feeders for fortified flour premixes is believed to be relatively low and these can usually

be installed without any major modification of existing milling equipment. In sharp contrast to rice milling, there are only 12 flour mills in the entire country and all of these are in the Manila area. Thus, the regulation of wheat flour fortification is greatly simplified. Because of the lower per capita consumption of wheat flour than in North America, it would be necessary to fortify to a higher level with iron but this should not present a major problem; pilot studies in which the existing level of iron fortification of flour in the USA is doubled or even tripled has apparently not posed major technical problems. Wheat flour is also a very effective vehicle for vitamin A enrichment and double enrichment with iron and vitamin is well established. Finally, the iodine content of many types of wheat imported to the Philippines is high and could be further enhanced by adding iodine although the feasibility of this is not firmly established. Wheat is therefore a candidate for fortification with all three micronutrients. The major limitation is that the consumption is limited to large urban areas and it would therefore have little impact on micronutrient deficiency at a national level.

The fortification a weaning foods is unquestionably the most important consideration because of the exceptionally high prevalence of IDA in infants between 6 and 18 months of age. While the widespread provision of liquid iron preparations to infants has appeal as an interim measure, the compliance with administration of liquid iron over several months may not be good and supplies of medicinal iron may be limiting as is often the case in pregnant women. The high cost and other major disadvantages of medicinal iron could be largely avoided by providing a low cost weaning food fortified with iron, vitamin A and even iodine. A prototype would be NutriPak in which the iron is added to the cereal component and vitamin A and iodine to the oil packet. Because this product contains isolated soy protein which strongly inhibits iron absorption, sodium iron EDTA which completely by-passes the inhibiting effects of soy would be the preferred form of added iron. Another possible weaning food is Insomix which is apparently prepared at the household level in many communities and sold at low cost. This weaning food could be readily fortified with the same rice premix that is proposed above for fortification of NFA rice. While much further thought and developmental work is needed to develop a satisfactory iron-fortified weaning food, investment in this area is strongly encouraged because of the serious and potentially long-lasting deleterious effects of IDA in this age group.

VII. Recommended Strategies for Integrated Control of Micronutrient Deficiencies

A good deal of effort was made during this visit to discuss and develop methods for closer coordination of micronutrient deficiencies in the Philippines. The efforts made by the Philippines at this point in time are very encouraging and there is no question that at higher levels of government, a strong effort is being made to package micronutrient deficiency as a single nutritional entity. At a practical level, however, the extent to which this coordination can be achieved is less promising. In reference to IDA, there is a continuing resistance to include blood sampling in the surveillance of micronutrient deficiency. For this reason, the national nutrition survey would appear to be a suitable means of monitoring IDA prevalence. There was a good deal of discussion about the pros and cons of doing a comprehensive micronutrient assessment at the time of entry to elementary school and there is no question that if blood sampling is included in this

assessment, hemoglobin determinations and serum ferritin measurements should be included. It is obviously difficult to integrate surveillance programs for micronutrient deficiency unless blood sampling is included in the assessment.

A number of options for double, or even triple, fortification of food vehicles were discussed but there is little or no experience with this approach. Double fortification of salt with iodine and iron has been much discussed by researchers in India but a clear strategy that is adequately documented in the scientific literature has yet to emerge from their many years of investigation. It is suspected that the major obstacle to double fortification is the purity of the salt available within the country. Adverse color reactions occur commonly when there is a high moisture content or a significant degree of impurity of the salt. Many studies have shown that iron added to salt with a high moisture tends to collect at the bottom of the storage container. The addition of iron that does not produce some degree of off-color is also a major obstacle in using salt as a vehicle for iron fortification. Despite these technical obstacles, some assessment of salt as a vehicle for double fortification with iodine and iron should be made because of the emphasis that is currently being placed on this vehicle for the delivery of iodine.

In other sections, the feasibility of using wheat as a vehicle for more than one micronutrient has been discussed. It is certainly feasible to use wheat for the delivery of additional dietary iron and the fortification of wheat with vitamin A is well established. Dr. Dunn has made the point that imported wheat often contains a relatively high content of iodine and it may be feasible to add more iodine in the process of wheat milling. The major limitation of wheat, as discussed elsewhere, is the fact that it does not reach a high proportion of the Filipino population.

The most obvious vehicle for the delivery of all three micronutrients is weaning foods. Double, or event triple, fortification will probably present no major obstacles although there is a good deal of technical work that will be required before a vehicle for infants that contains adequate amounts of all three micronutrients becomes available. As discussed elsewhere, the addition of vitamin A and iodine to the oil and iron to the cereal-based component of a weaning food would appear to be a very practical strategy at this time.

There is little opportunity for combining iron supplementation with either vitamin A or iodine supplements. The latter two micronutrients can be given at relatively infrequent intervals whereas iron must be given on a daily basis over an extended period of time. While there are some plans to focus attention on micronutrient deficiency in the Philippines by providing iron on "micronutrient day" this is not a very attractive approach for iron supplementation. Iron supplements should not be totally excluded from this effort for educational and publicity reasons but should be confined to the provision of iron tablets to pregnant women rather than attempting to supply iron supplements to a wider segment of the population. It is absolutely essential to provide iron supplements in child proof containers to avoid accidental iron poisoning.

