INACG

International
Nutritional Anemia
Consultative
Group

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INTERNATIONAL NUTRITIONAL ANEMIA CONSULTATIVE GROUP (INACG)®

ANNUAL REPORT

1 October 1988 to 30 September 1989

Cooperative Agreement DAN-5115-A-00-7114-00

I. Introduction

A. AID Support of the INACG secretariat

1. The Nutrition Foundation, Inc. was first granted financial support as secretariat for the International Nutritional Anemia Consultative Group (INACG) from the Agency for International Development (AID) in February 1977. Funding continued through a series of grants and extensions through the period 30 September 1987. On 25 September 1987 an agreement was made that allowed the Nutrition Foundation, Inc. to use remaining obligated funds until 31 December 1987 for continuation of INACG projects nearing completion.

2. On 30 September 1987 the Nutrition Foundation, Inc. (a division of the International Life Sciences Institute-Nutrition Foundation) entered into a five year cooperative agreement with AID to serve as the secretariat for the International Vitamin A Consultative Group (IVACG), INACG, and Sharing United States Technology to Aid in the Improvement of Nutrition (SUSTAIN). The total estimated amount of the program is $4,679,138 of which AID committed $2,998,693 and $1,680,448 was to come from non-federal sources.

3. Modification 01 to the above cooperative agreement was made effective on 30 August 1988, but was not available until this fiscal year. This modification provided an incremental increase in the obligated amount by $229,000. The total obligated funds were then increased to $1,410,938. Although not specifically designated, it was understood that this money was primarily intended to support the INACG program. For FY 89, the budget for INACG, not including indirect overhead costs, was $112,527.

The Nutrition Foundation, Inc., is a division of the
International Life Sciences Institute—Nutrition Foundation

B. INACG mission

1. The mission of INACG is to guide international activities aimed at reducing iron deficiency anemia in the world.

C. INACG functions

1. INACG sponsors international meetings and scientific reviews and convenes task forces to analyze issues related to etiology, treatment, and prevention of nutritional anemias. Examination of these issues is important to the establishment of public policy and action programs. INACG provides a forum to foster the interchange of ideas, the presentation of new research findings and survey data, and the discussion of action programs. INACG assists governments in the establishment of national programs to combat nutritional anemia through the development of strategies for the particular region and by furnishing consultants to these programs. All of these functions are carried out in close collaboration with the Office of Nutrition of AID.

D. Administration

1. Those familiar with INACG and its activities experienced a major loss with the death of INACG Chairman, Dr. Edouard DeMaeyer, in December 1988. Since the inception of INACG, Dr. DeMaeyer had provided keen insight and clear direction for the global activities combatting nutritional anemia. For most of the past year, INACG operated without a chairman. In the third quarter, the secretariat, with concurrence of the Office of Nutrition, S&T/N, invited Dr. Alberto Pradilla, Head of the Nutrition Unit at WHO headquarters, Geneva, to serve as chairman. While quite willing to do so, Dr. Pradilla learned that he was restricted from using that title, but could provide the same leadership under the title of Senior Advisor.

2. To further establish the leadership role that INACG plays in the area of nutritional anemia, a decision was reached to establish an INACG Advisory Committee to provide input to the Executive Committee. Five experts with backgrounds in clinical pediatrics, nutritional anthropology, operational programs, commercial iron fortification, and nutritional epidemiology have been invited to serve on the committee. It is anticipated that they will meet with the Executive Committee twice each year to help guide the development of INACG activities, recommend procedures for program implementation, review manuscripts prior to publication, and assist in identifying priorities for

action. Final decisions from them concerning their acceptance of this invitation are expected during the first quarter of FY90. A list of names and affiliations is shown in Appendix 1.

II. Activities

A. Initiation of an INACG Task Force on the relationship of anemia to mental and behavioral development

1. Dr. Ernesto Pollitt, in conjunction with WHO (Dr. Edouard DeMaeyer) developed a program to review current knowledge of the relationship between iron deficiency and mental and behavioral development. The secretariat, together with the INACG Secretary (Dr. Kahn, Office of Nutrition at AID) worked with Dr. Pollitt and Dr. DeMaeyer to plan a meeting sponsored by WHO that was held in Geneva on 10-12 October 1988. This "Conference on Iron Deficiency and Brain Function" served as a convenient opportunity to prepare an INACG monograph on the topic. INACG therefore provided financial support for this meeting in the form of assisting with travel expenses for some of the speakers who were asked to remain afterwards and draft a manuscript on this topic.

2. A task force meeeting was held on 13 October to begin writing a draft document that would summarize the current understanding of the complex relationship between brain function and iron nutriture. Dr. Pollitt chaired this task force which also included Dr. David Levitsky, Dr. Peter Dallman, Dr. Betsy Lozoff, and Dr. Moussa Youdim. The minutes of that initial task force meeting along with the summary of the 11-12 October conference are found in Appendix 2.

3. Chapter drafts were received from four of the task force members. One decided that his studies were not relevant to the thrust of the document, but agreed to edit the initial manuscripts. Following review by the secretariat and Dr. Samuel Kahn, INACG Secretary, it was determined that one of the four manuscripts needed to be rewritten to make it conform more closely to the style of the others. In addition, there was need for additional chapters that more fully described the operational issues raised by the data and suggest ways to utilize the findings to further efforts aimed at controlling anemia. Due to the heavy schedules of the four authors, a meeting in-person to discuss each other's manuscripts was impossible to schedule during this fiscal year. Instead, it has been recommended that the

secretariat obtain the services of another writer to continue the editing process and draft the additional chapters.

B. INACG Workshop on Maternal Nutritional Anemia

1. It is estimated that 1.2 billion people now suffer from anemia. Approximately half of all pregnant women are anemic. Recognizing this the INACG Executive Committee saw the need for a workshop to bring together those who have special expertise in the area of maternal nutritional anemia. The workshop's purpose was to review and discuss available information regarding implementation of prevention programs for the nutritional anemia of pregnancy.

2. During the first quarter of this fiscal year, plans were finalized for the workshop. This included inviting presenters, performing extensive literature searches to determine the active investigators in the field, selecting a date and place for the meeting to be held, and setting up the procedures for carrying out the meeting. The World Health Organization (WHO) agreed to host the meeting in Geneva, Switzerland, on 14-16 November 1988. Invitations were sent to 29 individuals representing organizations that were actively studying this field. The program, minutes, and list of participants for the workshop are shown in Appendix 3 of this annual report.

3. Following the workshop, a special task force meeting was held on 17-18 November to prepare a draft document that would state clearly the risks to the mother and unborn child when anemia is present during pregnancy. The four-member task force completed their writing within the 1 1/2 days allotted. Due to their commitment to rapidly review subsequent drafts, this document was able to be edited and submitted in final form to the printer before the end of the fiscal year. Members of the task force are listed in Appendix 3.

C. Administer a research project to determine the effectiveness of iron fortified infant cereal in prevention of iron deficiency anemia

1. A research study was begun during the previous year to determine whether infant cereal as it is presently fortified with electrolytic iron powder in North America is effective in preventing iron deficiency anemia. The proposal was submitted by Dr. Tomas Walter (Instituto De Nutricion Y Tecnologia De Los Alimentos) (INTA) located at the Universidad De Chile. The other principal investigator is Dr. Peter Dallman, University of California, San Francisco.

2. INTA has an excellent record of field trials related to iron nutrition of infants and is a widely recognized leader in this area. There is substantial evidence that iron fortified infant formula is a reliable means of preventing iron deficiency anemia. The evidence that infant cereal plays a similar role is presently not as strong. The study is directly measuring the bioavailability of iron added to infant cereals in order to determine the form of iron that is most bioavailable for infants.

3. The INACG secretariat was selected to serve as administrator for this study, with funding obtained exclusively from private sources. The secretariat was directed to monitor progress on the study protocol and to provide administrative and financial support for the investigators. This included working with baby food manufacturers to solicit their support in producing specially formulated products with and without iron. Considerable discussion occurred regarding difficulty in obtaining the proper infant formula to be used in the early stage prior to the introduction of weaning foods. Arrangements were finally made to purchase the unmarked formula from a local supplier. During the second quarter, a major order for laboratory supplies was organized, coordinated and shipped to Chile through a broker in Miami. Also, an annual financial installment was forwarded to the principal investigator.

4. The total level of funding for this project is $205,000 payable over three years. Subjects were first enrolled into this study in September 1988 and enrollment would continue over a two year period. The follow-up sampling and monitoring of subjects will continue during the following year. The first report from the Principal Investigator, Dr. Tomas Walter, on progress made in the study is included as Appendix 4.

D. Request for Background Paper for ACC/SCN

1. The chairman of ACC/SCN requested, through the INACG secretary, that INACG consider preparing a document that could be used as a working paper for developing strategies for controlling iron deficiency anemia. A comprehensive literature search was undertaken, with over 1130 citations to review. Further definition about the scope, length, and content of this document is being sought at this time.

E. Information Requests

1. During this fiscal year, the secretariat responded to 87 individual requests for publications and other information regarding nutritional anemia.

   Most of the information requests were from individuals and institutions in developing countries. The secretariat response to these requests included copies of INACG publications and information. Additional information was furnished to policy makers and officials concerned with health in developing countries.

2. In August 1989 AID sponsored a workshop for USAID Health, Population, and Nutrition Officers. An INACG display prior to the workshop provided an opportunity for workshop participants to discuss INACG activities and publications with the project manager and the program manager.

III. Publications

A. Conclusion of research project on "Comparison of iron bioavailability from three iron compounds in man, animals, and in vitro," published in American Journal of Clinical Nutrition 1989;49:225-38.

1. A study was conducted comparing iron bioavailability from ferric orthophosphate and electrolytically reduced iron of two particle sizes with that of ferrous sulfate in human, animal, and in vitro systems. Data for the clinical studies, the animal experiments, and in vitro characteristics were collected in 1986 with manuscripts prepared and received by the INACG secretariat in early 1987. Following the final meeting of investigators the manuscript was submitted in October 1987 to the American Journal of Clinical Nutrition for peer review and publication. Due to the complex nature of the studies and the interrelationships between the three components in the overall manuscript, the peer review process extended throughout most of FY88. The manuscript was finally published in the February 1989 issue of the American Journal of Clinical Nutrition. A copy is included as Appendix 5.

B. *Guidelines for the Control of Maternal Nutritional Anemia*

This six-page brochure was the product of a task force on the same topic described in section B.3. It provides clear guidance to government officials, field workers, and primary health care providers on how to reduce the likelihood of developing anemia during pregnancy thereby avoiding the serious consequences of impaired health of the mother as well as the newborn. Plans are already underway to translate this into French and Spanish. A copy is included as Appendix 6.

IV. FY 89 Expenditures and Funding

A. AID Reimbursable expenses

Personnel	$38,348
Meetings and Task Forces	
Int'l Travel	21,389
Int'l Per diem	14,810
Meeting Expenses	564
Consultants	2,126
Communications	5,817
Reports, Publications	4,120
Supplies	1,317
Fiscal Admin.	4,775
General Expenses	5,864
Total Expenses	$99,130
Overhead (12%)	11,896
Total	$111,026

B. Additional sources of funding

1. Many organizations provided support for INACG activities during FY 89. This support was in excess of $32,625 and represented funds allocated for travel, per diem, secretarial support, meeting rooms, office supplies, computer equipment, and expenditures not reimbursable by AID. Supporting organizations included WHO Nutrition Unit; WHO African Regional Office; UNICEF; The World Bank; Food and Nutrition Research Center of the Philippines; Ethiopian Nutrition Institute; Technion-Israel Institute of Technology; University of the Witwatersrand; University of California, Davis; Cornell

University; University of California, San Francisco, Medical Center; University of Florida; and International Life Sciences Institute-Nutrition Foundation.

2. During this reporting period, $111,500 was allocated from Nutrition Foundation funds to support the Chilean research project. These funds were donated for the project by Gerber Products Company.

V. INACG Work Plan and Budget 1 October 1989 through 30 September 1990

A. The FY 90 work plan is included in Appendix 7 of this report. The secretariat submitted the work plan to the project officer, Ms. Carolyn Coleman, on 25 August 1989. The director of the Office of Nutrition, the project officer, and the project manager discussed potential modifications of the scope of work of the cooperative agreement on 18 September 1989. The work plan and itemized budget for FY 90 are on hold until there is a resolution concerning cooperative agreement modifications.

APPENDIXES

1. INACG Advisory Committee

2. Minutes of the Relationship of Anemia to Mental and Behavioral Development Task Force meeting on 13 October 1988 and summary of "Conference on Iron Deficiency and Brain Function" on 10-12 October 1988

3. Program, minutes, and list of participants for the INACG workshop "Maternal Nutritional Anemia" and list of members of the Maternal Nutritional Anemia Task Force

4. First progress report for the research project "Effect of a Fortified Rice Cereal on Iron Status in Infancy"

5. "Comparison of iron bioavailability from three iron compounds in man, animals, and in vitro"

6. *Guidelines for the Control of Maternal Nutritional Anemia*

7. Proposed FY 90 work plan for INACG

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INACG Monograph Meeting
Task Force on the Relationship of Anemia to Mental and Behavioral Development
Thursday, October 13, 1988
Geneva, Switzerland

Present: Dr. C.O. Chichester, Dr. Peter Dallman, Ms. Evelyn Harris, Dr. David Levitsky, Dr. Ernesto Pollitt, Dr. Moussa Youdim

The meeting was called to order by Ernesto Pollitt at 9:00 am.

The meeting began with a general discussion of the purpose and distribution of INACG monographs. Dr. Chichester briefly informed us that the monographs are distributed to the general public through organizations such as UNICEF and AID, and are usually printed in English, French and Spanish. Distribution can be as high as 15,000 to 20,000 copies. The monographs usually run to about 40 pages, with numerous tables and figures as well as references. There should be a uniformity of style in each chapter and the writing should be non-technical and be easier to read than, for example, *Scientific American* magazine.

Dr. Chichester also informed us that INACG would like the monograph to come out before December 31, 1989.

Dr. Pollitt was unanimously elected to be editor of this project. He will take the responsibility of coordinating the writing of the monograph.

The following chapters were discussed and should be included in the monograph:

1. Introduction: What is iron and what is its effect on brain function? Definition of iron deficiency.

   Describe the stages of iron deficiency. Describe why and how people become iron deficient. Deficiency with anemia is what we have information about; other forms we may not.

2. What is the physiology of iron in the brain? Discussion of iron deficiency and dopaminergic receptors.

3. What role does iron play on peripheral systems that affect emotional and other states? Alternative biological pathways to explain observed cognitive and behavioral changes.

4. Functional effects of iron deficiency in infancy. A suggested title was "Infancy and growth: Implications of functional effects." Internal and external validity of studies.

5. Functional effects in preschool children. Internal and external validity of studies.

6. Public health and operational program implications. Prevention and treatment.

7. Future research areas This would be a short chapter.

8. Criticisms of hematology and methodology. This may be too technical; it may not be easy to understand by people with no background in the subject.

9. Possibly some case studies would be included.

A chapter may be included about various aspects of growth and development, including information about different demands an organism has, occurring with other things in the brain (this could be incorporated with information on stages and causes of iron deficiency).

Chapter assignments were made as follows:

1. Peter Dallman: Periods of vulnerability. He mentioned that there is little controversy on this subject, so it should not be difficult to write.

2. Moussa Youdim: Physiology and biochemistry of the brain

3. David Levitsky: Peripheral systems.

4. Betsy Lozoff: Functional effects of iron deficiency on infants. As she was not present we did not confirm this with her.

5. Ernesto Pollitt: Effects on preschool children.

Everyone will work together on the chapter on research needs and public health implications, bringing notes and ideas to the next meeting, at which time this chapter will be written.

Regarding the next meeting, Peter Dallman mentioned that he would be unable to write his chapter before February, and that he would prefer to wait until May or even June before the next meeting. David Levitsky also mentioned that his research and writing will take a considerable amount of time, and that with other commitments he could not have a chapter prepared before May. Given that and a general discussion about people's schedules, it was decided to hold the next meeting in May, rather than November. Also, since several members of the group live near San Francisco, it was decided that the meeting should take place there rather than Washington.

David Levitsky requested $200 to help pay for his library research.

The possibility of using the services of Evelyn Harris as copy-editor for the monograph was discussed. A decision will be made at a later date.

The meeting adjourned at approximately 11:00.

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Names and Addresses of People Attending the INACG Meeting Thursday, October 13, 1988 Geneva, Switzerland

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B

SUMMARY REPORT
Conference on Iron Deficiency and Brain Function
WHO Headquarters, Geneva, Switzerland
October 10-12, 1988

Ernesto Pollitt, Jere Haas and David Levitsky

Submitted to the United Nations University (UNU) and the
UN Administrative Committee on Coordination -
Subcommittee on Nutrition (ACC/SCN)

In keeping with the ACC/SCN and the UNU mandate the following four main questions were posed as guidelines to the participants at the beginning of the Conference:

1. Is there a statistical association between body iron status and behavior?
2. If such a relationship exists, is it causal?
3. What are the mechanisms by which this relationship is expressed?
4. How valid is it to generalize from the current findings to other populations and ecological settings?

Based on these questions the meeting focused on an in-depth critical evaluation of seven experimental human studies of iron interventions on samples that ranged from infants to school children. These presentations were preceded by reviews on behavioral developmental theory, the neurochemistry of iron deficiency, and animal models to assess the impact of iron deficiency on brain function. Two papers on methodological issues related to the assessment of iron status and its interpretation in populations followed the presentations of the human research studies. Discussions were facilitated by a background paper which included an algorithm to evaluate the degree to which causality can be inferred from the relationship between iron deficiency and behavior.

The human studies assessed the effects of iron deficiency on measures of psychomotor development, attention, learning, and school achievement. None of the individual studies contained *all* the elements required to establish causality. However, most were randomized, double blind, iron treatment trials, and the results consistently showed that the iron replete children obtained better scores than iron deficient anemic children before and after the experimental intervention. Two

studies (in Bandung, Indonesia and India) with clearly interpretable statistical findings reported that following iron treatment there was a significant improvement in test performance among anemic preschool- and school-aged children.

Based on the findings the following summary statement was approved at the end of the meeting:

> Many studies have shown an association between iron deficiency and less than optimal behavior in infants and children, as demonstrated by lower scores on tests of development, learning and school achievement. A problem with interpretation of past studies has been that iron deficiency anemia is associated with other adverse environmental and nutritional conditions.
>
> More recent studies using randomized designs with appropriate controls of environmental and nutritional variables have shown that iron therapy in preschool and school-aged children with iron deficiency anemia results in improvements in selective learning and school achievement tests.
>
> This evidence in humans and a number of animal studies suggests that iron deficiency anemia is causally associated with less than optimal behavior. For this reason and others it is important that iron deficiency anemia be prevented and treated in all children.
>
> Since the specific mechanism and functional significance of these behavioral changes are not completely understood, further studies are essential both to clarify the effects of iron deficiency anemia itself and to determine the importance of lesser degrees of iron deficiency in children.

The inference made on causality was based on both statistical probability and biological and behavioral plausibility. The effects of iron deficiency on behavior were clearest when subgroups of anemic preschool and school-aged children were identified, regardless of how the anemic group was defined. The evidence was weakest or non-existent in iron deplete children without anemia

regardless of age.

Critical questions raised during the discussions which remained unanswered and which therefore require further investigation fall into three general areas: (1) development of better measurement tools, especially in the field of psychology; (2) the search for mechanisms by which iron deficiency affects brain function and behavior; and (3) continued research into the causal relationship of iron deficiency and behavior. Each will be discussed briefly.