VIII. Evaluation of Mission Approach

The following is a few general comments relating to the advantages and disadvantages of joint micronutrient assessment as conducted during this two week period. There is no question that greater attention to micronutrient deficiency was achieved by organizing a joint micronutrient team. It was my impression that the total impact was greater than the sum of separate missions for each micronutrient. At the same time, the advantages of combining efforts for all three micronutrients has certain definite limitations.

The control of IDA presents a special problem in relation to the joint micronutrient approach. On the positive side, it will receive greater attention by integrating it with other micronutrients than it has in the past by considering it as an isolated problem. The overall effort directed to the control of IDA is likely to be greatly expanded under a joint micronutrient program. At the same time, prior efforts to develop programs to control IDA are seriously lacking as compared to the efforts invested in vitamin A and iodine deficiency. This inequity was clearly evident during this visit. There is a very active and energetic program for the control of vitamin A deficiency under the direction of HKI. Similarly, UNICEF has been extremely active in efforts to control iodine deficiency. There are no national or international agencies that have been concerned with the control of IDA, however. As a result, there were few, if any, contact points for nutritional anemia during this visit. Overall, I believe the emphasis in future coordinated visits of this type should be to allow more time for separate and independent assessment of each micronutrient problem during the main body of the visit. It is helpful to discuss the commonalities in micronutrient deficiency with government and nutrition officials at the outset of the visit and to summarize these in a coordinated fashion when the assessment is complete. Because the details of the assessment and interventions for micronutrients differ substantially at the practical level, more time should be allotted for independent evaluation of each micronutrient during the assessment.

APPENDIX B-4b

JOINT MICRONUTRIENT MISSION (JMM)
MANILA, PHILIPPINES
JUNE 13-26, 1993

Site Visit Team

Dr. Abraham Horwitz (Team chairman)
Dr. John Dunn (Iodine)
Dr. Keith West (Vitamin A)
Dr. James Cook (Iron)
Lynda Keiss

June 14, 1993 (Monday)

This first day of the JMM team began with a meeting at the Department of Health (DOH) with Dr. Juan Flavier, Secretary of Health. Dr. Flavier is a very outgoing, flamboyant man who generates a remarkable degree of energy and enthusiasm in regard to the evolving micronutrient program in the Philippines. Much of his motivation comes from a training meeting by PAMM in Atlanta earlier this year at which time he was briefed about the liabilities of micronutrient deficiency and apparently decided to place emphasis on this area of nutrition in the months to come. Some of the innovative strategies which will be detailed further in this report include the development of FVR which stands for fortified vitamin rice and are also the initials of President Ramos and, perhaps, because of this, has stimulated the President's interest and commitment to micronutrient malnutrition. A second innovative strategy fostered by Secretary Flavier is salt fortified with iodine referred to as Fidel salt, the president's first name. Much of the excitement and publicity in regard to micronutrient malnutrition was generated by a PAMM conference in the Philippines concluded on June 12, 1993 that President Ramos attended. He stated the objective of eliminating vitamin A and iodine deficiency by the year 1995 and, in fact, issued a mandate to all health ministries to give him a full update on the progress of the micronutrient program by the end of 1993. A video tape was seen later in the day including President Ramos' speech in which he mentioned vitamin A and iodine deficiency as being key goals of their nutrition program in the Philippines in the years to come. Secretary Flavier also outlined the plans to fortify rice with iron and a sample of this fortified product was seen later in the day. This will be discussed in further detail later in this report.

Much work has already been done in planning a micronutrient program. A national micronutrient action team (NMAT) has been formed that will be headed by Dr. Reodica,

Assistant Secretary for Special Concerns. From our session with Secretary Flavier, it is clear that no advocacy is needed for micronutrient interventions in the Philippines and, in fact, this program has been assigned a high priority by the government. Flavier indicated that he wants two types of reports. First, the written published report that he suspects will praise the effort in the Philippines for micronutrient malnutrition. However, he wants a second very frank report give directly to him, outlining the strengths and weaknesses of their effort. He is a very astute politician, it would appear, who has many acronyms for his approach to politics and health. One of these is MEDIC that stands for Media-friendly, Exciting, Doable Innovative, and Current. He also outlined some of the handicaps that the Philippines faces at the present time. In addition to a severe economic crisis, there is a current power crisis resulting in city-wide brown-outs for several hours daily. The Philippines had the world record for catastrophes in 1992 (over 700) including earthquakes, two volcanic eruptions, many floods, 21 typhoons, several rebellions, etc. Bangladesh was the country with the second highest record for catastrophes, numbering only 300 as compared to the Philippines. Thus, he appeals to international donor agencies for help in all areas of health but, in particular, the micronutrient initiative. Incidently, immunization has been very successful in the Philippines and this has also been a focus of the DOH. He made a commitment to the JMM to take the recommendations very seriously when we have completed our meetings.

The next session was held with the NMAT and was chaired by Dr Reodica. She mentioned that the Office of Special Concerns is concerned with several areas including family planning, AIDS, women's health, and nutrition, of which the micronutrient program has been given a high priority. A large number of individuals attended this conference, the purpose of which was to outline the schedule for the JMM in the days to come and discuss areas that may be site-visited. Dr. Solon, Director of the Nutrition Center for the Philippines (NCP) spoke at length and, at several points during this conference, indicated his commitment and involvement in the micronutrient initiative. This somewhat rambling discussion involved a description of various government agencies involved in the micronutrient initiative. A number of other agencies including DOH, Department of Education, Culture, and Sports (DECS), the Department of Trade and Industry (DTI), and Food and Nutrition Research Institute (FNRI) were mentioned.

Considerable discussion was made of the new commitment of the Philippine government to decentralization which is the transfer of government services to the provincial, municipal, and

barangay level. This process, also referred to as devolution, was passed into law approximately 18 months ago. The full impact of this process is still uncertain but it involves the transfer of money and manpower from the government level to the regional level. Under this new initiative, the number of workers in the DOH has been reduced from 7600 to 4600, creating a major transfer of manpower out of the central government offices. The role of DOH and other govemment agencies, in terms of the micronutrient nutrition, has been made less apparent but it is clear that the responsibility for this program is going to be transferred to the local level and become primarily the mayors. They, in turn, are going to request DOH to provide them specific guidelines on how to reduce the prevalence of micronutrient malnutrition. Dr. Solon is very clear in the ways that this transfer of power can be implemented with effectiveness, and I am certain that we will hear of these approaches later in our visit. Since he was mayor of Cebu at one time, he is very aware of how nutrition guidelines should be implemented at the lowest level of govemment. Some discussion was also made of the term "joint" which has been interpreted variously by the Filipinos as relating to the joint venture between the JMM and the NMAT team, and a joint venture in combining efforts for iron, vitamin A, and iodine.

Later in the morning, a visit was made to the AID mission to discuss their interest and perspective in relation to the micronutrient program. We met with Dr. V... who, for several years, has been Director of Health, Population, and Nutrition programs in the Philippines. He commented, at length, about the impact of devolution and what this process means in terms of micronutrient malnutrition. He indicated that the major question is how the program will develop under the new government organization for health initiatives. He mentioned that micronutrient, and indeed all, nutrition programs at USAID in the Philippines have been "focussed out", that is eliminated, about four years ago as the result of an effort to reduce the number of activities in the mission. He stated that he is a strong supporter of the micronutrient concept but is unwilling or unable to commit any resources to this effort. They have been aware of the OMNI program but have been unclear about the role of the Philippines mission in OMNI. Another interesting problem is that they cannot receive further funds from central AID because a cap has been put on the total amount of funds for their activities on an annual basis ($42 million). However, there are some ways to utilize funds that might derive from the OMNI project. Overall, it would appear that the USAID does not plan to obstruct any efforts in the micronutrient initiative but are not going to commit funds or manpower at the present time.

During the afternoon, we met with Rolf Clemm at the Helen Keller Institute (HKI), an institute that has played a major role in developing interventions for vitamin A deficiency since 1986. A three hour session was held in which the HKI program in the Philippines was described, emphasizing their efforts during the past year to broaden their program to include micronutrient malnutrition. They provided several educational materials that describe their current efforts for vitamin A as well as iodine and iron. The teaching section on iron deficiency emphasizes the history and physical examination as a means to detect iron deficiency anemia. There is a strong opposition to obtaining blood samples to assess anemia prevalence. He would like to see emphasis placed on large dose intermittent supplementation for vitamin A and perhaps iodine, but acknowledges that iron does not fit into this intervention strategy very effectively.

Their intervention approaches for iron consist of the provision of iron tablets to pregnant women (60 mg iron bid) and iron drops for infants <1 years of age. Apparently, the iron supplementation by iron drops is just beginning in certain regions and adherence to this program is not known at this time. The other major intervention is dietary modification and a number of food charts are being distributed at the local level to emphasize the importance of increasing the intake of green leafy vegetables and whole grain cereals. These food-based strategies appears more designed for combatting vitamin A deficiency than they do for IDA and certain of the recommendations such as whole grain cereals are ill-advised. On the other hand, the emphasis on eating fish and adding it to rice in infancy as a weaning food has been appropriately emphasized.

Some discussion was held about breast feeding practices. Although a large percentage of the women breast feed, they seldom continue breast feeding for more than two or three months. The major reason is the feeling that the infant is not getting adequate nutrition by breast feeding alone. As breast feeding is curtailed, either sugar water or rice water is introduced as an alternative which, obviously will have little impact on iron deficiency. Clemm emphasized his uncertainty about programs to either assess or combat nutritional anemia.

Some discussion was held about the utility of using children at the entry to school as the optimal time to assess micronutrient malnutrition (age 7). Physical examination would reveal the presence of goiter and the ophthalmologic signs of vitamin A deficiency (corneal scars, xerophthalmia, Bitot spots). They are reluctant to consider blood sampling to include anemia

in this assessment but the blood spot technology for TSH and retinol could conceivably be considered.

In summary, efforts by HKI have been expanded to include all three micronutrients. Rolf presented the group with a large list of questions but because of the limitations in time, few of these could be discussed at this session.

June 15, 1993 (Tuesday)

The morning meeting was held at the DOH and was chaired by Undersecretary Tan. In his opening remarks to this combined session of the micronutrient consultants and their counterparts, he emphasized that there was little knowledge about how to proceed with IDA and emphasized the need for help in this area. He stated that the Kiwanis, through UNICEF, has provided support for IDD, that HKI support for vitamin A but no donor agency has taken up support for IDA. He mentioned the need for assistance and consultancy in IDA.