1. Development of Better Measurement Tools

The need for infant tests better than those that have been generally used was recognized. The use of measures of specific information processes (e.g. attention and memory) and of social emotional development among iron deficient infants for detecting specific loci of effects was recommended. It was recognized that the Bayley Scales of Mental and Motor Development have contributed to an early identification of the effects of iron on infant behavior. However, the construct validity of global scales of mental and motor development was questioned.

Progress in this field might be accelerated by convening a workshop of developmental psychologists and neuroscientists to examine the problem and lay out guidelines for research. Steps in this direction were taken by participants of the Conference and officials from the Mental Health Division of WHO.

2. Investigation of Mechanisms

The participants recognized that considerable progress has been made in recent years in the study of brain neurochemistry as it relates to iron deficiency. The experimental evidence presented on the effects of iron deficiency on the dopaminergic system, and the suggestive reports that these effects may lie behind the behavioral alterations observed illustrated this progress. Neurochemical and behavioral developmental research should now be coordinated to draw more specific hypotheses on the processes involved, the loci of the effects, and mechanisms. A need to assess the possible involvement of peripheral biological systems as mechanisms involved in the behavioral alterations was also underscored.

Further research using animals models was considered urgent. However, the relevance of animal models in the assessment of the functional impact of iron deficiency to the human organism must be established. This includes: (1) cross-species verification of research results; (2) development of models that mimic the type and severity of iron deficiency found in humans; and (3) recognition of the concurrent development of the organism from biological, social and behavioral perspectives. The relevance of currently available indicators of iron nutriture in the progression of iron deficiency in regards to brain iron metabolism should also be investigated.

3. Continued Research

While most of the investigations used a randomized, double blind, iron treatment trial to establish causality, there were substantial differences in the application of this design such that some standardization in research methodology was recommended. This would greatly facilitate the evaluation of future research and strengthen conclusions about the generalizability of findings across diverse ecological settings. Some coordination of research might be facilitated through multicenter collaboration and communication.

Among others, the following are some of the critical issues that research designs need to address:

-- interactions between iron, on the one hand, and other nutrients and infection, on the other;

-- sensitivity and specificity of diagnostic strategies to minimize sample heterogeneity;

-- the impact of severity and duration of iron deficiency; and

-- reversibility of effects.

At the end of the meeting it was noted that the current findings already have direct implications for both clinical treatment and public health interventions. Although the treatment of iron deficiency anemia should not be justified solely on the basis of the current knowledge of its effects on behavioral development of children. The presence of this relationship provides further justification for continuing this line of research and considering the potential importance of a public health problem on other programs related to education and social and economic development.

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INTERNATIONAL NUTRITIONAL ANEMIA CONSULTATIVE GROUP (INACG)®

MINUTES OF THE WORKSHOP ON

MATERNAL NUTRITIONAL ANEMIA

14-16 NOVEMBER, 1988

WORLD HEALTH ORGANIZATION
GENEVA, SWITZERLAND

The Nutrition Foundation, Inc., is a division of the
International Life Sciences Institute—Nutrition Foundation

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MINUTES
INACG Workshop
Maternal Nutritional Anemia
14 - 16 November, 1988
World Health Organization
Geneva, Switzerland

The meeting was opened by Dr. Alberto Pradilla (Chief Nutrition Unit, WHO) who welcomed the participants and stressed the important contributions to international health of consultative groups such as INACG.

Dr. C. O. Chichester, representing the INACG Secretariat, ILSI-Nutrition Foundation, next expressed his appreciation to our WHO hosts for providing the excellent facilities for our meeting. He also thanked the AID Office of Nutrition for providing the support needed to hold this workshop. He pointed out that the purpose of the workshop was to concentrate on implementation of programs to prevent the nutritional anemia of pregnancy, using information that we already have available. The meeting was not intended as a research forum, although suggestions for further research would be welcome. He next introduced Dr. James Cook as chairman and Dr. Peter Dallman, as rapporteur.

Dr. James Cook reviewed the important contributions of Dr. Edouard M. DeMaeyer, Consultant, Nutrition Unit, WHO, to the development of international programs for the prevention of nutritional anemia and expressed regret that Dr. DeMaeyer would not be able to chair this meeting due to illness (Dr. DeMaeyer was warmly welcomed later in the day when he briefly attended the meeting).

Dr. James Cook next discussed the prevalence of anemia in women using the paper of DeMaeyer and Adiels-Tegman (Rapp. trimest. statist. sanit. mond., 38 (1985) 302) as background. It is estimated that 1.2 billion people now suffer from anemia. The percentage of women who are anemic during pregnancy is about 50%. Prevalence of anemia is highest in Asia and Africa. Although iron deficiency is the major factor responsible, Dr. Cook also stressed the important role of pilot evaluations to determine the major causes of anemia. He cited the example of a population in which thalassemia rather than iron deficiency turned out to be the major cause of anemia; this explained why an earlier iron supplementation program had been ineffective.

Dr. Chanarin discussed the folate needs of women based on an RDA of 3.1 ug/kg/d or 170 ug/55 kg woman. Pregnancy is estimated to increase the RDA by 100 ug/d for a total of 270 ug/d. These RDA values are felt to be on the generous side. Studies have shown pregnant women to be at increased risk of folate deficiency based on their red cell folate values often falling below 140 ug/ml and the occurrence

of megaloblastic bone marrow morphology. A dose of 300 ug/d was felt to be ample for most pregnant women and a dose of 500 ug/d for pregnancies complicated by malaria.

Dr. Bothwell discussed the pathogenesis of iron and folate deficiencies. He emphasized the importance of bioavailability in iron nutrition and the increased demands and losses associated with pregnancy. In folate nutrition, increased demand is also important but assimilation is likely to be decreased by loss during cooking rather than poor absorption, as in the case with iron. Data were presented on the effects of various fruits, vegetables and nuts in stimulating or depressing iron absorption.

The afternoon session was opened by Dr. McGanity, who discussed the particular vulnerability of young women in whom pregnancy occurs before growth is fully complete (< 5 yr. past menarche). Low birth weight and toxemia are highest in this age group and mothers must put on more weight per gram of fetal weight gain than at a later age.

Dr. Lind discussed the fall in hemoglobin concentration that occurs even in the course of a normal pregnancy and emphasized that this phenomenon must not be considered an abnormality. He stated his view that in areas where iron deficiency is relatively mild and uncommon (in some industrialized countries) pregnant women should not routinely be given iron supplements. In developing countries, this option was not felt to be applicable.

Dr. Fleming next spoke of populations in which iron deficiency and malaria or AIDS coexist, using Nigeria as an example. Malaria (Plasmodium falciparum) is particularly common and results in marked splenomegaly, with the risk of acute red cell sequestration by the spleen. This problem, in combination with iron deficiency results in major morbidity, and is an important cause of maternal and infant mortality. He described how this severe anemia progressed through the stages of being compensated, then uncompensated, and finally resulting in circulatory congestion.

Dr. Harrison discussed maternal anemia as a cause of mortality in Nigeria. Of 917 emergency admissions of anemic women to a university hospital, 11 with anemia died and 50 died with other conditions that were accompanied by anemia. In women with a hemoglobin below 4 g/dl, transfusion was often lifesaving.

The final session of the afternoon was devoted to the effects of iron deficiency on the newborn. Dr. Dallman started by reviewing the accumulating evidence that the fetus is not as effective a parasite on the mother as previously thought. Even though the fetus is to a large extent protected from depletion of iron stores and anemia, prematurity, low birth weight and perinatal mortality appear to be more damaging consequences of maternal anemia.

Dr. Lieberman reviewed her large study from Boston (NEJM 1987;317:743-48), which also came to the conclusion that maternal anemia is associated with prematurity. In her study, premature onset of labor was strongly associated with low maternal hematocrit at the time of delivery. This association may help to explain the common occurrence of prematurity among blacks.

Dr. Brabin reported on his experience in Papua New Guinea, where malaria and iron deficiency commonly coexist in pregnant women. Severe anemia (hemoglobin < 8.0 g/dl) in primigravida was associated with low birth weight.

The second day of the meeting opened with a review by Dr. Hallberg of the prevention and treatment of iron deficiency anemia in pregnant women. He outlined the substantial iron needs of the pregnant woman for delivery of iron to the fetus and placenta and for the expansion of red cell mass that occurs in the pregnant woman during the last half of gestation. He reviewed studies showing that iron is better absorbed as iron stores decline during pregnancy. However, this adaptation is insufficient to allow unrestricted hemoglobin production in many women, even in industrialized countries. This is shown by the fact that hemoglobin concentration remains relatively stable during the third trimester in unsupplemented women, whereas it normally rises by about 1 g/dl in women who are iron-supplemented.

Dr. Jackson next talked about how cultural attitudes and practices can determine the effectiveness of programs to prevent nutritional anemia. As an example, she described the situation in Liberia, where available prenatal services are poorly used whereas postnatal services are well utilized. This appears to be partly due to the cultural characteristic of placing more importance on the health of the infant than on the well-being of the pregnant woman. She emphasized the importance of being aware of local cultures, diets, and food taboos before planning a maternal-child health program.

The next part of the program consisted of brief presentations of the experience in several countries with programs to prevent iron deficiency anemia, particularly during pregnancy. Dr. Valyasevi described two programs that have been established in Thailand to prevent iron deficiency. Iron EDTA has been used to fortify fish sauce, which is an integral part of the diet in at least two-thirds of the Thai population. One year of fortification resulted in a significant rise in hemoglobin of 0.21 to 0.30 g/dl in all segments of the population except young adult men. Studies of pregnant women showed that 120 mg of iron as ferrous sulfate resulted in a satisfactory hemoglobin and ferritin response and 60 mg of iron was almost as effective in respect to hemoglobin. Side effects and drop out rates were between 10 and 15%. Folate had no additional effect on hemoglobin concentration. Programs to motivate women at the village level were felt to be essential. Obstacles to the success of programs of supplementation are that only one third of women attend prenatal clinics, and some

women fear that taking supplements will make the delivery more difficult by promoting greater growth of the fetus.

Dr. Sood described the national supplementation program in India, which uses tablets of 60 mg iron as ferrous sulfate and 500 mg folate. Limiting factors in the program are a distribution system which fails to reach much of the population and local health workers who are often uninformed about anemia. Prospects for success may be better with the plan to fortify salt with both iron and iodine. Salt fortification was found to be effective in pilot studies conducted in 3 rice-consuming areas. Iron absorption is better with a vegetarian diet based on rice than it is from wheat-based diets. A study of fortified salt in a wheat-consuming area of northern India is in progress.

Dr. Florentino described a program in the Philippines that is directed at high-risk pregnant women. It provides education and iron supplements of 120 mg iron as ferrous sulfate. It is estimated that 45% of the target population is reached. He also described a study, conducted in La Union province, in which the effectiveness of extra food, and administration of iron and folate supplements was compared. The combination of the two interventions was most effective in preventing both low birth weight of the infant and anemia in the mother.

Dr. Brabin described the circumstances of a northern coastal population in Papua New Guinea. The food intake is generally good because land and income availability are typically adequate. However anemia is common and use of iron dextran has been found to result in an increased number of falciparum malaria parasites in the placenta; incidence of malaria was unaffected by iron dextran.

Dr. Fleming next discussed his experiences with a Nigerian population in which about 50% of first deliveries were in women aged 16 years or less. Antimalarials alone virtually eliminated severe anemia (PCV, < 25%). Malaria appeared to promote folate deficiency as shown by a 56% prevalence of megaloblastic changes in women who received no antimalarials, 25% in those who received antimalarials, and 8% in those receiving both folate and antimalarials. Linear growth was significantly greater in young primiparas who received folate compared to those who did not. He strongly recommended using antimalarials, iron, and folate during pregnancy in tropical Africa.

Dr. Kaisi noted that anemia was a leading cause of maternal death in Dar es Salaam, Tanzania. He described an extensive experience with iron dextran use in pregnant women with hemoglobin concentrations ranging between 6 and 10 g/dl. About 25% of 632 patients had a reaction consisting of pyrexia and/or bone, joint or muscle pain. Severe reactions occurred in 4% and these were 3 anaphylactoid reactions. There have been 2 deaths related to iron dextran since the above study.

Dr. Bothwell concluded the country reports with a description of an effective fortification program in Durban, South Africa. The target population was an Indian population with a high prevalence of iron deficiency. Iron EDTA-fortified curry powder was effective in raising the mean hemoglobin concentration among women by 0.5 g/dl in a year and reducing the prevalence of anemia from 25% to 7% over a 2 year period.

Dr. Cook next outlined the plans for the remainder of the meeting with a focus on:

1. Specific recommendations for prevention of nutritional anemia during pregnancy that can be implemented immediately, and

2. Suggestions for applied research in developing countries.

The specific recommendations were considered in two categories: a. short term approaches - including primarily the use of supplements by pregnant women, and b. long term approaches - including modification of the diet and food fortification to improve the iron status of the population as a whole. Dr. Robert Cook, Family Health Division, WHO, emphasized the importance of making recommendations specific in terms of what should be done and how it should be done. This request was also expressed by Dr. Mark Belsey, Chief of Maternal and Child Health at WHO.

The remainder of the meeting was devoted to a discussion of the above topics by the group as a whole. These discussions were then incorporated on the following day into a brief statement by a writing group consisting of Drs. Bothwell, Dallman, Florentino and Jackson. This statement will be published and distributed separately from these minutes.

Respectfully submitted,

Peter R. Dallman

Peter R. Dallman,
Rapporteur

INACG

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Program

INACG Workshop

Maternal Nutritional Anemia

14-16 November, 1988

World Health Organization
Geneva, Switzerland

Room A

Monday, 14 November, 1988

0900-0930	Registration
0930-0945	Welcome Dr. Alberto Pradilla World Health Organization Dr. Samuel G. Kahn/Dr. C.O. Chichester Agency for International Development
0945-1015	Prevalence of Anemia in Women Dr. Edouard M. DeMaeyer/Dr. Jim Cook
1015-1030	Break
1030-1100	Iron Status of Women Dr. James Cook
1100-1130	Folate Status of Women Dr. I. Chanarin
1130-1200	Factors Responsible for Anemia in Women Dr. Thomas Bothwell
1200-1330	Lunch

1330-1530 Consequences of Anemia on Women: Pre-pregnancy/ Pregnancy/Parturition/Postpartum

Consequences and Prevention of Anemia in the Pregnant Adolescent
Dr. William McGanity

Does Normal Pregnancy Cause Anemia?
Dr. Thomas Lind

Consequences of Anemia in Pregnancy on the Mothers
Dr. Alan F. Fleming

The Effect of Severe Anemia on the Mother
Dr. Kelsey Harrison

1530-1545 Break

1545-1745 Consequences of Anemia on Fetal and Early Infant Development:

Effect of Maternal Iron Deficiency on the Newborn
Dr. Peter Dallman

Association of Maternal Hematocrit with Preterm Birth
Dr. Ellice Lieberman

Consequences of Maternal Anemia on Fetal and Early Infant Development in a Malaria Endemic Area: Perinatal Mortality and Morbidity
Dr. B. Brabin

1745-1800 Announcements

Tuesday, 15 November, 1988

0900-1030 Prevention and Treatment of Anemia in Women
Oral Supplementation with Iron
Parenteral Administration of Iron
Diet and Fortification
Dr. Leif Hallberg

Attitudes and Practices Toward Prevention and Treatment of Nutritional Anemia
Dr. Fatimah Linda Collier Jackson

1030-1100 Break

1100-1230	Case studies:
	Thailand Dr. Aree Valyasevi
	India Dr. S.K. Sood
	Philippines Dr. Rudolfo Florentino
	Papua New Guinea Dr. B. Brabin
	Nigeria Dr. Alan Fleming
	Tanzania Dr. Malise Kaisi
	South Africa Dr. Thomas Bothwell
1230-1400	Lunch
1400-1530	Case studies (continued)
1530-1545	Break
1545-1700	Issues to be Addressed in Combatting Maternal Nutritional Anemia
1730-1930	Reception, WHO Cafeteria

Wednesday, 16 November, 1988

0900-1015	Recommendations for Research
1015-1030	Break
1030-1200	Recommendations for Immediate Program Action
1200-1400	Lunch
1400-1700	Implementation Plan for Combatting Maternal Nutritional Anemia
1700-1715	Assignments

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Maternal Nutritional Anemia
14-16 November 1988
Geneva, Switzerland

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Reference Points for INACG

As a result of discussions during the INACG workshop "Maternal Nutritional Anemia" in Geneva, November 1988, the INACG secretariat believes the following should be considered in discussions of INACG's future plans:

1. INACG suggests that before any program of iron supplement distribution to women (especially pregnant women) is initiated, accommodations should be made for the cultural and behavioral characteristics of the population. Cultural anthropologists, in addition to pharmaceutical marketing experts, can be of significant assistance in this area.

2. If the results of field research on side effects related to new slow-release iron supplements are favorable, INACG should take an active role in evaluating the efficacy and cost-effectiveness of using these preparations in field programs.

3. INACG previously reviewed the role of injectable iron preparations in the treatment of anemia and did not recommend their use because of potential for increased vulnerability to infection. Evidence both pro and con the use of injectable iron (particularly in African settings) was presented during the workshop and this, in conjunction with earlier evidence, should be reconsidered in the future.

4. In the absence of routine screening of blood supplies for Human Immunodeficiency Virus (HIV) in Africa, transfusions of anemic women should be carefully considered. There is an urgent need to develop a reliable and rapid method for the determination of the presence of HIV in blood used for transfusion in severely anemic women in Africa. INACG should encourage the efforts of other organizations in this regard.

5. Programs utilizing Iron EDTA or EDTA as a fortificant for local foods should be initiated as rapidly as possible, particularly in the African region.

6. Because women in developing countries often suffer from both iron and iodine deficiency, and because salt can be effectively fortified with iodine, INACG should give consideration to joint fortification of salt with iron. The feasibility of fortification of salt with iron and iodine should be reviewed in conjunction with the International Council for Control of Iodine Deficiency Disorders (ICCIDD).

7. A program concerned with developing awareness of nutritional anemia should be developed in Africa, perhaps in conjunction with WHO.

8. INACG previously conducted a workshop on social marketing the concept that iron deficiency anemia is the major nutritional problem in the world. The emphasis was marketing to decision-makers at the policy level. In relation to maternal nutritional anemia, the importance of undertaking this effort became apparent. Implementation of a social marketing plan for increasing awareness of the worldwide significance of nutritional anemia should increase the resources available to develop national and regional programs directed to reducing the problem of nutritional anemia.

9. As part of a social marketing plan, INACG would work with the Agency for International Development (AID), Office of Nutrition, and its contractors such as the Academy for Educational Development, to develop information packets for AID missions, WHO field offices, UNICEF field offices, and other organizations involved in safe motherhood and child survival activities. The information packets could include documentation of the detrimental effects of nutritional anemia on work performance, mental development, school performance, pregnancy outcome, and infant and young child physical development, particularly as they affect the less developed countries.

refpts.min

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RECEIVED
JUN - 8 1989

C.O.Chichester, Ph.D.
The Nutrition Foundation
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Santiago, May 26, 1989

Dear Chi:

Unfortunately the report was sent off without the covering letter you will find now enclosed.

I'm taking the opportunity to make some further clarification about the report.

1) The number of infants at each time point for each group as depicted in Fig 1 and 2 is the same for all other Figures.

2) Table 5, as opposed to the text, reads as 3/37 infants of group 4 and 5/18 of Group 5 to be anemic. This is the correct number. Not a statistically significant difference. At 4 months, the 22 and 24 Hcts are the "screening" tests. Only 2 infants in G 4 and 3 in G 5 have had full reevaluations because of Hcts <32% as stated in the protocol. These infants were treated if repeat Hb were in fact <9.5 g/dl (our lower limit of normal for this age). In any event, we repeated the Hcts at 6 months, to ensure the fact that they were rising appropriately (data not shown). If the Hct remained low, we would treat with iron and remove from study.

3) The boys in G 4 (Fig 4b) weighed more than those in G 5, thus, the significant differences in the weight adequacy (Fig 6) is possible as of 5 months of age. We believe this is a mere chance event, since the main food (aside from breast milk) in these infants was the cereal which was consumed equally by both groups. More infants are needed to jump to conclusions.

I hope these further clarifications fill the possible gaps you may have already detected.

Sincerely,

Tomas Walter, MD.
Head, Hematology Unit.
INTA

c.c. Peter R. Dallman, M.D.
c.c. Guy Johnson, Ph.D.

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"EFFECT OF A FORTIFIED RICE CEREAL ON IRON STATUS IN INFANCY"

FIRST PROGRESS REPORT

May 1989

Instituto de Nutrición y Tecnología de los Alimentos
Universidad de Chile

Tomas Walter
Responsible Investigator

HEMATOLOGY UNIT

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General comments. The field study began in August 1988, after a two month period of personnel recruitment and training and preparation of field facilities, where a special examining room was built and furnished.

After several weeks delay for the milk formulas to be supplied, the decision was taken to begin with groups 4 and 5 (the exclusively breast fed infants) of the study as soon as the cereal arrived on site. The purpose of this decision was to obtain experience regarding the acceptance of the cereal and to solve unexpected problems that could arise in the field study once it began at full speed.

However, the delays in obtaining the milk formulas after 6 months of the onset of the study have prevented the further accrual of the milk fed infants. The accrual of groups 4 and 5 was, therefore, stalled in December coming to a complete halt in February 1989. In our field trials it is important to run all groups in parallel, since during the 2 years of study many unexpected changes (environmental, nutritional or socioeconomic) could occur, affecting the results of the study rendering it uninterpretable. With the groups in parallel, any change would affect all groups equally.

Thus far, we have not encountered unexpected events. The only design change is that the cutoff birth weight for eligibility was raised from 2500 to 3000 grams, since those infants from 2500-3000 grams are now enrolled in a special program by the clinic with nutritional supplements which would complicate their follow up.

We have prepared some tables and figures to illustrate the results thus far. No attempt can be made at their interpretation with such small numbers, however, it may provide an idea of our work thus far. Additionally, it can be of assistance in defining future projections.

Accrual data. The global statistics of clinic visits and accrual are shown in table 1. The new infants entering the clinic monthly (at 2 mo of age) have ranged from 114 to 150, with no new appointments made from December to February for the above stated reasons and a shy start in March. Admission into the study began in October, when infants became 4 months of age, however, only a total of 61 have been actually admitted for reasons presented above. Of these, 14 have dropped out; 4 because of change in address, i.e., migration from city sector, 5 for mother's work, so that there would be no more breast-feeding or consistent caretaker (nursery) and 5 for protocol refusal after

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initial acceptance. This attrition rate is in accordance with our previous experience (20-25%). Once the formula is made available, we expect to be recruiting 40-50 infants per month according to these initial results.

Dietary results. The cereal has been well accepted in this breast fed population. The recommendation is to prepare a gruel with cow milk, juice or fruit and offer it twice daily as tolerated aiming for 30 g of cereal at 6 months of age. As of 24 weeks of age over 95% of infants are consuming 80% of the suggested 30g of cereal (Fig 1). The number of standard spoonfuls (2.5 g of cereal per spoon) in shown in table 2 for every single infant recorded at each weekly visit in a questionnaire filled by the mother every day. Thus, the number of spoonfuls shown is the daily mean for that week.

The actual average amount of cereal consumed is shown on table 3 and depicted in Fig 2 showing again that at cereal is consumed in increasing amounts as the infant grows, with an average of 25 g at 6 months and a peak of 30-32 grams at 34 weeks. No difference in acceptability is detected between groups 4 and 5.

Nutritional results. Groups 4 and 5 have shown adequate and identical growth in height (Fig 3), weight (Fig 4) and head circumference (Fig 5). When these figures are adjusted as 50th percentile of the National Center for Health Statistic's tables (Fig 6 and 7) our previous experience is repeated. Weight of breast fed infants reaches a mean of 115% at 4-6 months of age with a gradual fall to 100 percent of the 50th percentile by age 9-10 months. Height does not vary staying within 99 to 101%. We believe , as was discussed with Dr. Ray Yip from the CDC in a meeting in Dallas last December, that this is due to the fact that NCHS tables were not drawn with breast fed infants, who are "Chubbier" in the first 6 months of life.

Morbidity. The number of episodes per infant per 6 months is shown in Fig 8. The bars to the left correspond to episodes detected during the house visits and the right bars to those seen by the physician in the clinic. Since these are breast fed infants, diarrhea as perceived by the mother was more frequent that seen at the clinic. This discrepancy usually decreases as our mothers learn what is the normal bowel habit of a breast fed infant and as the infant begins consumption of solids. There were no significant differences in the number of episodes or their severity between groups 4 and 5 (Table 4).

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Hematologic data No significant differences between groups 4 and 5 (Table 5) are detected as yet, however, at 8 months of age there is a tendency toward better hemoglobin in group 4. More subjects are necessary to confirm these tendencies. At 4 months of age there are no differences. Two of 22 infants in group 4 and 3/24 infants in group 5 have shown anemia at 4 or 8 months (Table 6).

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TABLE 1

STATISTICS FROM THE OUTPATIENT CLINIC

	1988 AUG	SEP	OCT	NOV	DEC	1989 JAN	FEB	MAR	APR	TOTAL
TOTAL INFANTS SEEN	130	239	229	275	80	77	66	91	110	1022
FIRST APPOINTMENT	130	119	114	150	0a	0a	0a	28	50	591
MORBIDITY (SICK CALLS)		23	35	42	43	37	45	42	51	318
ADMITTED			19	27	11	4	0b	0b	0b	61
DISCHARGED			0	0	3	2	4	3	2	14c

a) Eligible infants were not appointed

b) Infants were not admited to study

c) Discharged infants because:

Mother's work = 5

Change of address = 4

Refuse protocol = 5

TABLE 2

Cereal consumption all subjects (mean spoonfuls/week)

Nro	Nombre	Est	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
			Age (weeks) (standard spoon=2.5 g)																										
1	Arriagada Contr	4	*			10	11	16	11	11	11						12		12		12	12	6	0	0	8	14	12	12
4	Merino Elguata	5		5									13	13	16	16	17	25	22	22	22	22	22	11	11	18		7	15
5	Rivera Araya Ne	4		4	0	0	0	0	0	8	8	8	8	8	6	6	6	12	12	12		5	12	12		12	8	12	12
6	Catalan Rubio T	5	8	8	0	0	0	0	0			8	8	8	2	12	12	12	12	12		14		10	10	10	12	10	10
7	Sepulveda Guerr	4		4	5	6			6	10	10	10	8	10	10	4	8	8	14	12	12		12	12	12	12	11	11	12
8	Ruiz Varas Rica	5		10	10	10	10	10		10	0	12	12	10	0	12	12	5	12					12	12	12	12	0	0
9	Suarez Letelier	4		6	8	10		10	8	10	10	12	12		12				11	11	0	10	12	12	12	12	12	12	12
10	Espinoza Pizarr	5		6	6	6		6	3	3	4	12	12	12	12	12							12	12	17	15	9	10	11
11	Alarcon Vera Ka	4		8	4	4	4	0	3	2	3	2	5		5	6	8	7	8	6	3		0	3	0	3	2	3	
15	Garrido Labrana	4		0	0	0	3	3	6	6	0	10	10	12	12	12	12	12		12	12	13	13	12	12	12	12		
16	Jauregui Nahual	5	0	0	0	0	0	0	0	0	7	7	10	10					12	7	12	12	4	7	9	11			
17	Cea Perez Carlo	5	8	8	6	6	8	8	8	8		9	6	6	12	10	10		12	12	12	12	12	12	12	12			
20	Olguin Escobar	4		10	10	10	5	10		13	6		4	10	1	17	18	12	12	12	12	12	12	12					
23	Albornoz Muille	5	4	4	0	0	8	10	10	9	10			12	11	16	16	16	12	10	10								
24	Catalan Martine	4	0	0	0	0	0	0	5	5	6	12	12	12	12	12	9	12	12	12	12	12	12						
25	Saavedra Valdiv	5		4	8	8	8	10	10	10	10	12	12	12	16	12	12	12	12	12	12	12	12	8	14				
27	Pastrian Cordov	5	8	10	10	10	12	12	12	12	12	12	12	12	12	12	12	13	16	16	16	16	16	16					
28	Cabaras Ibanez	4	6		6		6	10		10	10		12	12	12	12	12	12	12	12	12	12	12						
29	Flecher Nieto J	5	0	0	3	3	3		8	8	8	10	10			8	10	10	10	4	10								
30	Velasquez Iriba	4	6	6	6	6	6	8		10	10	12	12	12	12	12	12	0	12	12	12	12	13						
31	Aguilera Vargas	5			8	8	8	8	8	12	12	12	12	12	12	13	12		24	15	18	18	10	14	14				
32	Calfuala Ormeno	4	4	4	2	2	4	2		6	8	8	8	8	8		12	12	12	12	12	12	12	12					
33	Fuentes Vasquez	5	0	0	5	6	8	6	6	6	6	8	20	6	7	6	3	0	0	4	10	3							
34	Amestica Solar	4	0	6	6	6	6	8	8	9	11	10	12	12	12	12	10	14	14	14	14	14	15	15					
35	Contreras flore	5		6	4	2	6		10	12	12	12	12	12	12	12	0	14	12	10	8	2	0						
38	Ramos Novoa Pab	4	0	0	0	0	0	0	0	0	2	2	3	4		1	0	12	13	13	14	8	0						
39	Opazo Castillo	5	3	6	4	4	6	6	5	8	5	9	10	10			12	3	4	12	9	9	13						
40	Erazo Pavez Ros	4	4	6	6	6	4	8	12	12	12	12	9	9	7	12	12	2	9	12	10	15	14	14					
41	Parra Vilchas J	5		0	0	0	0	5	9	12	7	6	6	6	0	0	0	0	5	12	16	16	6						
42	Gutierrez Medin	4	4	4	6	5	4	7	12	12	12	12		12	12	10	12	9	12	10	14	12	9						
43	Plaza Estrada R	5		0	3		6	8	6	6	6	0	4	2	1	5	4	0	0	0	3	7							
44	Saez Castro Mil	4		0	0	0	3	3	1	3	4	6	6		6	12	12	12	12	6	3	9							
45	Montecinos Flor	5	0	0	0	0	8	8	9	9	7			10	12	11	10	11	11	4									
46	Moreno Bustaman	4	0	0	0	2	3	3	2	7	7	7	4	2	3	11	6	0	6	10									
47	Soto Torres Jul	5	1	1	4	2	8	6	10	10	10	10	12	12		12	12	12	12	12	12								
48	Bastida jofre F	4		0	0	0		0	3	4	6	6	4	8	9	7	4	6	2	2									
50	Bulboa Contrera	4		0	0	0	0	0	6	6	3	8	12	12	12	16	16	16	12	12	16								
51	Campos Anaribar	5			0	0	1	5	5	6	10	11	14	12	13	10	14	6	9										
52	Matus Castro Ma	4		0	0	0	6	6	6	6	6	12	12	12	12	12	12	12	12										
53	Levinao Bravo A	5	0	0	0	1	4	4	5	4	4	12	12	0	0	7	10	11											
55	Miranda Vidal M	5			0	0	1	8	7	7	8	5	0	2	9	10	10		5										
56	Rayes Garcia Ja	4		0	0	0	6	6	6	6	6	6	12	12	12	12	12	12											
57	Maldonado Padil	5	0	0	0	0	3	8	8	8	11	12	12	12	12	12	12												
58	Castillo Perada	4		3	6	7	8	10	10	10	11	12	12	12	12	12	12												
59	Obando Colipan	5			4	0	8	12	12	10	12	12	12	12	12	8	12	12											
60	Bobadilla Rique	4	8	8	8	8		8	10	12	12	12	7	12	12														
61	Pena Jorquera P	5	4	8	8		12	12	12	12	5	9	6	9	12														

* Daily [illegible]n spoonfuls per week of age

TABLE 3

Cereal consumption of breast fed infants (g/day)

	Age (weeks)										
	17	18	19	20	21	22	23	24	25	26	27
Group 4	10[a]	21	22	22	19	22	19	23	23	20	21
	8[b]	8	8	9	10	13	15	19	19	22	22
p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Group 5	13	20	23	21	22	21	22	22	21	21	22
	7	10	9	8	15	18	19	21	20	24	26

	Age (weeks)										
	28	29	30	31	32	33	34	35	36	37	38
Group 4	19	21	19	21	19	19	18	16	14	15	10
	25	24	26	26	24	28	27	27	28	26	26
p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Group 5	23	20	21	21	17	19	16	14	12	10	9
	23	23	26	25	24	27	26	30	30	27	28

a Number of infants

b Mean grams of cereal consumed

TABLE 4

Episodes of morbidity in breast fed infants in the field and the clinic

		FIELD (ep/infant/6 mo)			OUTPATIENT CLINIC (ep/infant/6 mo)				
		WO*	Dia	IR	WO*	URI	LRI	Dia	Other
Group 4	*X*	20	0.90	2.51	32	2.48	0.90	0.14	1.21
	SD	3	1.29	1.71	5	1.83	1.53	0.38	1.26
	p	ns	<0.05	ns	ns	ns	ns	ns	ns
Group 5	*X*	20	1.89	2.09	32	2.00	0.70	0.35	0.88
	SD	5	1.94	1.75	4	1.14	0.92	0.49	0.73

* Weeks of observation

TABLE 5

Hematologic data in breast fed infants of group 4 and 5

4 months

		Hto (%)	E (10^{12}/L)	VCM (fL)	Hto2 (%)	Hb (g/L)	L (10^{9}/L)
Group 4	N	22	2	2	2	2	2
	X	35.4	4.1	78.5	32.5	111	10.8
	SD	2.8	0.2	0.5	0.7	2	0.8
	p	ns	ns	ns	ns	ns	ns
Group 5	N	24	3	3	3	3	3
	X	34.4	4.1	74.7	31.0	103	13.9
	SD	2.4	0.1	4.1	0.8	6	3.8

8 months

		E (10^{12}/L)	VCM (fL)	Hto (%)	Hb (g/L)	L (10^{9}/L)	Hb<110 g/dl
Group 4	N	12	11	17	17	12	
	X	4.7	73.9	36.0	120	11.8	
	SD	0.4	4.0	2.8	9	3.1	
							3/17
	p	ns	ns	ns	ns	ns	
Group 5	N	16	121	18	18	16	
	X	4.6	73.8	34.7	113	10.8	
	SD	0.4	3.6	2.8	12	2.2	
							5/18