Dr. Florentino then proceeded to describe the results of the National Nutrition Council (NNC) meeting held on the previous day in which the Philippine Plan of Action for Nutrition (PPAN) was outlined; a document describing this program was supplied to the conference participants. The goal of PPAN, which spans the time from 1993 to 1998 is focussed on two aspects: increasing household food security and eliminating micronutrient malnutrition. The goals include a plan to reduce the prevalence of PEM by 50%, to virtually eliminate vitamin A deficiency (VAD) and IDD and to reduce, by a modest 10%, the prevalence of IDA in infants and women in the childbearing age. The definition of elimination in regard to VAD and IDD is based on clinical signs, that is the absence of eye signs and goiter. Thus, PPAN, as outlined in an accompanying diagram, is composed of the following five programs: 1. Micronutrient supplementation and fortification; 2. Home and community food production; 3. Nutrition education; 4. Credit assistance for livelihood; and 5. Food entitlement. The **enabling mechanisms** likewise consist of the following five elements: 1. Manpower development; 2. Nutrition advocacy for legislation, sectoral policy formation; 3. Overall planning, management coordination, and surveillance; 4. Research; and 5. Resource generation.

The proposed iron plan includes the provision of iron supplements to pregnant women and iron drops and/or syrup to infants. The efforts for VAD and IDD intervention are outlined

in the strategy. In regard to food fortification, the fortification of salt with iodine, margarine with vitamin A, and rice with iron (FVR-iron fortified rice) are the targets for the initial strategy.

Dr. Florentino briefly discussed the plan to fortify rice with iron. Rice will be prepared in a premix by coating with ferrous sulfate and then mixing the coated grain 1:200 with whole rice. Initially, this rice will be distributed through the National Food Authority (NFA) that represents approximately 10% of the rice production in the country, targeted primarily to the urban poor. Although Dr. Florentino is not overly enthusiastic about this, he indicates that it is at least a start. Because of the lower cost of this subsidized rice, it is believed that it will reach the lower economic groups. Other components of the program that may impact on IDA include an effort to increase livestock production at the local level as well as increase fish intake.

The discussion of this program was followed by a detailed review of the monetary aspects of this ambitious program (PPLAN). The program will be complemented with features such as nutrition education using the teacher/child/parent approach (TCP) in which the teacher provides the child with nutrition information which is then transferred to the parent in the home. This will also be accompanied by a growth monitoring promotion (GMP) which seeks to weigh all newborns at monthly intervals for the first year followed by every three months until the age of 5. One aspect of this program in regard to iron that is not especially well conceived is the plan to provide iron supplements to children identified as having PEM. Although many of these children are anemic, it is unlikely that the cause is iron deficiency. There was some discussion of the total budget for the five year program. The total projected costs (details in monographs) total 14.2 billion pesos or approximately $500 million US. In terms of the breakdown (pesos), a large portion of this (approximately 8 billion) is for food entitlement, roughly 2 billion will be used for micronutrient intervention with estimates of 119 million for vitamin A, 947 million for iron, and 1 billion for iodine. It was emphasized that these figures are very uncertain and there has been a request by Secretary Flavier for more detailed breakdown of the projected costs together with identification of potential sources of funding.

This meeting also included considerable discussion about the potential for double fortification of either rice (vitamin A + iron) or salt (iodine + iron). Although there was limited enthusiasm by Dr. Solon and Dr. Florentino for double fortification, they were not aware that this technology was available and felt the need to press on with separate fortification of rice with iron and salt with iodine. They pointed out that their efforts to doubly fortify rice have been

unsuccessful because of the rapid deterioration of vitamin A in the presence of iron. Apparently, the Bon Dente rice fortification project with both iron and vitamin A has also proved unsuccessful because of the deterioration of vitamin A and darkened color resulting when iron is added. Although this fortification strategy was initially targeted as a program for FVR, it became apparent that the royalties requested by Bon Dente were exorbitant. Their estimates were on the order of 2.50 pesos/kg for the first year, 1.75/kg for the second year, and 0.75/kg for the third year (licensing fees). In order to retain the concept of FVR, which was torpedoed by these high prices, it was decided to use the term FVR for rice fortified with any nutrient and to designate the nature of the nutrient fortified. Thus, the new fortified rice is being called "FVR-iron fortified".

The afternoon session was held at the Department of Agriculture (DA) at which time Undersecretary Dr. Fernandez outlined his perspective of the NNC program (PPAN). This session was not too helpful but some preliminary discussions were held in regard to the use of wheat as a vehicle for fortification. Apparently, the amount of wheat imported has increased sharply in recent years but Dr. Fernandez thinks that a large proportion of this is used for animal feed. He did not know whether the wheat is imported as flour or milled within the country. Because of increasing wheat consumption, it is presently estimated that roughly 20% of cereal intake is derived from wheat. He also believed that there was a roughly even distribution of intake by all segments of society. Wheat clearly deserves further evaluation as a vehicle.

Our meeting at the NFA, the final meeting of the day, was rather disappointing in regard to rice fortification with iron. The Director of this agency (Mr. David) believes that the small increment in price that will result if NFA attempts to fortify their rice with iron will be unacceptable to the target population. The current price of rice is 9.75 pesos/kg of rice which will go to 10.50 pesos, retail. The fortification process would add 25 centavos to this, raising the total price to 10.75 pesos/bag and even this small increment would probably not be acceptable to the consumers. However, none of the arguments against rice fortification by this agency were especially compelling. Mr. David emphasized the potential advantages of using sugar, salt, or wheat as a vehicle because of the potential embarrassment to his agency if a large quantity of rice is fortified and then cannot be sold on the local market. There have been virtually no studies of stability of the fortified product nor any studies known to him about the bioavailability of the fortified rice.