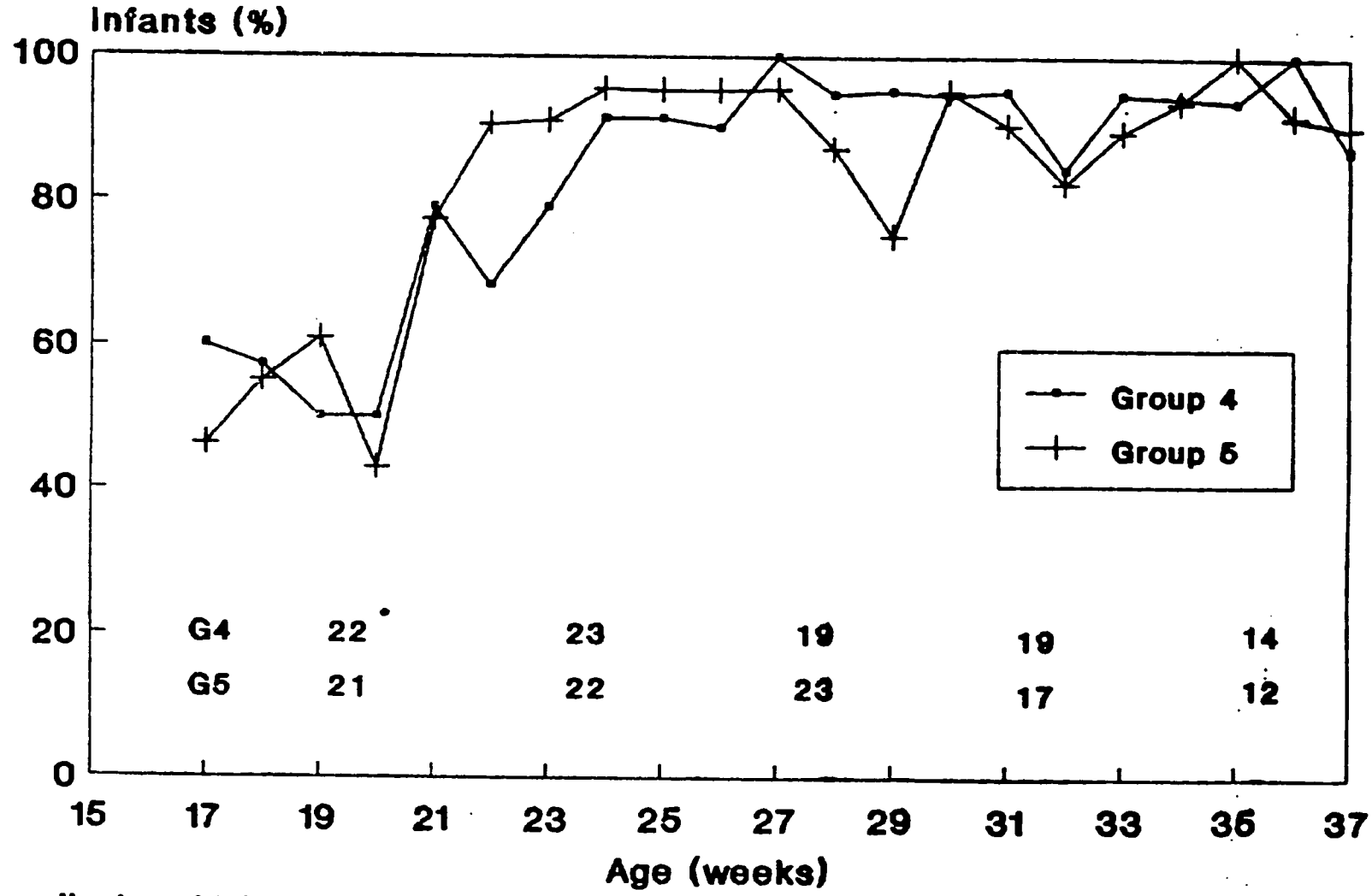
TABLE 6

Hematologic values all subjects

No	Name	St	Sex	BW	Hto	4 mo E	4 mo MCV	4 mo Hto	4 mo Hb	4 mo Leu	8 mo E	8 mo MCV	8 mo Hto	8 mo Hb	8 mo Leu
1	Arriagada Contr	4	2	3200	41,1						4,4	77	34,2	11,2	10600
4	Merino Elgueta	5	1	3990	35,4						4,7	76	36,4	12,2	10550
5	Rivera Araya Ne	4	1	4020	36,8						4,9	71	31,5	11,8	9394
6	Catalan Rubio T	5	2	3470	33,9										
7	Sepulveda Guerr	4	2	3040	32,5										
8	Ruiz Varas Rica	5	1	3461	35,0						5,3	70	37,6	12,2	9283
9	Suarez Latelier	4	2	3810	41,0						5,1	74	38,0	12,2	15900
10	Espinoza Pizarr	5	1	3280	40,0										
11	Alarcon Vera Ka	4	2	3710	39,4										
15	Garrido Labrana	4	1	3900	34,2						4,6	72	32,7	10,9	12150
16	Jauregui Nahuel	5	1	4000	34,2						4,8	72	34,7	11,1	13650
17	Cea Perez Carlo	5	1	3700	32,6										
20	Olguin Escobar	4	1	3350	34,2						4,3	72	31,6	10,6	20400
23	Albornoz Muille	5	1	3300	34,9						4,4	69	30,7	9,9	9795
24	Catalan Martina	4	1	3700	37,1						5,3	67	36,0	11,7	11100
25	Saavedra Valdiv	5	2	3190	38,3						4,4	79	35,4	11,7	11400
27	Pastrian Cordov	5	1	3400	30,3	3,9	80	31,6	10,6	8777	4,3	73	32,0	10,9	5805
28	Cabezas Ibanez	4	2	3150	34,4								36,0	11,8	
29	Flecher Nieto J	5	2	3580	36,1								37,5	12,2	
30	Velasquez Iriba	4	2	3480	31,8	4,3	78	33,2	11,3	10050	5,2	73	38,5	13,1	9476
31	Aguilera Vargas	5	1	3500	36,3						5,4	71	38,9	12,8	10500
32	Calfuala Ormeno	4	2	3400	38,8								40,0	13,0	
33	Fuentes Vasquez	5	2	2900	35,6								30,0	10,0	
34	Amestica Solar	4	2	3380	36,0								38,0	12,1	
35	Contreras flore	5	1	3700	35,9						4,4		35,0	11,6	10900
38	Ramos Novoa Pab	4	1	2920	32,3								37,5	12,6	
39	Opazo Castillo	5	2	4270	33,3						4,4		36,0	11,0	9000
40	Erazo Pavez Ros	4	2	3000	34,3						4,7		39,0	13,1	12300
41	Parra Vilches J	5	2	3100	28,3	4,2	70	29,9	9,6	14750	4,0	70	28,0	7,5	10800
42	Gutierrez Medin	4	1	3200	32,3								36,0	11,4	
43	Plaza Estrada R	5	1	2790	33,9						5,0		35,0	10,5	13100
44	Saez Castro Mil	4	2	3400	35,0										
45	Montecinos Flor	5	2	4550	34,8										
46	Moreno Bustamen	4	1	3750	36,6										
47	Soto Torres Jul	5	1	4000	34,5						4,4		36,0	12,0	8600
48	Bastida jofre F	4	1	3640	30,9	3,9	79	31,7	10,9	11600					
50	Bulboa Contrera	4	1	3350	33,4						4,2	71	34,0	10,7	10600
51	Campos Anaribar	5	2	3450	33,0						4,3	80	35,7	12,7	15500
52	Matus Castro Ma	4	1	3100	37,9						5,0	82	41,0	13,8	11200
53	Levinao Bravo A	5	1	3000	33,9						5,1	74	38,0	12,5	11400
55	Miranda Vidal M	5	1	3100	33,0						4,5	78	35,0	11,5	9600
56	Reyes Garcia Ja	4	1	4290							4,1	79	33,5	11,2	9117
57	Maldonado Padil	5	1	3800	36,9										
58	Castillo Pereda	4	1	3410	35,6						4,6	75	34,9	12,3	9748
59	Obando Colipan	5	1	2800	31,3	4,2	74	31,6	10,8	18100	4,5	74	33,0	11,1	12500
60	B [illegible] illa Rique	4	2	3900	33,5										
61	P[illegible] [illegible] ara	5	[illegible]	30[illegible]	34[illegible]										

Fig 1

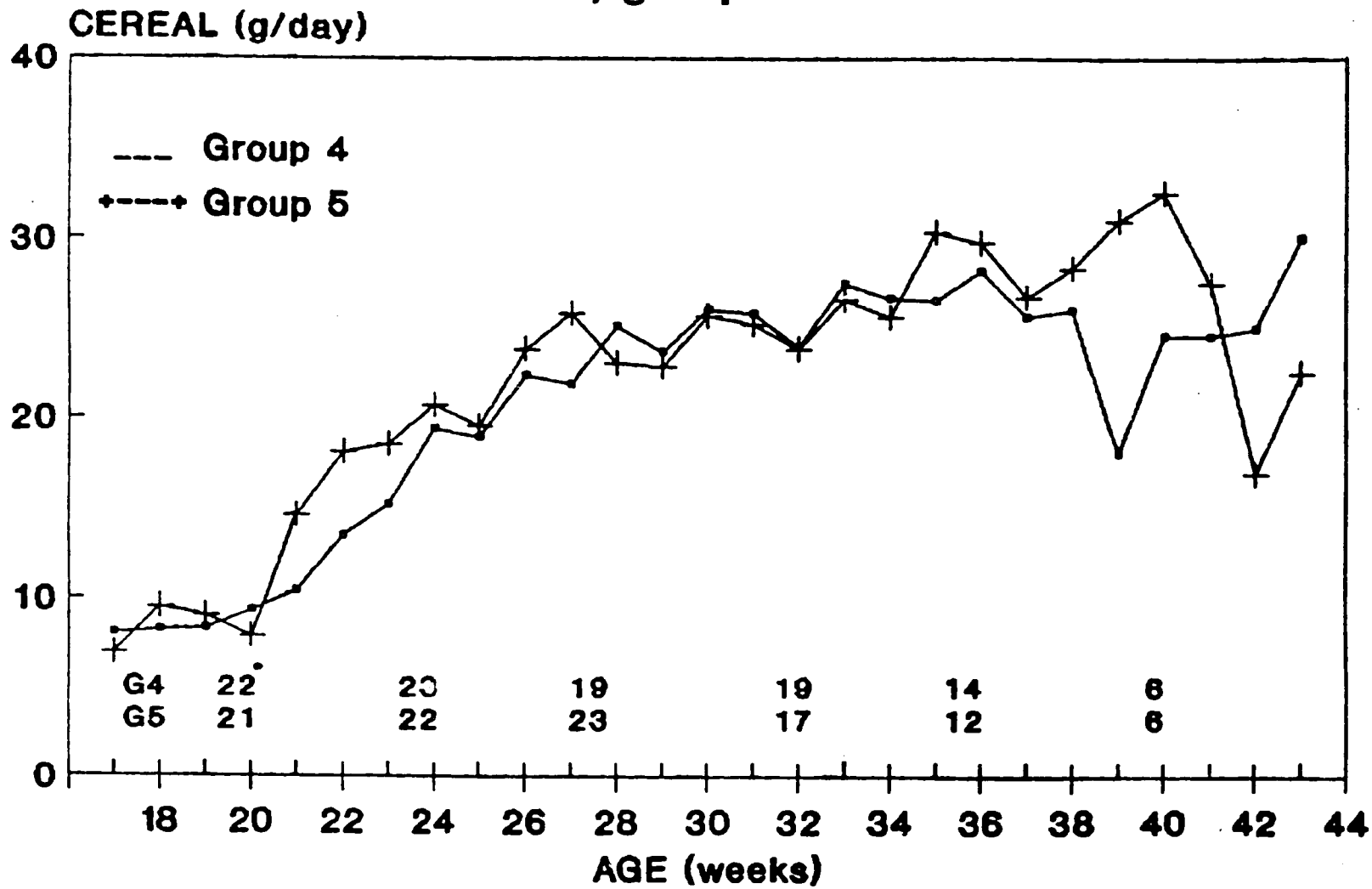
Mean percent of infants consuming more than 80% of cereal recommended



• Number of infants

Fig 2

Average consumption of rice cereal (g/day) in breast fed infants, groups 4 and 5



* Number of infants per group

Fig 3

Heigth gain (cm) in breast fed group 4 and 5

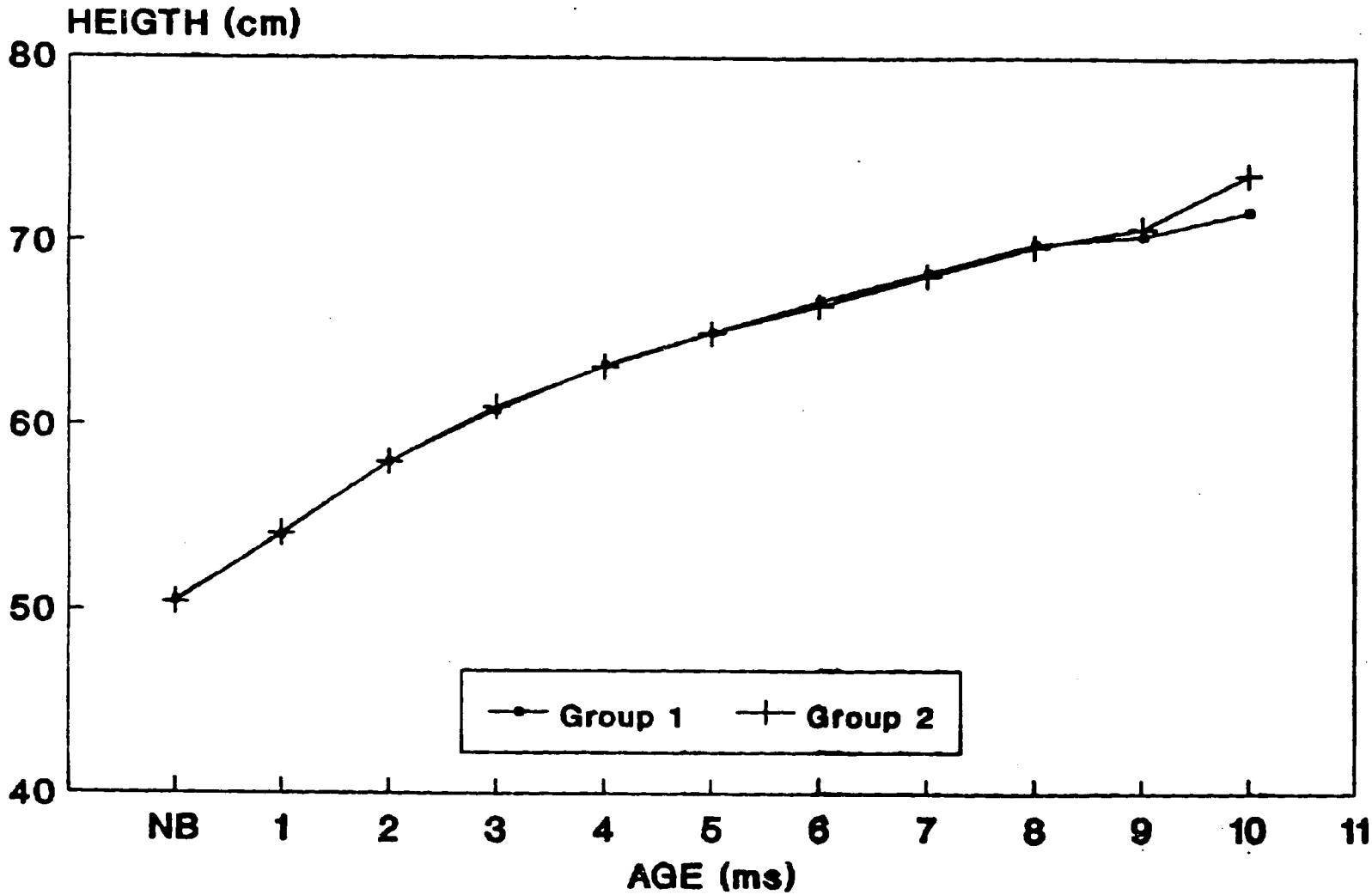


Fig 4a

Weight gain (Kg) in breast fed infants, groups 4 and 5 (girls)

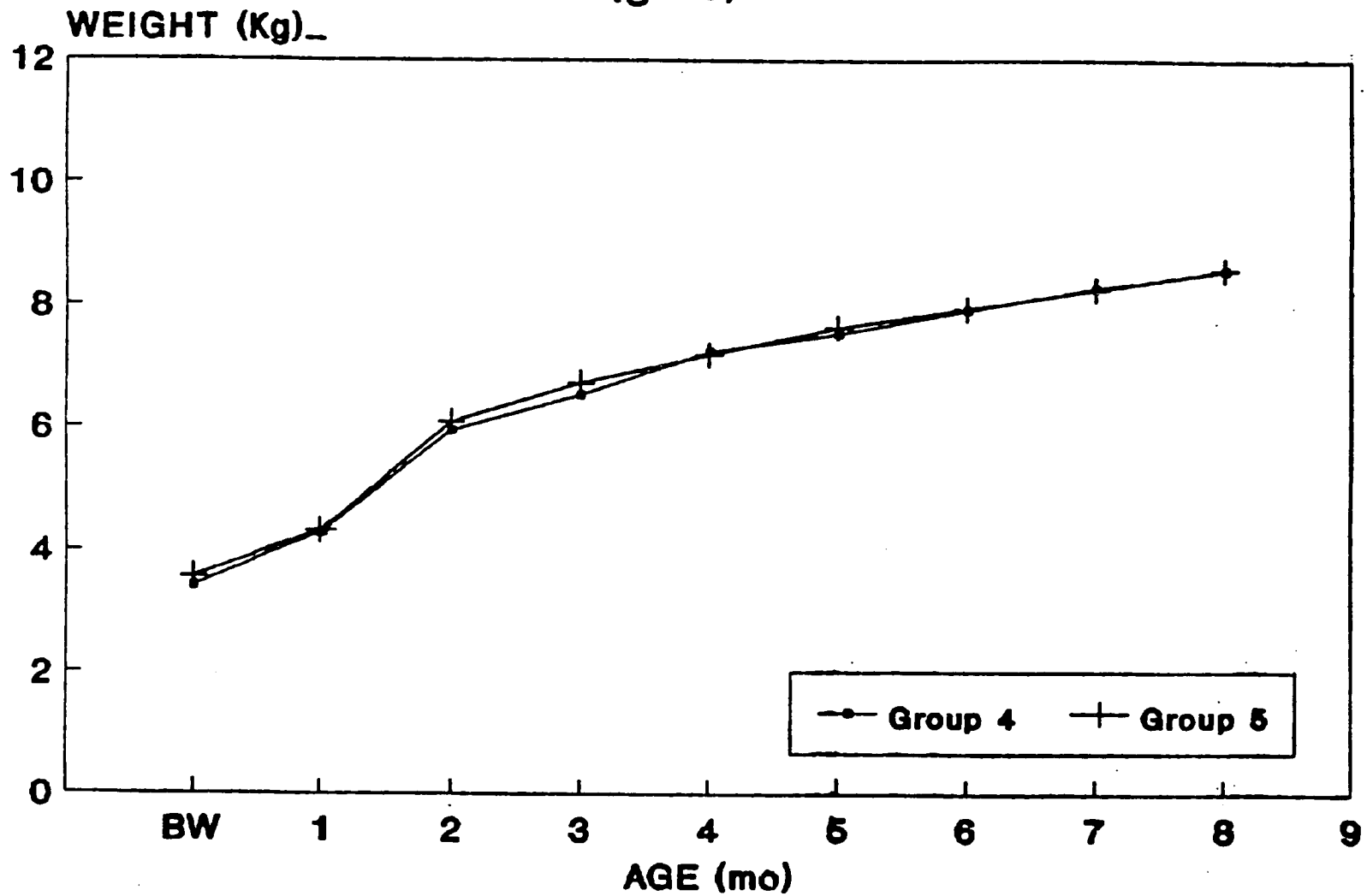


Fig 4b

Weight gain (Kg) in breast fed infants, groups 4 and 5 (boys)