The NFA mills approximately 500,000 metric tons annually which would translate to a cost, if fortified, of approximately 12.5 million pesos annually. This represents only 10% of the 5 year budget allotted for iron so that it does not seem unreasonable to subsidize the fortification process. However, consumption of this fortified product by the population would not exceed 10%. An additional 40% could be reached if they could require all commercial rice millers to fortify their product. However, at least 30% of the population living in rural areas would not be reached by this strategy because they grow their own rice and mill it in the household rather than having it centrally milled. Whether or not the NFA could stimulate the use of the fortified product by commercial producers is unclear but regulation of the commercial fortification process would be very difficult. It is estimated that there are roughly 38,000 rice millers in the country. There are approximately 100 national millers that account for the bulk of rice fortification.

Therefore the major obstacles to rice fortification with iron as viewed by Mr. David a.): 1. A small, but meaningful, increase in cost that will reduce the sales of the fortified product; 2. Limited distribution to the population at need (10% if all NFA rice is fortified); 3. Uncertainty about the storage, quality, and acceptability of the fortified rice; 4. Difficulty in extending the fortification program to cover a larger percentage; and 5. Ceiling coverage of no more than 50-60% of the population if all commercial millers agreed to the fortification process.

June 16, 1993 (Wednesday)

The morning meeting began at the NFA facility with representatives from the Flour Millers Association and the major salt producer in the Philippines (Pacific Farms Inc.). The meeting was also attended by Mr. Vincente Lim, President of the Philippine Chamber of Food Manufacturers Inc. Mr. Lim was quite knowledgeable about possible food vehicles and had several interesting suggestions about nutrient delivery.

Much of the initial discussion focussed on salt and its suitability for iodinization. This section of the discussion will be summarized elsewhere and was only of peripheral interest in regard to iron fortification. It appears that the purity of the salt is below the level that would be suitable for double fortification with iron and iodine although, because the technology for double fortification is not adequately defined, salt cannot be excluded with complete confidence as a possible vehicle for iron. The discussion was focussed on the mechanics of iodination of salt which is planned to be undertaken within the next few weeks, at least for the 25% of the salt

produced by PFI. There are a large number of logistic problems in undertaking the iodinization of salt but the CEO of PFI seemed confident that these problems could be solved and was generally enthusiastic about the iodinization of salt.

During the latter part of the morning, discussion turned to the possibility of using wheat as a vehicle for enrichment of either iron or vitamin A. The representative of the Philippine Association of Flour Millers (PAFM) was not very knowledgeable about the technical aspects of fortification and, in fact, was ignorant about what nutrients are currently added to flour at the mill. A very strong feature in the support of wheat fortification is the fact that there are only 12 flour mills in the Philippines, all of them concentrated in the Manila area. Eight are under the jurisdiction of one miller's association and the remaining 4 are separate. All wheat is imported as such and milled within the country. It is estimated that 1.6 million tons are imported yearly and, assuming an extraction of approximately 80%, this should generate roughly 1.2 million tons of flour annually. The director of PAFM did not know what percentage of imported wheat goes to feed, only that the millers would know that value. Wheat has increased its appeal as a nutrient vehicle during the past 10 years. In 1980 it was estimated that only approximately 10% of cereal consumption was in the form of wheat flour. This figure has more than doubled in the interim and is now believed to represent about 20% of cereal consumption (as compared to 6 million tons of rice consumed in the country annually, 1.2 million tons of flour represents about 20%). Most of this wheat is distributed in Manila, and contrary to a decade ago when most of this wheat was consumed at the upper level of the economic spectrum, it is currently believed to be consumed by all socioeconomic segments. It is, however, consumed largely in urban areas with very little rural consumption. A large proportion of the wheat flour is sold to fast food vendors and there is a large and growing consumption as noodles. Packaged noodles are not directed to the lower socioeconomic segments of the population whereas biscuits and low quality baking products are consumed by low income families. Unfortunately, there is probably little, if any, intake of wheat products by preschoolers. Nevertheless, given the limited technical problems in fortifying wheat and the possibility that more than twice the amount of iron could be added to offset the lower consumption, this vehicle remains a real possibility for iron fortification in the future.

The afternoon was spent at FNRI reviewing past studies but focussing, primarily, on the use of FVR fortified with iron. There has been enormous interest in this product. Even this week, President Ramos ordered 500 small packets to be sent to him for distribution by the end of the week. The process of fortifying rice is identical to that described by Florentino in the early 1980's, the details of which are outlined in their earlier studies of iron. Rice is coated in a mixture with ethyl cellulose and ferrous sulfate. Following coating by tumbler, the preparation is air dried. It imparts a slightly yellow color to the coat but when mixed with regular rice, actually has whiter and more opaque appearance than the normal rice grain. It is planned to add sufficient iron to this product to bring the iron concentration to 6 mg iron/100 g of rice when mixed 1:200 with unfortified rice. This surprisingly high concentration of iron is based on a consumption of approximately 100 g rice/day for a school aged child giving about 6 mg of iron. However, this would provide an average of 18 mg/day to adults using an average per capita figure of 300 g of rice/person/day. This would give a figure of roughly 70% of dietary iron derived from fortification, a value that is inappropriately high.