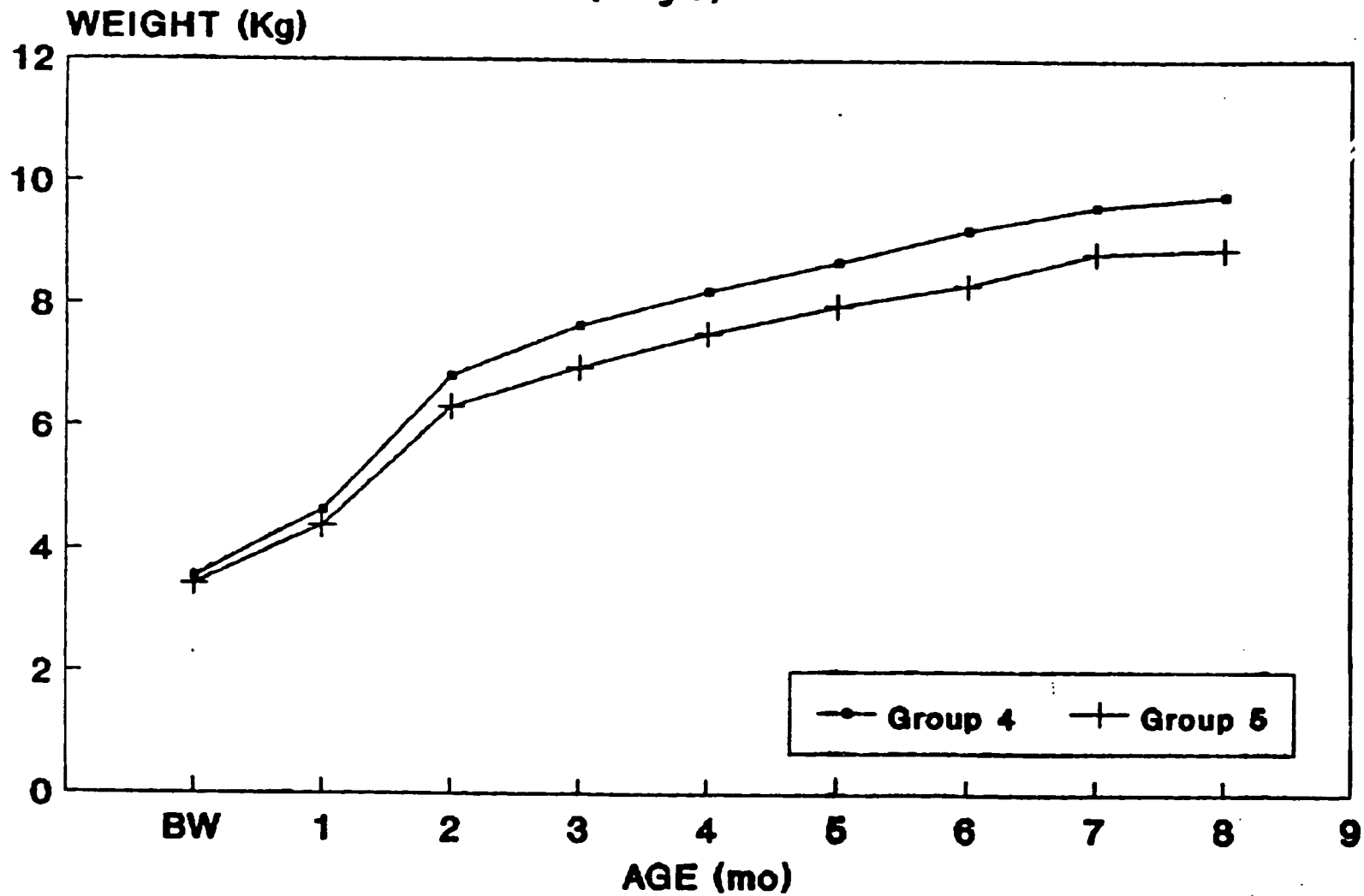


Fig 5

Head circumference (cm) gain in breast fed infants, groups 4 and 5

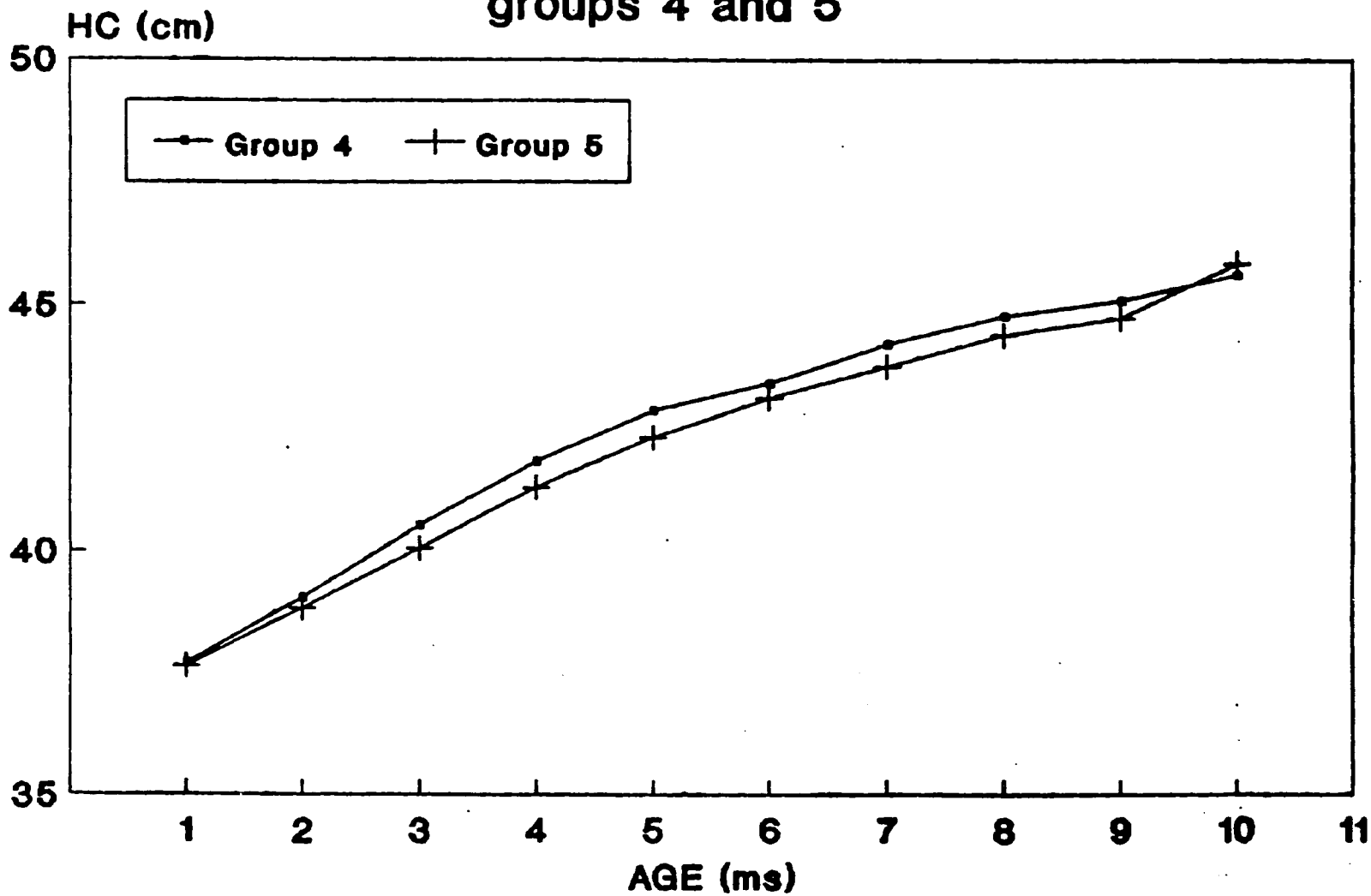


Fig 6

Weight adequacy as p50 of NCHS in breast fed infants, groups 4 and 5

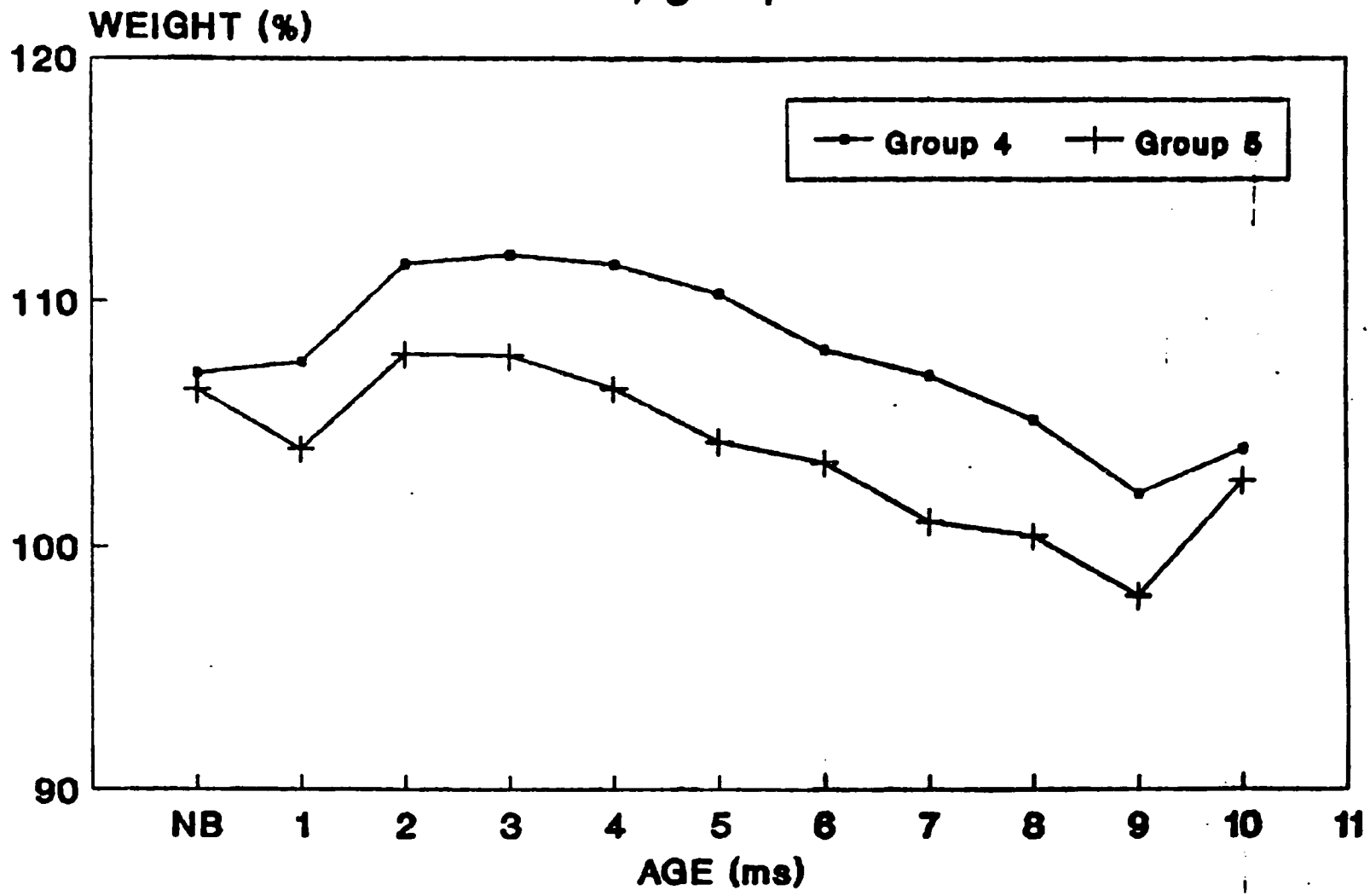


Fig 7

Height adequacy as p50 of NCHS in breast fed infants groups 4 and 5

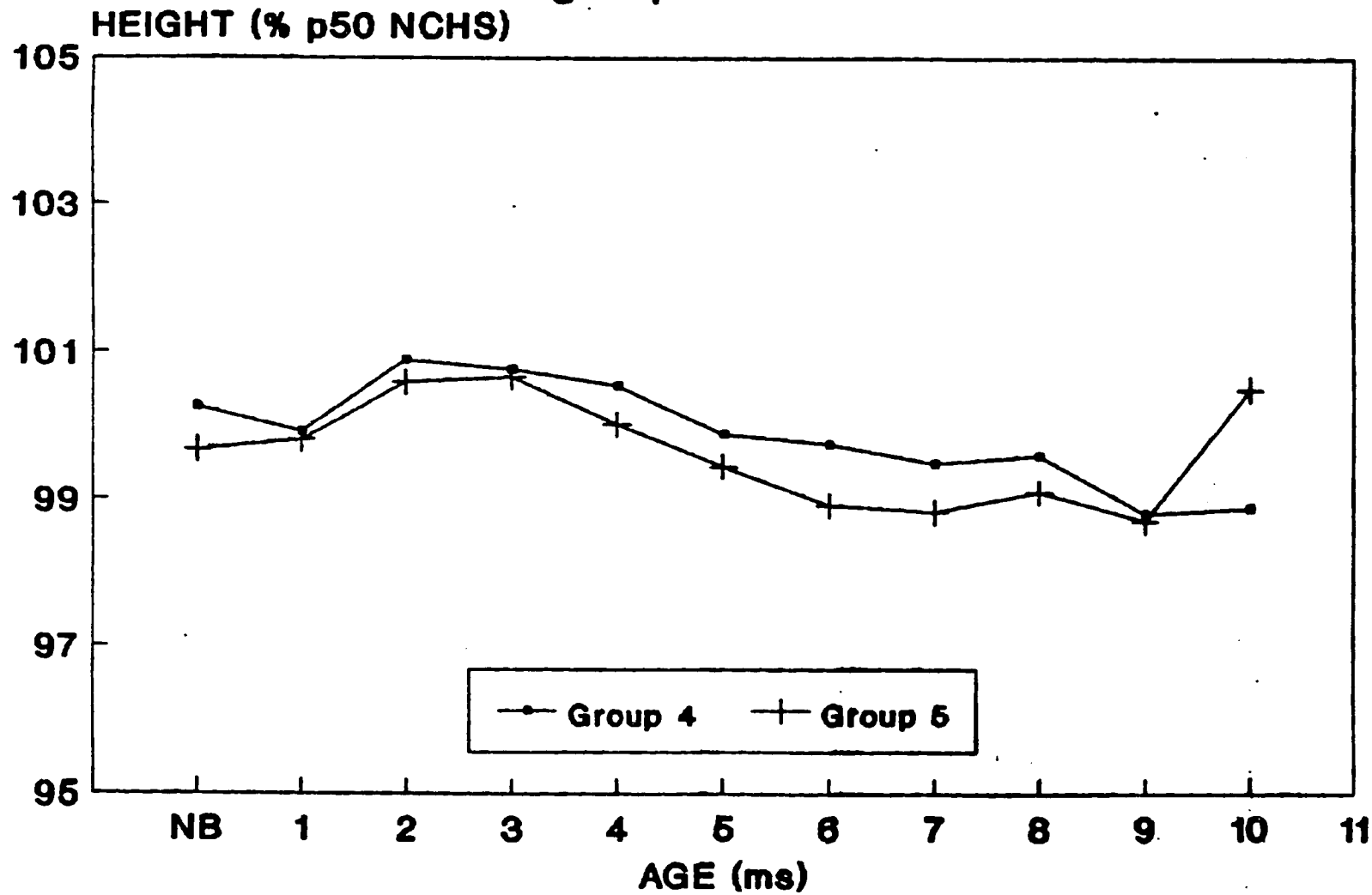
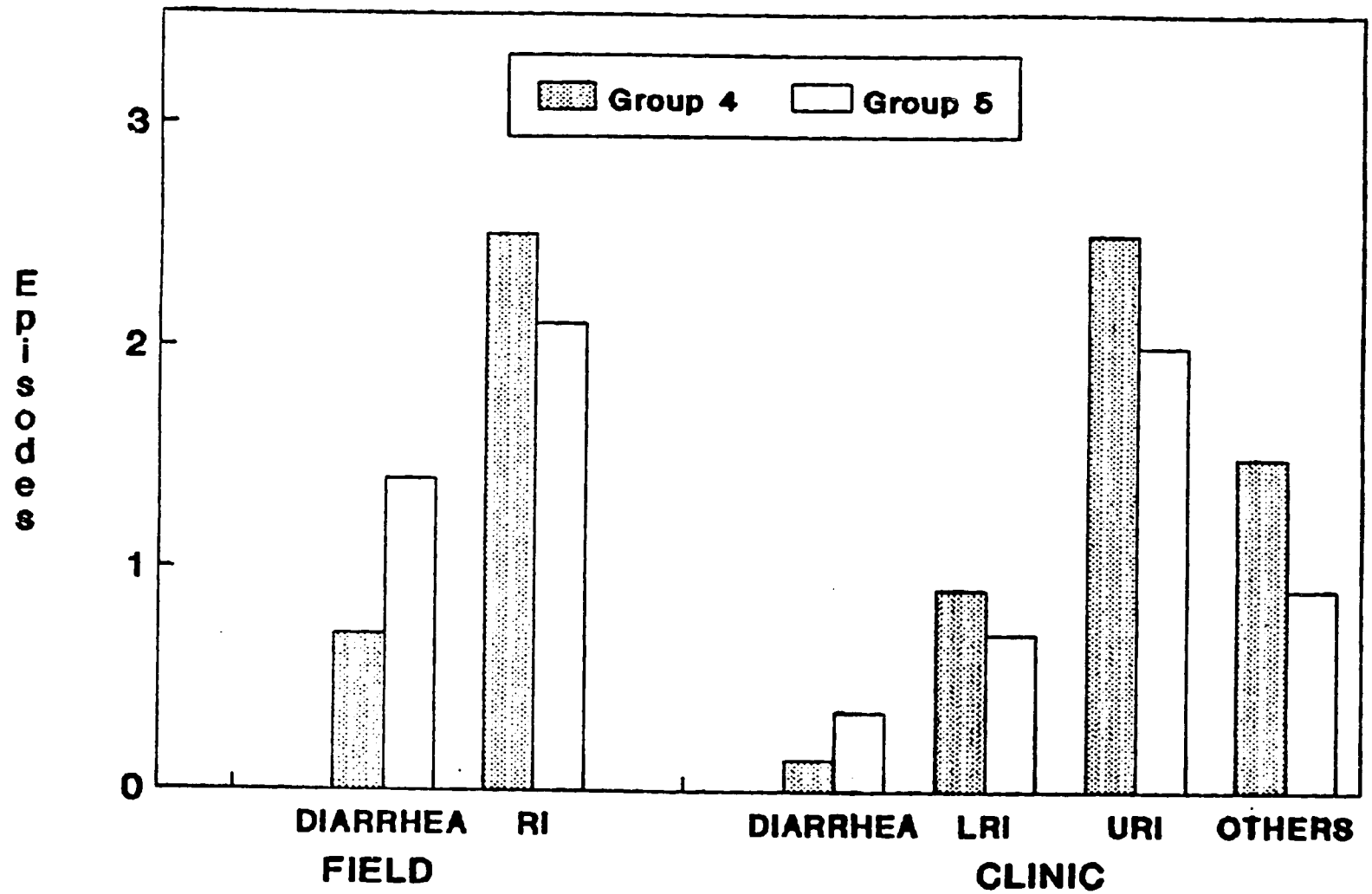


Fig 8

EPISODES OF MORBIDITY IN THE FIELD AND CLINIC

(EPISODES/INFANT/6 MOMTHS)



Special Article

Comparison of in vitro, animal, and clinical determinations of iron bioavailability: International Nutritional Anemia Consultative Group Task Force report on iron bioavailability[1-3]

Allan L Forbes, Catherine E Adams, Maurice J Arnaud, CO Chichester, James D Cook, Bertha N Harrison, Richard F Hurrell, Samuel G Kahn, Eugene R Morris, James T Tanner, and Paul Whittaker

ABSTRACT Relative bioavailability of two iron fortificants, electrolytic Fe and ferric orthophosphate, was related to that of the reference ferrous sulfate with in vitro and rat model depletion-repletion methods in four laboratories to compare values directly with those obtained in a parallel human study. In vitro testing was performed on Fe compounds with both solubility and dialysis in a simulated in vitro gastrointestinal digestion system. Two depletion-repletion techniques, hemoglobin-regeneration efficiency (HRE) and an official method of the Association of Official Analytical Chemists (AOAC), were examined. AOAC relative biological values (RBV) of electrolytic Fe were 0.66 and 0.78 and of $FePO_4$ were 0.25 and 0.34. HRE values were 0.78 and 0.58 for electrolytic Fe and $FePO_4$, respectively. When compared with $FeSO_4$ in a radiolabeled farina-based meal fed to humans, the RBV of $FePO_4$ was 0.25 and electrolytic Fe 0.75. Results obtained with the AOAC method serve as the most reliable prediction of Fe bioavailability in the human although in vitro dialysis is a promising screening technique. *Am J Clin Nutr* 1989;49:225–38.

KEY WORDS Iron, in vitro, iron bioavailability, ascorbic acid

Introduction

The present series of studies is a result of objectives established since 1978 by the International Nutritional Anemia Consultative Group (INACG®) and the protocols that were followed to compare iron bioavailability methods (**Fig 1**). INACG® was organized to promote international efforts aimed at reducing the prevalence of Fe-deficiency anemia.

The importance of Fe in nutrition has been a topic of concern in Third World countries as well as in maternal and child health care in developed countries. The practical regulation of Fe-fortification compounds would be aided by the establishment of reliable methods for predicting Fe-compound bioavailability in humans.

Although Fe bioavailability has long been measured by the rat bioassay, no studies adequately assess Fe bioavailability utilizing the same test Fe compounds in clinical, animal, and in vitro experiments to establish a viable comparison of results of the three approaches. The purpose was to establish a laboratory method for accurately predicting human bioavailability of Fe compounds commonly used in fortification. Electrolytic Fe, ferric orthophosphate, and ferrous sulfate as a reference were tested concomitantly. The absorption of the fortification Fe compounds relative to ferrous sulfate in the clinical studies produced an index that was used to compare the relative biological values obtained in animal studies and in

[1] From the Food and Drug Administration, Washington, DC; the International Life Sciences Institute-Nutrition Foundation, Washington, DC; the Nestle Research Centre, Lausanne, Switzerland; the University of Kansas Medical Center, Kansas City, KS; the Agency for International Development, Washington, DC; and the US Department of Agriculture, Beltsville, MD.

[2] Supported in part by the Bristol-Myers Co, Evansville, IN; Carnation Co, Los Angeles, CA; General Foods, White Plains, NY; General Mills, Inc, Minneapolis, MN; Gerber Products Co, Fremont, MI; The Glidden Co, St Louis, MO; HJ Heinz Co, Pittsburgh, PA; Hoffmann-LaRoche, Inc, Nutley, NJ; Kellogg Co, Battle Creek, MI; Kraft, Inc, Glenview, IL; Nabisco Brands, East Hanover, NJ; Nestle Food Co, Purchase, NY; Procter and Gamble Co, Cincinnati, OH; The Quaker Oats Co, Barrington, IL; Roman Meal, Tacoma, WA; and Ross Laboratories, Columbus, OH and by the Food and Drug Administration, Washington, DC; US Department of Agriculture, Beltsville, MD; World Health Organization, Geneva, Switzerland; and Agency for International Development, Washington, DC (AID cooperative agreement DAN-0227-A-00-2104-00).

[3] Address reprint requests to P Whittaker, Food and Drug Administration, 200 C St SW (HFF-268), Washington, DC 20204.