Very little consideration has been given to the manner in which this imminent fortification process will be expanded. In fact, there has been only a few small batches of the pre-mixed, coated rice prepared to date. If 6 million tons of rice are consumed in the country, this would require 30,000 tons of pre-mix assuming that all the rice was fortified with iron. In fact, the real question is what proportion of commercially sold rice could be fortified. The initial objective would be to fortify the rice provided by NFA which represents 8-10% all the rice consumption in the country. Florentino did not agree that there was a significant cost problem in fortifying the rice. He believed that because the NFA rice is much cheaper than commercial rice the cost of fortification could be absorbed. Surprisingly, there are virtually no facilities to prepare the pre-mix. It is hoped, by Florentino, to attract an entrepreneur who may be given space in the new FNRI building and equipment until he or she can expand the process to commercial level.

Apart from the major focus of our afternoon visit, namely FDR, some discussion was also held about other strategies that are being proposed for iron. An extended discussion was held about iron supplementation for pregnancy. It is currently estimated that no more than 40% of pregnant women can attend maternal health clinics and therefore can be reached for iron supplementation. Of this 40%, it is uncertain what percent actually is supplied iron in maternal health clinics but is probably less than half. If compliance is 50% of this figure, then only 10%

of pregnant women in the Philippines are taking iron supplements. There is a general agreement that this is not satisfactory coverage but there are few other options for providing iron in pregnancy.

A discussion was also held about supplying medicinal iron to infants. This is presently not a practice in the country although liquid preparations of iron are available commercially. It would seem that the cost will limit the extension of the program to the entire country. Moreover, it is likely that major compliance problems will be encountered when attempting to supply liquid iron to all infants under the age of 12 months. A more attractive possibility would be to provide a heavily iron-fortified rice mixture that would provide 6 g or more/100 g of rice. This could be labelled as "For Infants Only" and, if the mother is willing to prepare it separately from the rice being prepared for the remainder of the family, it would provide a more effective and less expensive coverage of iron for infant children. There seemed to be some enthusiasm for this suggestion by Dr. Florentino.

In summary, there are many uncertainties related to FVR and my personal feeling is that it will encounter many problems in the early stages of implementation. It is not known whether problems will arise when mixed with other foods. Off-color changes may occur during storage, even if preliminary organoleptic studies conducted in the early 1980's did not identify this as a problem at room temperature. There is a surprising loss of iron in fortified rice with storage, presumably because the iron coat is dissolved in the increased humidity. The rice is traditionally washed at least twice before boiling but iron loss from the coated grains is said to be minimal. The iron in the pre-mix is completely dissolved when the rice is boiled and another potential loss can occur from the water used to prepare the rice, although it is believed that almost all of the water is reabsorbed into the rice grain. There are no reliable, nor recent, figures on the percentage recovery of added iron in the food served at the table.

June 17, 1993 (Thursday)

This day was devoted to a visit of an urban area in metro-Manila referred to as the Malabon region. After a briefing at the NCR, we traveled to this region in northern Manila where we spent considerable time with the Director of Health Services, Dr. Soriano. Much discussion was held about the major health problems in the Malabon area and the effect of devolution which has transferred increasing funds and responsibility to the local mayor. After a brief

meeting with Mayor Oreta, and a luncheon, we visited several areas in the Malabon region including a public market where food items relevant to fortification were examined such as the availability of iodized salt, food items rich in vitamin A content, iron fortified foods (none were seen!), etc. We then visited a regional health center (Panghulo Health Center), a small hospital built approximately 3 years ago that is staffed by one physician (a radiologist). We visited the laboratory where hematocrit determinations were being performed and visited a ward where a patient was receiving a blood transfusion for a "blood disorder". There was virtually no laboratory information or knowledge about the nature of his anemia problem. We then visited a large public school where many families were camping because of a fire that destroyed several homes in the region. Temporary housing was provided in the school and the Red Cross was distributing food supplies to this indigent population. Overall, the amount of information gained in our visit to Malabon was rather limited in relationship to the JMM.

June 18, 1993 (Friday)

On this morning we travelled north to the Cordillera Administrative Region (CAR). In the latter part of this five hour drive north of Manila, we climbed to an elevation of 6000 feet. We were lodged in the John Hay Camp, a military R&R facility that was transferred to the Filipinos when the military bases were closed about two years ago.

Shortly after arriving in Baguio City where the major hospital and health facilities were located, we travelled to Tuba, a municipality of Baguio City, that was selected for more detailed information dealing primarily with salt fortification. We visited with the town council in this region who made a strong pitch for acquiring one of the salt iodinization facilitates that will be placed in the region in an attempt to iodinize all salt transported into the CAR.

Some discussion was held during this meeting about iron problems. Anemia is not considered a major problem in this region, presumably because the higher elevation increases the level of hemoglobin above the usual cut-off level for anemia. Apparently, no revision of anemia criteria have been made on the basis of higher altitude. In discussing the potential of food vehicles for iron, it was learned that about 20-30% of rice used locally is red rice and is home milled. The remaining two-thirds of the rice entering the area comes from the lowlands. It was felt that FNA rice is not used extensively in the region despite lower cost because the quality is considered low. It is conceivable that some FNA rice is used by the very low income

groups in the region. Overall, the municipal leaders believe that FVR would be accepted, even with the 25 centavo price difference, if advertised as being nutritionally beneficial. Some discussion of Insomix was held which is comprised of rice, mung beans, and oil, that are cooked separately in a few kitchens, repackaged, and sold as weaning foods. The cost of these food packages is very low (5 pesos). Insomix is widely used within the region but not in other areas of the country. Wheat and flour consumption in the region is minimal.

Following the meeting of the leaders in the Tuba region, we travelled to a health clinic in Camp 6, a small barangay a few miles outside of Baguio City. Several women had been brought to the health clinic; all had goiters ranging in size from very large to barely detectable. I discussed the anemia problem with the lady in charge of the health clinic who had been previously trained in laboratory measurements. She stated that she did hemoglobin measurements regularly in pregnant women although the hemoglobinometer was currently in her home where she had been checking the hemoglobin levels of her children. When asked to see the available iron preparations, a small bottle with a few tablets was offered for inspection but she stated that the availability of iron tablets had been sharply curtailed in recent months as part of the devolution process. She mentioned that there was a strong preference for "chocolate" coated tablets that are purchased locally rather than provided by the health service. It was said that these coated tablets had less side effects and I suspect that the reason is the low availability of the iron they contain. Much of the discussion during this visit related to the acceptance of iodinized salt and the willingness of these women to pay a slightly higher cost (actually 50% increase) for this product. The following day involved another visit to a health clinic with focus on goiter, a major problem in this mountainous region. Dr. Dunn also visited with a Dr. Chang, Director of the Baguio Filipino/Chinese Hospital, who has been very committed to addressing nutritional problems in the region. In the evening, the group had dinner with the governor, Jaime Panganiban, who seemed to have a rather peripheral interest in the salt iodinization problem. He is governor over a large region in the CAR and was a generally pleasant man who was quite open about the problems in the region. Early on the morning of June 20, 1993, the group returned to Manila for an evening meeting with the JMM participants.

June 21, 1993 (Monday)

This day began with a visit to the PAMM alumni at the BRL facility (Bureau of Research Laboratories) which, I believe, is under the DOH. This large regional laboratory performs many of the more detailed laboratory studies for metro Manila. After meeting with the PAMM alumni, the laboratory facility devoted to studies of iodine deficiency was visited. Most of these individuals are graduates of the Atlanta program and, accordingly, their emphasis was on iodine measurements. They had established a laboratory for the measurement of urinary iodine but the number of measurements seemed rather limited. A relatively new plate reader had been installed for TSH measurements but, once again, the number of determinations of TSH appeared to be limited. These workers purchase their TSH kits commercially and, as I remember it, the cost per individual TSH measurement was relatively low, about $0.50/assay.

The afternoon began with a meeting with a health economist, a Mr. Gamboa, who was previously the Undersecretary for Finance in the country. He is now employed by a company devoted to concerns about health economics and I found this meeting uninformative.

We then traveled to the HKI facility where a group meeting was held with a large number of NGO (non-government organizations) representatives. This meeting was organized and orchestrated by Rolf Clemm, the country director for HKI in the Philippines. It was pointed out that more than 60,000 NGO's operate within the Philippines but many of these organizations are transient and operate only at the barangay level. A number of organizations were represented at this meeting such as Catholic Relief Fund, Save the Children, etc. In general, there seemed to be a great deal of enthusiasm, but a lack of knowledge, about the micronutrient initiative among the NGO's with the exception of HKI under Rolf Clemm. Some discussion was held about the benefit of organizing NGO's more closely to address the micronutrient problem and whereas the members attending this conference were generally enthusiastic, specific details of how this organization might occur were lacking. There is, nevertheless, a strong capability in the Philippines of implementing programs with the assistance of the NGO's. The NGO representatives are strongly supported by the government in contrast to the situation in many developing countries.

June 22, 1993 (Tuesday)

We traveled to the Nutrition Center of the Philippines (NCP) where our meetings were orchestrated by Dr. Florentino Solon. The NCP has survived the revolution and is again on the upswing despite near collapse of the institution, after fall of the Marcos regime. For example, a large number of food processing plants that were placed in the country to produce NutriPak and various other packaged foods had been closed because of lack of governmental support. Dr. Solon presented his typically strong media-oriented approach to nutrition involving video, publications, etc. The NCP is considered a private sector institution and appears to be well funded from commercial firms that are interested in specific nutrition problems. The NCP was involved in the failed trial of MSG fortification with vitamin A. They are currently interested in looking at the potential beneficial effect of fortifying a chocolate beverage with iron that will be directed to school-aged children. Dr. Solon was very interested in the use of erythrocyte protoporphyrin measurements and has received advice from Tomas Walter in Chile.

A variety of agencies displayed their wares during this session at the NCP. The meeting began with representatives from the DECS with their emphasis on nutrition education. Dr. Solon described his success in implementing a strong nutrition curriculum in the elementary school and a variety of the manuals and information developed in connection with this program were presented. Dr. Solon is a strong advocate of the so-call TCP approach to nutrition eduction in which the Teacher transfers information to the Child who, in turn, takes the information home and presents it to the Parent. Most of the early afternoon session was devoted to a review of the activities of the NCP under Dr. Solon but much of this presentation was historical in nature with regrets about the decline in the NCP in the early 1980's.

During our discussion with Dr. Solon, I raised the question of the rather limited objective in relation to the IDA control program. Their framework monograph describes a target reduction of 5 percentage points in the prevalence of anemia in the entire population and a somewhat more optimistic reduction of 10% in the prevalence of IDA among infants, pregnant and lactating women, preschoolers, and school children. Dr. Solon said that these limited objectives were based, primarily, on their rather disappointing experience in reducing the prevalence of anemia in pregnant women using combined iron and food supplementation programs. He agreed that these limited objectives should, perhaps, be revised upwards with a plan to control a larger proportion of IDA in the country. No specifics were given.