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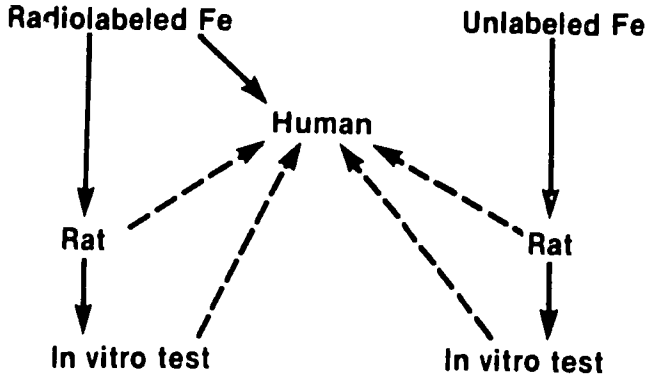

FIG 1. Basic study design for comparing different methods for the prediction of iron bioavailability in the human.

vitro experiments. Research protocols were developed and the project was designed as a collaborative effort involving industrial, academic, and governmental agency laboratories.

There is a continuing need for reliable methods to assess the bioavailability of Fe-fortification compounds especially in countries where fortification is extensively practiced. Ferrous sulfate is the most bioavailable Fe source but produces an organoleptic effect in many food vehicles. This has resulted in the use of more inert forms of Fe, such as phosphate compounds or elemental Fe powders. Although undesirable changes in color and taste can often be avoided by using these forms of Fe, there is commonly a parallel reduction in their gastrointestinal absorption. Because different commercial batches often vary markedly in this respect, reliable methods to assess bioavailability in humans are required.

Several approaches have been used to estimate the bioavailability of Fe sources. A variety of physical chemical methods have been used to evaluate elemental Fe powders by measuring particle size, solubility in hydrochloric acid, and reactive surface area. The most widely used animal method compares the hemoglobin (Hb) response in anemic rats given graded quantities of the fortification Fe source with that obtained from ferrous sulfate. The degree to which these animal measurements predict bioavailability in human subjects is uncertain because there have been no systematic comparisons of bioavailability measurements in animals and humans. The present study was undertaken to examine the accuracy of several methods to assess bioavailability of Fe compounds. Ferric phosphate and electrolytic iron were chosen to provide a wide spectrum of bioavailability. Assessment of the bioavailability of these compounds in humans is the subject of the present report.

The following sections describe production of the Fe compounds and report the results of in vitro, animal, and clinical experiments to measure relative bioavailability. Recommendations are given as to the best methods for predicting the bioavailability of fortification Fe sources in the human. A summary of the study participants and experiments is given in **Table 1**.

Production procedures

Fe compounds for use in the study were produced by New England Nuclear (NEN, North Bellerica, MA). All products were manufactured approximately 2 y before their use in the experiments. Fe compounds were produced according to specifications given to NEN by the INACG® Secretariat, who required that the radioactivity level be 0.12 MBq/mg Fe and that radiolabeled and nonradiolabeled Fe compounds have similar physical and chemical properties and resemble commercially available forms as closely as possible. The nonradioactive and radioactive compounds were manufactured in a similar manner except for scale. The nonradioactive compounds were manufactured and sized in batches that were 4000 times greater than those for the radioactive compounds.

Nonradioactive ferric orthophosphate. Four kilograms ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, designated as $FeSO_4$ hereafter) was dissolved in 1 L warm water. The solution was filtered through glass wool into a 4-L beaker containing a stirring bar. Concentrated phosphoric acid (H_3PO_4) was added with continuous stirring until the solution was blue and a 5% excess of H_3PO_4 had been attained. Sodium hypochlorite (NaOCl) solution was added until the solution turned green with a 5% excess of NaOCl to oxidize the Fe^{+2} to Fe^{+3} ions. The NaOCl was slowly added with constant and vigorous stirring to avoid precipitating Fe as ferrous phosphate. Tribasic sodium phosphate ($Na_3PO_4 \cdot 12H_2O$), dissolved in distilled water to saturation, was slowly added to the 4-L beaker containing the Fe^{+3} ions produced above with constant stirring to precipitate the Fe^{+3} ions as ferric phosphate ($FePO_4$). The solution was covered and allowed to stand overnight. The supernatant was removed and fresh distilled water was added with stirring. $FePO_4$ was again allowed to settle and the water was removed. The $FePO_4$ was washed until the supernatant pH was ~7 for three consecutive washings. The resulting precipitate was filtered through a coarse frit and washed twice using distilled water with continuous suction. Suction was maintained until the precipitate cake cracked. The $FePO_4$ cake was transferred to crystallizing dishes and dried in a 90 °C oven, after which it was crushed to a powder with a mortar and pestle. The $FePO_4 \cdot 2H_2O$ (designated as $FePO_4$ hereafter) was then weighed and transferred to shipping vials, which were sealed under nitrogen. Total yield of $FePO_4$ was 800 g.

Radioactive ferric orthophosphate. Two hundred mg $^{55}FeSO_4$ was produced in a 50-mL centrifuge cone using ^{55}Fe chloride ($^{55}FeCl_3$) (NEN) (specific activity = 3.7 MBq/mg Fe; total activity = 37 MBq) as the starting material. The $^{55}FeCl_3$ was evaporated to dryness and dissolved in 0.05 mol sulfuric acid (H_2SO_4)/L. Sulfur dioxide gas (SO_2) was bubbled through the solution to reduce the Fe^{+3} to Fe^{+2} ions. The solution was then heated to expel all SO_2 and the solution was cooled. $FeSO_4$ (165 mg) was added as the carrier Fe source to provide a total of 200 mg $^{55}FeSO_4$ in 0.05 mol H_2SO_4/L (sp act ~0.15 MBq/mg Fe). This solution was then used as the starting material in the production of $^{55}FePO_4$ following the process outlined above for the nonradioactive compound, producing a final yield of 197 mg.

Nonradioactive electrolytic iron compound. A lucite plating cell (24 × 20 × 28 mm) was immersed in a 50 °C water bath. $FeSO_4$ (50 g) was dissolved in warm water and filtered. The filtered solution was then added to the plating cell, which was maintained at pH 5 by the addition of ammonium hydroxide.

An Fe anode (Armco Inc, Middletown, OH) was placed at one end of the plating cell with a titanium cathode at the other end. Electrodes were connected to a power supply and a constant current density of 215 A/m^2 was maintained through-

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TABLE 1
Summary of iron bioavailability study participants and experiments

Lab	Institution	Experiments			
		Human	Rat hemoglobin repletion	Rat iron absorption	In vitro
1	Nestle Research Lausanne, Switzerland		•	•	•
2	US Department of Agriculture Beltsville, MD			•	•
3	University of Kansas Medical Center Kansas City, KS	•	•	•	
4	Food and Drug Administration Washington, DC		•		•

out the plating process. Each plating cycle was 4–8 h, after which plated Fe was removed from the cathode. The plated Fe was then pulverized with a hand grinder to ~6-mm pieces and passed through a ball mill purged with N_2. Fe was removed from the ball mill at 4–8-h intervals and was passed through brass sieves (sizes 30 and 10 μm) to select particles with an average size of 20 μm. (Average size particles of 20 μm were those that passed through the 30-μm sieve but were caught in the 10-μm sieve.) The sized electrolytic Fe, total yield 1000 g, was then weighed and dispensed into shipping vials sealed with N_2 (Fig 2).

Radioactive electrolytic iron compound. Radioactive $^{55}FeCl_3$ (sp act = 3.7 MBq/mg Fe; total activity = 0.74 MBq) was converted to the sulfate form as described in the process for $^{55}FePO_4$. Subsequent to plating, radiolabeled electrolytic ^{55}Fe was produced according to the procedure for the manufacture of nonradioactive electrolytic Fe, yielding a total of 1.5 g. The Fe content of both the radioactive and nonradioactive electrolytic Fe was 997 g/kg.

I Iron bioavailability by in vitro tests

Eugene R Morris, Richard F Hurrell, and Paul Whittaker

Materials and methods

Fe sources were investigated for their solubility in dilute hydrochloric acid (HCl) and for their dialyzability in an in vitro simulated gastrointestinal digestion system (1). Laboratories 1 and 4 compared Fe sources by solubility. Laboratories 1, 2, and 4 made the dialyzable Fe determinations.

FIG 2. Electrolytic Fe of 20 μm average size at 12 000× magnification.

Solubility in dilute HCl

The method used for nonradioactive Fe sources was based on that of Shah et al (2). An aliquot calculated to contain 20 mg Fe was weighed into a 500-mL conical flask and 250 mL 0.02 mol HCl/L (pH 1.2), preheated to 37 °C, was added. The flasks were placed in a shaking water bath at 37 °C and gently shaken at a rate of 1 Hz. Two 2-mL aliquots were taken at 30 min and centrifuged for 5 min at 760 × g. One milliliter of supernatant was transferred to a 25-mL volumetric flask and diluted to volume with 0.02 mol HCl/L. Fe content was measured by atomic absorption spectroscopy (AAS) (3).

Dialyzable iron

The method of Miller et al (1) as modified by Hurrell et al (4) was used: Dialyzable Fe passes from an in vitro enzymatic digestion of a test meal into the interior of a dialysis bag containing 25 mL 0.5 mol $NaHCO_3$/L. The test meal was a scaled-down version of the farina meal fed in the human study. The nonradioactive test Fe sources and $FeSO_4$ were premixed separately with powdered sucrose in a #45 sieve or a coffee mill to a concentration of 0.6 mg Fe/g sucrose.

The test meal was prepared as follows: 50 mL distilled water containing 0.1 g NaCl was brought to a boil in a 250-mL Erlenmeyer flask or 150-mL wide-neck conical flask. Farina (8 g) was added and the mixture was boiled for 1 min with constant stirring. Whole milk (24.0 g), 2.8 g butter, 3.33 g sucrose-Fe premix (containing 2 mg Fe), and 1.47 g powdered sucrose were added and mixed thoroughly. When the mixture was cool, the pH was adjusted to 2 with 6.0 mol HCl/L and the final weight of the test meal was adjusted to 100.0 g with 0.01 mol HCl/L. Twenty-gram aliquots were measured in triplicate for dialyzable Fe as described by Hurrell et al (4) with bathophenanthroline used to measure Fe in the dialysate.

Results

Solubility in dilute HCl

The solubility of the Fe sources was determined in duplicate using 20-mg Fe samples in 250 mL 0.02 mol HCl/L at 37 °C. The electrolytic Fe was much more soluble than the $FePO_4$. After 30 min shaking 75% of the electrolytic Fe was dissolved in laboratory 1 and 60% in labora-

TABLE 2
Dialyzable iron from farina meal fortified with nonradioactive iron*

Fe source	Laboratory 1		Laboratory 2		Laboratory 4	
	Dialyzable Fe (% of dose)	Relative dialyzability†	Dialyzable Fe (% of dose)	Relative dialyzability	Dialyzable Fe (% of dose)	Relative dialyzability
$FeSO_4$	2.63 ± 0.04	1.00 ± 0.02	3.40 ± 0.31	1.00 ± 0.09	1.39 ± 0.11	1.00 ± 0.08
Electrolytic Fe	1.96 ± 0.01	0.75 ± 0.01	1.98 ± 0.13	0.58 ± 0.04	0.99 ± 0.01	0.71 ± 0.01
$FePO_4$	0.74 ± 0.03	0.28 ± 0.02	0.91 ± 0.17	0.27 ± 0.05	0.64 ± 0.04	0.46 ± 0.03

* $\bar{X}$ ± SEM.
† Relative dialyzability = dialyzable test Fe (% of dose)/dialyzable Fe of $FeSO_4$ (% of dose).

tory 4. $FePO_4$ was very insoluble with a range of 3–4% solubility after 30 min.

Dialyzable iron

The amount of Fe dialyzed from the in vitro enzymatic digestion of the $FeSO_4$-fortified farina meal ranged from 1.39 to 3.40% of the dose in the three participating laboratories (**Table 2**). Dialyzability relative to $FeSO_4$ was 0.58–0.75 for electrolytic Fe and 0.27–0.46 for $FePO_4$.

Discussion

Solubility in dilute HCl

Although the solubility of an Fe source varies with time, a single value was necessary to make a direct comparison with the human and animal relative bioavailability data. Consequently, a 30-min shaking period was chosen as the basis of preliminary tests to compare solubility of Fe and its relative bioavailability as measured in the Hb-repletion studies. As expected electrolytic Fe solubility was intermediate and $FePO_4$ was rather insoluble compared with $FeSO_4$.

Dialyzable iron

The dialyzable Fe values were low and variable among laboratories. The low values are presumably due to inhibitory substances present in cereals that bind Fe and prevent its dialysis. The variable relative dialyzability values indicate the need for rigorous standardization of the method. Time expended in different manipulations is critical if the method is to be used to predict the bioavailability of Fe sources. The longer an Fe source remains in a liquid system at low pH, the more it will dissolve and be dialyzed. If sufficient time is allowed for the Fe source to dissolve, it will be dialyzed similarly to $FeSO_4$ or other soluble Fe sources. In the present study each laboratory used an identical method but may not have adhered to a strict time schedule. Nevertheless, $FePO_4$ was found to be a relatively poorly bioavailable Fe source by all laboratories and the electrolytic Fe a fairly good one with about two-thirds the dialyzability of $FeSO_4$. The $FePO_4$ was dialyzed to a greater extent than would be expected from its poor solubility in dilute HCl possibly because as the soluble Fe is removed by dialysis the equilibrium reaction is shifted to dissolve the insoluble $FePO_4$ to replace the dialyzed Fe.

II Assessment of bioavailability of fortification iron compounds in the rat model

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Materials and methods

Evaluation of the hemoglobin repletion tests in rats

Bioavailability tests of the nonradioactive Fe sources were based on Hb repletion in anemic rats as measured by two different methods. Laboratories 1 and 4 used an official method of the Association of Official Analytical Chemists (AOAC) (5, 6), whereas laboratory 3 used a technique similar to that of Mahoney and Hendricks (7), the Hb-regeneration efficiency (HRE) method. Laboratory 4 used the HRE method to compare nonradioactive and radioactive Fe sources.

Modified AOAC method—laboratories 1 and 4. One hundred forty male weanling Sprague-Dawley rats, individually housed in stainless steel cages, were fed a low-Fe diet and deionized, distilled water ad libitum for 24 d. The low-Fe diet containing 1 mg Fe/kg and 3 mg Fe/kg for laboratories 1 and 4, respectively, as verified by atomic absorption spectroscopy (AAS) (Perkin-Elmer model 5000, Norwalk, CT) with wet ashing as described by Boline and Schrenk (3) contained (g/kg): 684.8 glucose·H_2O, 200 vitamin-free casein, 50 corn oil, 20 monosodium phosphate, 20 calcium carbonate, 5 potassium chloride, 5 sodium chloride, 2.7 trace-element premix, 1.5 choline chloride, 10 vitamins (AIN 1976), and one DL-methionine. The trace-element premix contained (g/kg): 738.2 $MgSO_4$, anhydrous; 196.6 $ZnSO_4 \cdot 7H_2O$; 57.3 $MnSO_4 \cdot 7H_2O$; 7.3 $CuSO_4 \cdot 5H_2O$; and 0.6 KIO_3. After the depletion period the rats were weighed and ~100 μL blood was drawn from the orbital venous plexus (8) for Hb determination with a commercial kit (Hoffman-La Roche Diagnostica, Basel, Switzerland) following the cyanmethemoglobin procedure (9). One hundred ten rats with Hb values between 30 and 60 g/L were selected for the repletion test. Eleven groups of 10 rats, housed according to a randomized block design, received 1 of 11 experimental diets, which were prepared by fortifying the low-Fe diet with $FeSO_4$ or with one of the test Fe sources. $FeSO_4$ was added at 0, 6, 12, 18, and 24 mg Fe/kg diet; electrolytic Fe and $FePO_4$

were added at 12, 24, and 36 mg Fe/kg. Diet levels were verified by AAS (3).

The animals were fed the Fe-supplemented diets ad libitum for 14 d, after which they were weighed, blood was drawn, and Hb level was measured as before. Food intake was recorded throughout the repletion period. The bioavailability of each test Fe source relative to $FeSO_4$ was calculated by comparing gain in Hb with the Fe level in the diet by the slope ratio procedure (10, 11).

HRE method—laboratory 3. Thirty male weanling Sprague-Dawley rats, individually housed in stainless steel cages, were fed ad libitum a low-Fe diet (US Biochemical Co, Cleveland, OH) containing 9 mg Fe/kg of diet as verified by AAS (3) and deionized, distilled water for 8 d. Approximately 1 mL blood was drawn from the orbital venous plexus of each animal into a heparinized capillary tube on days 4 and 6 (8). After 8 d of depletion, the animals were weighed and blood was again drawn for Hb determination by the cyanmethemoglobin method (9). The animals were randomized into three groups of 10 rats each with approximately equal average body weight and Hb concentration. Each group was fed one of three experimental diets over a 10-d repletion period. The low-Fe diet contained (g/kg diet): 560 cornstarch, 140 hydrogenated vegetable oil, 270 vitamin-free casein, 30 salt mixture without $FePO_4$ (12), and 4 total vitamin supplement (US Biochemical Co).

The experimental diets had the same composition as the low-Fe diet and were supplemented with 35 mg Fe/kg as $FeSO_4$, electrolytic Fe, or $FePO_4$. Dietary levels were again verified by AAS (3). Food intake was recorded. After a 10-d repletion period the animals were again weighed and blood was drawn for Hb determination. HRE values measure the efficiency of converting dietary Fe into Hb relative to the amount of dietary Fe consumed during repletion.

Hb Fe (mg) is determined as (7, 13)

$$\text{Hb Fe, mg} = \text{body wt, kg} \times \frac{0.075\ \text{L blood}}{\text{body wt, kg}} \times \frac{\text{Hb, g}}{\text{blood, L}} \times \frac{3.35\ \text{mg Fe}}{\text{Hb, g}} \quad (1)$$

HRE values were then calculated for each animal as follows (7):

$$\text{HRE} = \frac{(\text{Hb Fe, mg})_{\text{final}} - (\text{Hb Fe, mg})_{\text{initial}}}{\text{Fe consumed, mg}} \quad (2)$$

HRE method laboratory 4. The HRE method was also used in laboratory 4 although it was modified because of a limited quantity of fortification Fe. Fifty male weanling rats were randomized into five groups of 10 animals each so that mean body weight and Hb concentration were approximately equal among the groups. The procedure was identical to that used in laboratory 3 except that animals were depleted for 7 d and repleted for 9 d and five diets were prepared for the repletion period containing 20 mg fortification Fe/kg diet, verified by AAS (3). Each diet was fed during the 9-d repletion period and supplemented with one of the nonradiolabeled or radiolabeled Fe compounds as follows: *1*) $FeSO_4 \cdot 7H_2O$; *2*) electrolytic Fe; *3*) $FePO_4 \cdot 2H_2O$; *4*) electrolytic ^{55}Fe; and *5*) $^{55}FePO_4 \cdot 2H_2O$. Dietary consumption was recorded daily with correction made for spillage. The HRE values were calculated as in laboratory 3.

Evaluation of the iron absorption test in rats

This assay was developed to resemble human Fe absorption studies as closely as possible. Rats aged 7–8 wk were fed a single meal containing both $^{59}FeSO_4$ and one of the ^{55}Fe-labeled test Fe sources to obtain simultaneous comparisons in the same animal. The level of incorporated red cell activity was measured 2 wk later by scintillation counting and by calculating absorption of the test Fe sources relative to $FeSO_4$ (14, 15). Three laboratories participated in the Fe-adequate rat study. Laboratories 1 and 2 used basically the same methodology whereas laboratory 3 used a slightly modified experimental approach.

Each laboratory was provided with identical aliquots of 5 mg electrolytic ^{55}Fe (0.61 MBq, 8 March 1985) and 25 mg $^{55}FePO_4$ (0.93 MBq, 8 March 1985) but used its own $^{59}FeSO_4$. The latter was produced by dissolving nonradioactive $FeSO_4$ in water and adding enough $^{59}FeSO_4$ to yield a specific activity of 0.14–0.19 MBq/mg Fe. The solution was freeze-dried to a powder of ~0.33 as Fe, which was confirmed by AAS (3).

Laboratories 1 and 2. Twenty male weanling Sprague-Dawley rats were housed individually in stainless steel cages and fed ad libitum an Fe-adequate diet containing 35 mg Fe/kg as $FeSO_4$ for 4–5 wk. The diet was identical to that used in the Hb repletion study but with a higher Fe content. Deionized, distilled water was provided throughout. After this preliminary period the rats were divided into two groups of 10 rats and were trained to eat by meal. Each rat was offered 20 g diet between 1800 and 2000 h and any remaining diet between 0700 and 0900 the next morning. At 1800 on the third day, rats weighing an average of ~250 g were fed one meal consisting of 10 g diet containing both $^{59}FeSO_4$ and one of the labeled test Fe sources. Each group of 10 rats received a different ^{55}Fe-labeled test Fe source. The meal was removed after 2–2.5 h, by which time most of it had been consumed. Food consumption was measured. The rats were placed in a whole-body counter immediately after the meal to determine emitted gamma radiation (16, 17). The Fe-adequate diet was continued the next morning and was fed ad libitum with deionized, distilled water for 14 d. On day 14, after whole-body counting to confirm the level of ^{59}Fe absorbed, rats were anesthetized with ether and blood was drawn by cardiopuncture for radioactivity measurements.

In laboratory 1 the radiolabeled meal contained ~400 μg Fe, half of which came from $^{59}FeSO_4$ and half from one of the ^{55}Fe experimental sources. Meals were prepared by thoroughly mixing 1 g of a glucose premix containing the radioactive Fe sources with 9 g of the low-Fe basal diet (as used in the Hb-repletion test) from which an equivalent quantity of glucose had been omitted. The glucose-Fe premixes were prepared in a coffee mill and homogeneity and Fe content were confirmed by AAS (3).

Laboratory 2 prepared and fed radiolabeled meals similar to those in laboratory 1. The meals were prepared by thoroughly mixing 2 g glucose-Fe premix with 8 g low-Fe basal diet. The glucose-Fe premixes were produced by mixing and pulverizing until homogeneity was assured. Each 10-g meal contained ~230 μg Fe as $^{59}FeSO_4$ plus one of the following: 370 μg Fe as electrolytic ^{55}Fe or 650 μg Fe as $^{55}FePO_4$.

In laboratory 1 ^{59}Fe and ^{55}Fe activity in 100 μL blood was simultaneously measured by liquid scintillation counting after digestion in 1.5 mL Protosol® (DuPont NEN, Boston) and ethanol (1:2) and decolorization with 0.5 mL 30% H_2O_2 for 30–60 min in a shaking water bath at 60 °C (15). Background counts were determined in identical quantities of nonradioactive blood. To ascertain the amount of ^{59}Fe and ^{55}Fe consumed, 1 g of each glucose premix was digested with 2 mL concentrated HNO_3 and 2 mL concentrated HCl (in quintuplicate) and was diluted with deionized, distilled water to 100 mL. One hundred microliters digested premix and 100 μL nonradioactive blood were digested, decolorized, and counted as above. Laboratory

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2 used the same method with 200 μL blood. The glucose-Fe premixes (0.5 g) were ashed, dissolved in HCl, and diluted to 25 mL with water.

Absorption of each isotope was calculated by dividing cpm into total blood volume (body wt, kg × 0.075 L/kg) (13) by cpm in the food ingested. No adjustment was made for the portion of absorbed dietary Fe not present in the blood. The retention of ^{59}Fe was also determined by whole-body counter measurement.

Laboratory 3. Laboratory 3 performed the same experiment but with the rats that had previously been used for the Hb-repletion test and had received an Fe-adequate diet containing 35 μg Fe/g for 10 d. The animals were randomized into two groups of mean body weight 136 ± 4 and 136 ± 2 g and Hb concentration 117 ± 5 and 116 ± 6 g/L and were then fed a meal containing both $^{59}FeSO_4$ and one of the ^{55}Fe-labeled test sources.

Two diets were prepared for the Fe-adequate test, each containing 33 μg Fe/g fortification Fe as confirmed by AAS (3). Each animal received 20 g diet containing 330 μg Fe (26 kBq ^{55}Fe) from the ^{55}Fe-labeled compound ($FePO_4$ or electrolytic Fe) and 330 μg Fe (19 kBq ^{59}Fe) from $FeSO_4$.

The meal was fed during an 18-h overnight period. Rats and fecal excretion from the 18-h interval were placed in a whole-body counter to measure $^{59}FeSO_4$ consumed (16, 17). Rodent laboratory food (5001, Ralston Purina Co. St Louis, MO) was then fed ad libitum with deionized, distilled water for 14 d. On day 14, rats were again measured in the whole-body counter for verification of $^{59}FeSO_4$ absorption. Animals were weighed and blood was drawn from the orbital venous plexus (8) into a heparinized capillary tube for blood variable and radioactivity measurements.

^{59}Fe and ^{55}Fe activity in 100 μL blood was simultaneously determined by liquid scintillation counting (15). A mixture of Soluene-350® (United Technologies Packard, Downers Grove, IL) and isopropanol 1:2 (vol:vol) was added and the vial was swirled gently; within 0.5 h the blood was solubilized. Five-tenths milliliter 30% H_2O_2 was then added dropwise to increase the bleaching effect. To dissociate the peroxides vials were placed in a 40 °C water bath for 30 min. After the vials were cooled to room temperature, a mixture of 15 mL Insta-Gel® (United Technologies Packard) and 0.5 mol HCl/L (10:1, vol:vol) was added to the vial, vortex mixed, and counted. Background counts were determined using the same quantity of nonradioactive rat blood. To determine the amount of ^{59}Fe and ^{55}Fe consumed, 20 g of each diet was first wet-digested and counted using the above procedure.

Absorption of each isotope and retention of ^{59}Fe were determined by the same procedure as in laboratories 1 and 2.

Results

Relative biological values (RBV) of electrolytic Fe determined by the modified AOAC method in laboratories 1 and 4 as compared with the reference $FeSO_4$ are shown in **Table 3.** Laboratory 3 used the HRE technique to calculate RBV (**Table 4**). Laboratory 4, using the HRE method, compared bioavailability values of radiolabeled and nonradiolabeled electrolytic Fe and $FePO_4$ (**Table 5**).

The absorption values of $^{59}FeSO_4$ and electrolytic ^{55}Fe in the rat as reported by the three participating laboratories are shown in **Table 6.** The elemental ^{55}Fe source was not as well absorbed as $^{59}FeSO_4$. In each laboratory the retention of ^{59}Fe on day 14 measured by the whole-body counter closely resembled ^{59}Fe absorption values calculated from blood radioactivity measurements.

Fe absorption values for rats simultaneously fed $^{59}FeSO_4$ and $^{55}FePO_4$ are shown in **Table 7.** Absorption of ^{59}Fe was similar to that in the above experiment; however, the $^{55}FePO_4$ was approximately half as well absorbed as the electrolytic ^{55}Fe source.

Discussion

Using the modified AOAC technique, laboratories 1 and 4 reported similar RBV for the test Fe sources of 0.66 and 0.78 for the electrolytic Fe and 0.25 and 0.34 for $FePO_4$, respectively. HRE-derived relative biological values reported by laboratory 3 were 0.78 and 0.58 for the same two compounds. Hb gain in the experimental rats averaged 86 g/L in those fed $FeSO_4$, 64 g/L in those fed electrolytic Fe, and 44 g/L in those fed $FePO_4$. By use of Hb gain to calculate RBV, values of 0.75 and 0.51 were derived for the electrolytic Fe and $FePO_4$, respectively. These values were slightly lower but of the same order as those calculated from the HRE ratio. In this laboratory the RBV for $FePO_4$, the lesser bioavailable compound, was higher than that reported from the AOAC method; however the RBV for electrolytic Fe was similar.

The apparent overestimation of RBV of $FePO_4$ using the HRE method (Tables 4 and 5) as compared with AOAC values (Table 3) is probably a result of differences in the length and method of depletion and subsequent repletion. In laboratories using the modified AOAC method, weanling rats were depleted to an Hb level of 30–60 g/L over 24 d solely by dietary means. In the HRE method weanling rats were depleted within 7–8 d by a combination of phlebotomy and low-Fe diet. The anemic rats were then fed diets containing a single level of Fe (20 or 35 mg Fe/kg diet) for 9–10 d. Fe stores of animals used in the HRE studies were therefore not as severely depleted (18) as those used in the AOAC protocol. The shorter depletion period of the HRE method resulted in anemic rats being ~2 wk younger when repleted with the test Fe sources. The HRE method also accounts for the body weight-gain factor in calculating relative bioavailability.

Test values obtained in laboratory 4, which utilized the HRE method in comparing radiolabeled and nonradiolabeled iron compounds (Table 5), were similar to those of laboratory 3, which tested nonradiolabeled Fe compounds by the same method. HRE values for nonlabeled forms of $FePO_4$ were 0.46 and 0.44, of electrolytic Fe were 0.65 and 0.59, and of $FeSO_4$ were 0.76 and 0.75 for laboratories 4 and 3, respectively. Results in laboratory 4 indicated that values for radiolabeled and nonradiolabeled test compounds were not significantly different (Table 5).

Mean percent absorption of Fe from $^{59}FeSO_4$ by rats in the different laboratories varied from 20 to almost 70% although within the same laboratory mean absorption of

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TABLE 3
Relative biological values (RBV) of test iron sources as measured by the AOAC method*

Iron source	Supplemental Fe	Body wt gain	Hb gain	Slope	RBV†
	mg Fe/kg diet	*g*	*g/L*		
Laboratory 1					
	0	38 ± 5	−10 ± 1		
$FeSO_4$	6	69 ± 7	5 ± 1	0.2203 ± 0.0077	1.00
	12	86 ± 7	15 ± 2		
	18	102 ± 6	32 ± 4		
	24	105 ± 7	42 ± 1		
Electrolytic Fe	12	65 ± 5	8 ± 1	0.1461 ± 0.0053	0.66 ± 0.02
	24	81 ± 6	26 ± 2		
	36	97 ± 6	42 ± 3		
$FePO_4$	12	46 ± 6	−3 ± 2	0.0560 ± 0.0053	0.25 ± 0.02
	24	57 ± 5	2 ± 2		
	36	71 ± 3	12 ± 2		
Laboratory 4					
	0	55 ± 13	−10 ± 3		
$FeSO_4$	6	58 ± 15	6 ± 6	0.2360 ± 0.0342	1.00
	12	84 ± 13	20 ± 4		
	18	89 ± 13	42 ± 5		
	24	95 ± 13	49 ± 5		
Electrolytic Fe	12	77 ± 12	17 ± 3	0.1832 ± 0.0312	0.78 ± 0.03
	24	69 ± 15	42 ± 7		
	36	86 ± 9	58 ± 5		
$FePO_4$	12	63 ± 12	3 ± 3	0.0803 ± 0.0269	0.34 ± 0.03
	24	60 ± 11	13 ± 3		
	36	79 ± 10	21 ± 4		

* Ten animals per group were fed the test diets for 14 d. $\bar{x}$ ± SEM.
† Slope of test Fe source (Hb gain vs level of supplementation) relative to slope of $FeSO_4$.

$^{59}FeSO_4$ from the two different meals fed was almost identical. The high absorption of iron by rats in laboratory 3 was due to their being slightly anemic (mean Hb 117 g/L). The ^{55}Fe-labeled test Fe sources were also better absorbed by rats in laboratory 3, and consequently the absorption of these Fe sources relative to the absorption of $^{59}FeSO_4$ was similar among the laboratories. This is especially true for $^{55}FePO_4$ (~25% that of $^{59}FeSO_4$).

TABLE 4
Relative biological value (RBV) of test iron sources as measured by the HRE method*

	$FePO_4$	Electrolytic Fe	$FeSO_4$	*p* value	LSD†
Laboratory 3					
Body wt, initial (g)	88 ± 3	88 ± 4	89 ± 3	0.952	NS
Body wt, final (g)	135 ± 4	137 ± 4	135 ± 2	0.875	NS
Body wt gain (g)	47 ± 3	49 ± 2	46 + 3	0.615	NS
Iron intake (mg)	4.83 ± 0.06	4.90 ± 0.06	4.71 ± 0.06	0.111	NS
Hb, initial (g/L)	55 ± 2	55 ± 1	56 ± 2	0.959	NS
Hb, final (g/L)	99 ± 3	119 ± 3	142 ± 4	<0.001	9
Hb gain (g/L)	44 ± 3	64 ± 3	86 ± 3	<0.001	8
Hct, initial	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.984	NS
Hct, final	0.38 ± 0.01	0.44 ± 0.01	0.47 ± 0.01	<0.001	0.02
Hct gain	0.17 ± 0.01	0.22 ± 0.01	0.26 ± 0.01	<0.001	0.03
HRE ratio	0.44 ± 0.02	0.59 ± 0.03	0.75 ± 0.02	<0.001	0.07
RBV ratio‡	0.58 ± 0.03	0.78 ± 0.04	1.00 ± 0.03	<0.001	0.09

* Ten animals per group were fed the test diets containing 35 mg fortification Fe/kg diet during the 10 d repletion period. One animal died in the $FeSO_4$ group. $\bar{x}$ ± SEM.
† Mean differences must equal or exceed the least significant difference value to be significantly different at the 0.05 probability level. NS signifies not significantly different.
‡ Hb regeneration efficiency of test Fe source divided by mean Hb regeneration efficiency of $FeSO_4$.

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TABLE 5
Relative biological value (RBV) of test iron sources as measured by the HRE method*

	$FePO_4$	Electrolytic Fe	$FeSO_4$	$^{55}FePO_4$	Electrolytic ^{55}Fe	*p* value	LSD†
Laboratory 4							
Body wt, initial (g)	86 ± 2	86 ± 2	86 ± 1	86 ± 2	85 ± 3	0.977	NS
Body wt, final (g)	118 ± 2	117 ± 2	119 ± 1	122 ± 2	120 ± 2	0.491	NS
Body wt gain (g)	32 ± 2	31 ± 2	33 ± 1	35 ± 1	36 ± 2	0.204	NS
Iron intake (mg)	2.47 ± 0.00	2.46 ± 0.01	2.46 ± 0.01	2.37 ± 0.02	2.42 ± 0.03	0.003	0.06
Hb, initial (g/L)	74 ± 2	74 ± 2	75 ± 2	75 ± 2	75 ± 2	0.999	NS
Hb, final (g/L)	93 ± 2	109 ± 4	116 ± 2	88 ± 1	101 ± 2	<0.001	7
Hb gain (g/L)	19 ± 3	35 ± 2	41 ± 2	14 ± 2	27 ± 3	<0.001	7
Hct, initial	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.29 ± 0.01	0.956	NS
Hct, final	0.37 ± 0.01	0.42 ± 0.01	0.46 ± 0.01	0.34 ± 0.01	0.42 ± 0.01	<0.001	0.02
Hct gain	0.07 ± 0.01	0.12 ± 0.01	0.17 ± 0.01	0.04 ± 0.01	0.12 ± 0.01	<0.001	0.02
HRE ratio	0.46 ± 0.03	0.65 ± 0.05	0.76 ± 0.03	0.46 ± 0.02	0.61 ± 0.03	<0.001	0.09
RBV ratio‡	0.61 ± 0.04	0.86 ± 0.06	1.00 ± 0.03	0.60 ± 0.03	0.80 ± 0.05	<0.001	0.12

* Ten animals per group were fed the test diets containing 20 mg fortification Fe/kg diet during the 9 d repletion period. $\bar{x}$ ± SEM.

† Mean differences must equal or exceed the least significant difference value to be significantly different at the 0.05 probability level. NS signifies not statistically significant.

‡ Mean individual Hb regeneration efficiency of test Fe source divided by mean Hb regeneration efficiency of $FeSO_4$.

The agreement among laboratories was not as precise concerning the relative absorption of electrolytic ^{55}Fe. Laboratories 1 and 4 reported absorption ratios of 0.48 and 0.47, respectively, whereas laboratory 2 reported a value of 0.69. The reason for this is unclear but could be related to lower absorption of $FeSO_4$ in the rats in laboratory 2 (confirmed by whole-body counter).

III Assessment of bioavailability of fortification iron compounds in human subjects

Paul Whittaker and James D Cook

Subjects and methods

Subjects

Absorption was measured in 25 normal volunteers, 12 men and 13 women, aged 18–38 y. None of the subjects gave a history of disorders that might affect the absorption of Fe from the gastrointestinal tract. All subjects were in good health although two had depleted Fe stores as reflected by a serum ferritin level < 12 μg/L. All subjects gave written informed consent before participating in the studies, which were carried out in accordance with the procedures of the Human Subjects Committee at the University of Kansas Medical Center.

Radiolabeled compounds

A limited amount of electrolytic ^{55}Fe and $^{55}FePO_4$ was prepared together with a larger quantity of unlabeled material under identical conditions. The radiolabeled and unlabeled Fe were mixed at the time of administration to attain the desired specific activity.

Experimental design

The bioavailability of each of the two radiolabeled forms of Fe was evaluated in two separate studies by comparison with

TABLE 6
Absorption (% of dose) of electrolytic ^{55}Fe

	Laboratory 1			Laboratory 2			Laboratory 3		
Rat	$^{59}FeSO_4$ (A)	^{55}Fe (B)	Ratio (B:A)	$^{59}FeSO_4$ (A)	^{55}Fe (B)	Ratio (B:A)	$^{59}FeSO_4$ (A)	^{55}Fe (B)	Ratio (B:A)
1	39.2	22.1	0.56	24.7	15.6	0.63	63.0	27.0	0.43
2	34.0	15.7	0.46	16.5	11.3	0.68	77.5	30.5	0.39
3	32.9	16.8	0.51	19.0	15.6	0.82	46.3	20.7	0.45
4	35.6	17.5	0.49	14.4	9.8	0.68	70.8	31.3	0.44
5	31.0	14.8	0.48	28.7	17.5	0.61	64.8	26.6	0.41
6	22.2	9.9	0.45	21.0	14.9	0.71	74.1	44.5	0.60
7	29.4	12.8	0.44	22.6	15.7	0.69	46.5	20.6	0.44
8	36.9	18.1	0.49	15.2	11.4	0.75	53.3	21.9	0.41
9	28.5	14.0	0.49	22.9	17.8	0.78	54.1	28.0	0.52
10	37.2	17.8	0.48	12.2	7.8	0.64	70.0	33.3	0.48
11	—	—	—	—	—	—	67.5	34.9	0.52
12	—	—	—	—	—	—	60.1	34.2	0.57
13	—	—	—	—	—	—	77.5	37.4	0.48
Mean	32.7	16.0	0.48	19.7	13.7	0.69	63.5	30.1	0.47
±1 SEM	1.6	1.1	0.01	1.6	1.1	0.02	3.0	1.9	0.01

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TABLE 7
Absorption (% of dose) of $^{55}FePO_4$, dihydrate

Rat	Laboratory 1			Laboratory 2			Laboratory 3		
	$^{59}FeSO_4$ (A)	^{55}Fe (B)	Ratio (B:A)	$^{59}FeSO_4$ (A)	$^{55}FePO_4$ (B)	Ratio (B:A)	$^{59}FeSO_4$ (A)	$^{55}FePO_4$ (B)	Ratio (B:A)
1	32.6	7.2	0.22	24.0	6.8	0.28	48.2	9.4	0.20
2	33.8	6.5	0.19	24.6	6.3	0.26	68.1	10.4	0.15
3	27.1	8.8	0.32	13.1	3.8	0.29	64.7	11.5	0.18
4	41.5	11.3	0.27	15.9	5.3	0.33	73.0	19.5	0.27
5	40.1	10.0	0.25	20.9	8.2	0.39	63.6	18.3	0.29
6	32.9	7.9	0.24	19.1	3.9	0.20	79.5	20.3	0.26
7	30.4	9.5	0.31	22.3	4.8	0.22	71.7	22.6	0.32
8	44.8	13.2	0.29	26.6	4.6	0.17	72.4	14.6	0.20
9	24.7	6.4	0.26	24.2	6.2	0.26	61.7	18.6	0.30
10	39.1	10.7	0.27	—	—	—	71.4	11.8	0.17
11	—	—	—	—	—	—	69.5	16.3	0.23
12	—	—	—	—	—	—	79.4	11.9	0.15
13	—	—	—	—	—	—	71.2	16.3	0.23
Mean	34.7	9.1	0.26	21.2	5.5	0.26	68.8	15.5	0.22
±SEM	2.1	0.7	0.01	1.5	0.5	0.02	2.3	1.2	0.01

$FeSO_4$. An identical farina-based meal was employed in both studies. The first test meal contained 3 mg Fe as $^{59}FeSO_4$ and the same quantity of Fe from the alternate Fe source labeled with ^{55}Fe. To determine whether relative bioavailability was influenced by level of absorption from the meal, the same absorption study was performed 14 d later from meals containing added ascorbic acid. Four separate radio-Fe absorption tests were performed in each subject from two separate doubly labeled meals.