The group then travelled to the Food Development Center (FDC) housed in a beautiful facility constructed by the Japanese and opened in the 1989. The FDC is now part of the NFA but has not been involved with rice or corn. They apparently are funded by industries that wish to facilitate the marketing of various food products. This is an extremely well-equipped facility with every conceivable instrument for the development of products in the food industry. They plan to become involved in upscaling FVR but have no specific plans, as yet, and it is not clear that Dr. Florentino will transfer the responsibility for expanding the preparation of the rice premix to the FDC. However, the facilities and capability of this institute far exceed those of FNRI and it would be unfortunate not to have this institute become involved with rice fortification. The FDC can accommodate 140 technical personnel but have an existing staff of only 77 because of the difficulty in locating qualified food technology personnel. With the construction of the FDC in 1989, their space has, nevertheless, grown from 2000 to 8000 feet^2 and it is clear that this institute can play a major role in the development of fortified products for fortification with iodine, iron, and vitamin A.

The group then travelled to the Bureau of Food and Drug (BFAD) equivalent to the USFDA. This facility was also built by the Japanese in 1987 and is devoted to analysis of food and drugs, drugs comprising about two-thirds of their activities. The tasks to which this facility is assigned far outweighs their capability, although their laboratory facilitates appear to be relatively up-to-date. They apparently will assume responsibility for certifying the content of nutrients as the micronutrient initiative expands to involve fortified foods. I raised the question of analysis of pharmaceutical preparations of iron available commercially. They said that they monitor the bioavailability by solubility studies but are not in a position to conduct any *in vitro* measurements.

June 23, 1993 (Wednesday)

This meeting was held at the HKI facility; the purpose was to meet with clinical counterparts (hematologists, ophthalmologists, and thyroid specialists). However, it was a group meeting that was focussed on the organization of governmental efforts for micronutrient intervention with relatively little opportunity to discuss the clinical perspective of micronutrient deficiency. The hematologist representative was Dr. Cavilles, a pediatric hematologist who has recently retired from the University of the Philippines and is employed at a large children's

hospital in Manila. Only a few brief questions were posed in relation to the anemia problem. One interesting facet is that thalassemia is present in a significant proportion of the Philippine population although the exact percentage is unknown. He stated that he looks after 50 families of children with thalassemia which represents about 1% of the population of the population from which his hematological practice is derived. He could not describe the forms of thalassemia other than to say it was a mixture of "β and α". Others believed that thalassemia was not a significant problem within the country but there was no precise information about the true frequency.

Another important comment that emerged was the problem of malaria within the Philippines that is believed to involve a large geographic proportion of the country. The problem of malaria infection is expanding in the Philippines, as it is in other countries in Southeast Asia. No one knew what agency is involved with malarial control programs, but almost certainly, there is another agency in DOH that is concerned with this problem. The extent to which malarial infection is being confused with IDA is unclear and it will be important to get more comprehensive data about malaria prevalence and efforts at its control in the Philippines. Dr. Cavilles presented a clinical perspective about iron deficiency in the country. He believes that parasites are relatively uncommon at the present time and commented on the sharp decline in hookworm infection in recent years. He does, however, routinely practice de-worming procedures in his pediatric population. Someone commented that trichuris infection is an increasing cause of blood loss (rectal infection) because apparently the anti-helminthic preparations are reasonably effective controlling ascaris but do not eradicate trichuris. Overall, however, parasitic infection is not believed to be a major cause of IDA. Dr. Cavilles felt that iron solutions would be given by mothers if an ample supply is provided by the health service. However, he said that it is unusual for mothers to return to get another bottle of liquid iron preparation and, if routine supplementation with medicinal iron is implemented in pre-school children, it would have to be coupled with an energetic educational program to promote its use. This meeting closed with the sense that much more information needs to be obtained about the cause and prevalence of non-iron deficiency anemia in the Philippines.

As an addendum to these various meetings, we were given a correspondence by Romeo David, Administrator of the NFA. A letter was addressed to Secretary Flavier in relation to the rice fortification project. This letter communicated his overall support of the rice fortification

initiative and his commitment to working with any government agency that will promote this mandate. He outlined the issues related to rice fortification and acknowledged that the NFA can play a critical role in creating mass awareness of the FVR iron-fortified rice. He also felt that the NFA could promote the expansion to the private sector by demonstrating feasibility of the project and encouraging the fortification of all commercial rice. He pointed out the importance of a simultaneous program to direct the fortified rice to the target population and indicated that NFA rice is currently distributed on the basis of supply need. Thus, fortification of NFA rice will not necessarily mean consumption by the women and children who require it but every women and child in any area where, for any reason, rice is not available. He also pointed out that the fortification of NFA rice will require a mandate from the DA and/or President Ramos. He pointed out that the technology for rice premix preparation has not been commercialized. He did mention the potential involvement of the Bon Dente Company, a small R&D firm in the Seattle area that is promoting the use of vitamin A fortified rice.

Mr. David emphasized the importance of initially carrying out rice fortification on a pilot scale so that the potential variables can be better controlled. He recommended the formation of a rice fortification task force to establish a program and implement the activities required to introduce rice to the private sector. He indicated that NFA, if mandated by the DA, can organize and lead the task force. He emphasized the need to acquire adequate funds and manpower resources to enable the task force to achieve its objectives by the target date of 1995. He mentioned the critical need for international funding and information about the most appropriate technologies for rice fortification. Finally, he recommended the development of a nutrition education campaign, especially among school children, to promote the use of nutrient-rich fruits and vegetables.