Absorption tests

All test meals were served between 0700 and 0900 after an overnight fast. Only water was allowed for the subsequent 3 h. After blood for measurement of background radioactivity and serum ferritin (19) was obtained, the first test meal, containing 37 kBq ^{59}Fe and 0.11 MBq ^{55}Fe, was administered. Blood was obtained 14 d later for assay of incorporated red-cell radioactivity. The second test meal containing the same quantity of ^{55}Fe and ^{59}Fe was then consumed and 14 d later a final blood sample was drawn. Absorption was determined from the radioactivity incorporated into circulating red cells for the first pair of tests and from the increase in radioactivity from days 14 to 28 for the second test meal. All measurements of ^{55}Fe and ^{59}Fe were performed on duplicate 10-mL specimens of whole blood (14). Absorption of Fe was calculated on the basis of the blood volume estimated from weight and height of the subject (20, 21). Red-cell incorporation of absorbed radioactivity was assumed to be 80% (22).

Test meals

The test meal consisted of unfortified farina (supplied by Carl Hoseney, Kansas State University, Manhattan, KS). Each meal contained 40 g farina, 120 g whole milk, 0.5 g salt, 14 g butter, 24 g sucrose, and 250 mL water. Sufficient $^{59}FeSO_4$ to provide 3 mg Fe and the same quantity of the alternative Fe compound tagged with ^{55}Fe were thoroughly mixed with the meal immediately before serving. $FeSO_4 \cdot 7H_2O$, analytical grade, was used. The second meal, given 2 wk later, was identical to the first except that it contained 100 mg freshly dissolved ascorbic acid.

Statistical analysis

Absorption data were converted to logarithms for statistical analysis because of the highly skewed distribution of Fe absorption measurements in humans (23). The results were reconverted to recover the original units. When absorption from any pair of test meals was compared within the same subject, a paired t test was used to determine whether the logarithmic absorption ratio differed significantly from zero.

Results

The geometric mean absorption of $FePO_4$ in a meal containing no ascorbic acid was only 0.83% (**Table 8**). The absorption ratio of $FePO_4$ to $FeSO_4$ was relatively constant (**Fig 3**). The mean ratio of 0.25 indicated that the absorption of $FePO_4$ was only 25% that of $FeSO_4$. Although the addition of 100 mg ascorbic acid resulted in a striking increase in Fe absorption of about fourfold, the absorption ratio was unchanged, averaging 0.25 (ratio C:D, Table 8). This indicates that the facilitating effect of ascorbic acid on these two forms of fortification Fe was identical.

The absorption of electrolytic Fe relative to $FeSO_4$ was much higher than relative to $FePO_4$ (**Table 9**). Absorption averaged 0.75 relative to $FeSO_4$. Ascorbic acid produced a sharp increase in absorption of both Fe forms although the relative increase was slightly less with electrolytic Fe ($p = 0.007$). As a result, relative availability decreased from 0.75 to 0.62 (Fig 3).

Discussion

One of the earliest clinical studies of the availability of fortification Fe compounds using radioisotopic techniques was that of Steinkamp et al (24), who could detect no obvious differences among the several preparations

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TABLE 8
Bioavailability of ferric orthophosphate

Sex	Age	Packed cell volume	Serum ferritin	Iron absorption: Without ascorbic acid $FePO_4$ (A)	Without ascorbic acid $FeSO_4$ (B)	With ascorbic acid $FePO_4$ (C)	With ascorbic acid $FeSO_4$ (D)	Absorption ratio A:B	C:D	C:A	D:B
	y	%	μg/L	%							
M	32	43	109	0.22	0.75	1.32	4.72	0.29	0.27	5.99	6.29
M	23	47	76	1.98	4.47	3.20	10.28	0.44	0.31	1.61	2.29
F	23	42	50	0.51	1.70	3.92	12.87	0.29	0.30	7.68	7.57
F	21	36	42	0.58	3.11	1.57	5.91	0.18	0.26	2.70	1.90
M	26	44	40	1.12	3.46	1.10	7.01	0.32	0.15	0.98	2.02
F	24	39	33	0.12	0.98	1.20	9.28	0.12	0.21	10.00	9.46
F	24	40	28	0.26	1.03	0.82	4.78	0.25	0.17	3.15	4.64
F	21	40	23	0.46	2.76	4.23	12.46	0.16	0.33	9.19	4.51
M	21	44	19	0.71	3.15	4.76	14.07	0.22	0.33	6.70	4.46
F	22	41	14	7.07	24.88	21.11	49.33	0.28	0.42	2.98	1.98
F	25	41	13	1.67	7.13	7.50	30.78	0.23	0.24	4.49	4.31
F	23	38	5	6.33	17.36	17.72	76.56	0.36	0.23	2.79	4.41
Mean*	24	41	28	0.83	3.30	3.29	13.00	0.25	0.25	3.95	3.94
			−1 SEM	0.58	2.41	2.41	10.03	0.23	0.23	3.21	3.37
			+1 SEM	1.20	4.52	4.48	16.85	0.28	0.28	4.86	4.61

* Arithmetic mean for age and packed cell volume; geometric mean for all others.

tested. However, each compound was studied in a separate group of subjects and even large differences in absorption would be masked by subject-to-subject variability in absorption. The sensitivity of Fe availability studies has been greatly enhanced by employing dual radioiron tracers to obtain simultaneous comparisons in the same individual. Even with this approach day-to-day variations in absorption within the same subject, typically 20–30%, can still mask appreciable differences in availability. For this reason we compared absorption of different forms of fortification Fe with $FeSO_4$ by adding both isotopes to the same meal. Theoretically, some interaction may occur between two Fe sources present in the same meal. For example, $FeSO_4$, which served as the reference Fe compound, may promote the solubility of less available forms of Fe, or perhaps the absorption of $FeSO_4$ is reduced by adsorption to the surface of less-soluble particulate forms of Fe. This possibility was carefully excluded in previous studies (25, 26) that showed that the bioavailability of fortification compounds relative to $FeSO_4$ is the same whether the two radiolabeled sources are administered on different days or added to the same meal.

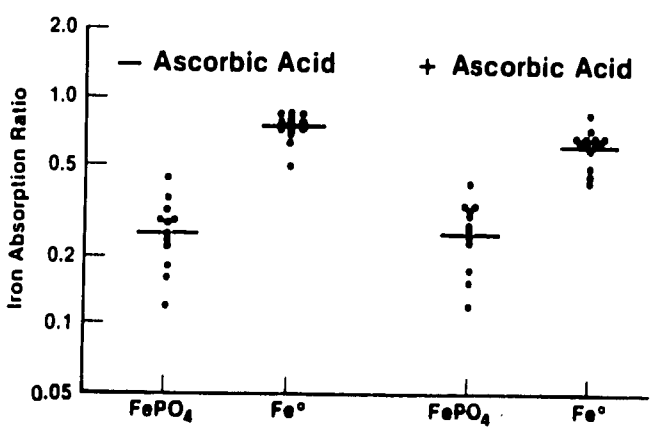

FIG 3. Absorption of fortification iron compounds expressed as a ratio of ferrous sulfate absorption from a farina meal with and without ascorbic acid.

The absorption of nonheme dietary Fe in humans varies widely depending on the nature of the meal. This could pose a problem when bioavailability is evaluated. Highly soluble forms of Fe, such as $FeSO_4$, undergo complete isotopic exchange with the common nonheme iron pool (27, 28) but the degree of exchange of less-soluble Fe sources may vary with different meals. To examine this possibility the various Fe compounds in the present study were evaluated with test meals with and without added ascorbic acid to obtain a wide difference in nonheme Fe absorption. With $FePO_4$, relative bioavailability was identical despite a dramatic threefold increase in absorption when ascorbic acid was added to the farina meal. This indicates that, at least with this form of fortification Fe, the nature of the meal does not influence the estimation of relative bioavailability. On the other hand, the increase in absorption of electrolytic Fe and $FeSO_4$ with ascorbic acid was somewhat less than with $FePO_4$ (Fig 4). A possible explanation is that the presence of

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TABLE 9
Bioavailability of electrolytic iron

Sex	Age	Packed cell volume	Serum ferritin	Iron absorption: Without ascorbic acid: Electrolytic Fe (A)	Without ascorbic acid: $FeSO_4$ (B)	With ascorbic acid: Electrolytic Fe (C)	With ascorbic acid: $FeSO_4$ (D)	Absorption ratio: A:B	C:D	C:A	D:B
	y	%	μg/L	%							
M	19	49	79	2.38	2.83	3.41	5.20	0.84	0.65	1.43	1.83
M	18	47	72	1.20	2.38	3.71	5.47	0.50	0.67	3.09	2.29
M	19	48	69	2.98	3.78	6.30	8.68	0.78	0.72	2.11	2.29
M	22	43	62	1.01	1.35	2.35	3.60	0.74	0.65	2.32	2.66
M	19	48	51	6.68	8.41	9.96	15.48	0.79	0.64	1.49	1.84
M	18	46	50	1.21	1.63	13.73	20.35	0.74	0.67	11.34	12.48
F	20	36	25	1.20	1.41	4.12	6.21	0.85	0.66	3.43	4.40
F	21	43	24	12.45	16.15	12.72	18.98	0.77	0.67	1.02	1.17
M	20	48	20	6.66	7.85	16.61	33.71	0.84	0.49	2.49	4.29
M	18	46	18	3.76	5.88	11.61	13.77	0.63	0.46	3.08	4.21
F	22	37	15	4.57	5.86	13.68	15.81	0.77	0.86	2.99	2.69
F	29	14	14	15.67	21.72	15.88	35.57	0.72	0.44	1.01	1.63
F	23	40	11	3.82	5.16	9.31	15.40	0.74	0.60	2.43	2.98
Mean*	21	44	28	3.37	4.52	7.97	12.76	0.75	0.62	2.37	2.83
			−1 SEM	2.62	3.53	6.63	10.37	0.72	0.59	1.99	2.40
			+1 SEM	4.33	5.78	9.59	15.71	0.78	0.66	2.82	3.34

* Arithmetic mean for age and packed cell volume; geometric mean for all others.

ascorbic acid, a reducing agent, would cause an increase in Fe^{+2} concentration, thereby enhancing Fe absorption. Ascorbic acid may therefore enhance Fe absorption of $FePO_4$ by reducing Fe^{+3} ions available from an existing equilibrium reaction. Nevertheless, the differences in estimates of bioavailability at the extremes of nonheme Fe absorption were minor and it can be concluded that the nature of the meal has little influence on bioavailability measurements in humans.

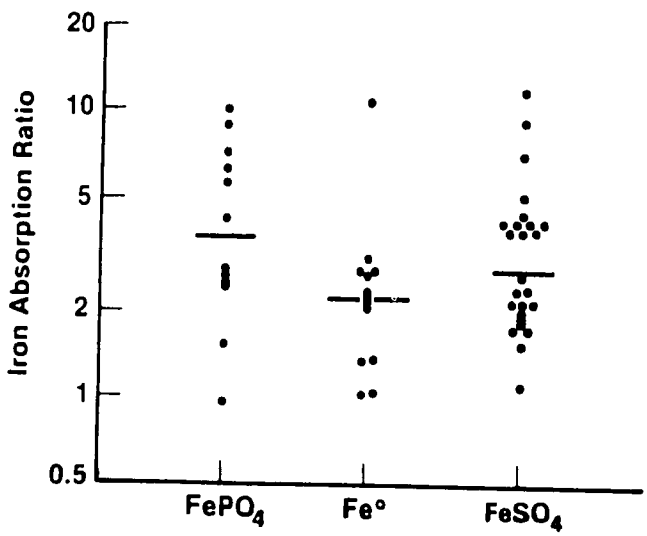

FIG 4. Increase in absorption of various iron compounds after the addition of 100 mg ascorbic acid to the meal.

There is a remarkable similarity in the bioavailability of $FePO_4$ determined in the present study and that observed in a previous study (28) in which this form of Fe was baked into wheat rolls. The mean absorption ratio for $FePO_4$ relative to $FeSO_4$ in the earlier report was 0.31 as compared with 0.25 in the present study. These estimates are consistent with rat studies (29, 30) in which bioavailability relative to $FeSO_4$, referred to as RBV, ranged between 0.07 and 0.32. The difference in reported RBV for $FePO_4$ is similar to that observed by Harrison et al (31), who obtained values between 0.06 and 0.46. The wide range of bioavailability values in animal studies is possibly a reflection of particulate differences in the sources of $FePO_4$.

IV Summary of the iron bioavailability study

Clinical study

The clinical measurements of Fe absorption in this study serve as the reference point against which other animal and physical chemical methods for assessing Fe bioavailability are compared. The reliability of absorption tests in human subjects has improved dramatically in recent years with the use of double-labeled meals in which labeled compounds do not interact. In the present study all Fe compounds were assessed within the same subject by calculating absorption ratios of either $FePO_4$ or electrolytic Fe to $FeSO_4$.

Another consideration in clinical studies is whether es-

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timates of Fe bioavailability are influenced by the nature of the test meal. To explore this possibility we decided to examine two levels of food Fe availability by administering the test meal with and without ascorbic acid. The addition of vitamin C increased absorption of $FeSO_4$ from 3.3 to 13% in the study with $FePO_4$ (Table 8) and from 4.5 to 13% in the study with electrolytic Fe (Table 9). With ferric orthophosphate the estimate of bioavailability remained identical despite a threefold increase in absorption. Obviously, the bioavailability of this form of fortification Fe is not influenced by the level of absorption from the test meal. With electrolytic Fe the increase in absorption with ascorbic acid was less than with $FeSO_4$; as a result relative availability was reduced from 0.75 to 0.62. These findings suggest that the facilitating effect of ascorbic acid may be somewhat diminished with elemental Fe powders as compared with Fe salts although the difference is minor. For comparison with the animal and in vitro measurements of availability, we have chosen the value of 0.75 obtained with the farina test meal containing no added ascorbic acid.

All of the volunteers in this study were hematologically normal although serum ferritin levels indicated a wide range in Fe status in both study groups. This variation is also reflected in differences in Fe absorption from $FeSO_4$, which ranged from 0.8 to 25% in the study with $FePO_4$ and from 1.4 to 22% in the study with electrolytic Fe. However, there was no obvious difference in the bioavailability ratio between subjects with low and high serum ferritin levels. It is important to note that these results may not therefore be extrapolated to every clinical situation.

Comparison of methods

Although animal studies may serve as useful predictors, in practical terms one must recognize that the human diet is mixed, containing components that potentially enhance or inhibit Fe absorption, whereas the experimental animal diet is more homogeneous in nature.

The most widely accepted method for predicting Fe bioavailability in human subjects is Hb repletion. This technique measures the Hb response to graded amounts of Fe in rats with induced Fe-deficiency anemia. Excellent agreement was observed in the present study between clinical measurements and a slightly modified AOAC method. With electrolytic Fe, RBV values of 0.66 and 0.78 were observed in rats as compared with 0.75 in humans. Excellent agreement was also obtained with $FePO_4$, which gave RBV values of 0.25 and 0.34 in rats as compared with 0.25 in humans.

A modified Hb-repletion technique, the HRE method, was also evaluated. There was excellent agreement between the RBV of 0.78 and the clinical value of 0.75 with electrolytic Fe using the HRE method. $FePO_4$ availability was overestimated, however, with an RBV of 0.58 as compared with the clinical value of 0.25. The major differences in this method are that only one level of dietary Fe is tested and calculations are based on the efficiency of converting dietary Fe into Hb. The method is more economical because only one-quarter of the number of animals are required to test an Fe compound. This was a major advantage because it permitted a direct comparison of bioavailability from the unlabeled and radiolabeled Fe sources; there was not enough labeled material to perform the standard AOAC method. The depletion period is only 7 d for the HRE method as compared with ≥ 28 d for the AOAC method; the repletion period is 10 d for the HRE method and 14 d for the AOAC method. The results obtained with the radiolabeled sources were virtually identical to those obtained with the unlabeled Fe sources. Thus, the RBV of radiolabeled electrolytic Fe was 0.80 as compared with 0.86 using the unlabeled material. With $FePO_4$, a value of 0.60 was obtained as compared with a value of 0.61 using the unlabeled source.

The bioavailability of radiolabeled Fe sources was also evaluated in Fe-replete rats using an isotopic method similar to that employed in clinical studies. With $FePO_4$ a mean RBV of 0.25 was obtained, identical to the clinical value. Results in different laboratories were much more variable in the case of electrolytic Fe: RBV values of 0.48, 0.69, and 0.47 were obtained as compared with a clinical RBV of 0.75. We could find no explanation for this interlaboratory variation. However, in one of the laboratories (laboratory 3), the rats were mildly Fe-deficient when studied. Nevertheless, the RBV of 0.47 for electrolytic Fe was nearly identical with the value of 0.48 obtained in laboratory 1 in Fe-replete rats. This indicates that, as in human studies, Fe status does not influence estimates of bioavailability. A radioactive animal method is impractical in the assessment of fortification Fe compounds because of the difficulty in preparing radiolabeled Fe sources.

In vitro tests of solubility in dilute acid showed variation in measuring bioavailability of electrolytic Fe and tended to underestimate $FePO_4$. Electrolytic Fe relative solubility values were 0.60 and 0.75, which compares well with the clinical value of 0.75. With a more insoluble source such as $FePO_4$, in vitro solubility appeared to underestimate bioavailability as measured in human subjects. Values were 0.03 and 0.04 compared with the clinical value of 0.25. As suggested in previous studies, solubility in dilute acid provides a screening method for assessing Fe fortification compounds.

Studies were also performed with an in vitro technique that has not previously been used to assess the bioavailability of fortification Fe. The major difference is that availability is measured in the presence of food by determining the proportion of dialyzable Fe after simulated gastric digestion. In laboratory 1 the results obtained with this technique were almost identical to those obtained in clinical studies. However, there were appreciable differences between laboratories. For electrolytic Fe, RBV values ranged from 0.58 to 0.75 and for $FePO_4$, from 0.27 to 0.46. We believe that some of this variability is due to the fact that technical experience with the method was limited in some laboratories. Even relatively small variations in incubation time can lead to pro-

TABLE 10
Summary values for human, rat, and in vitro studies

Model	Method	Laboratory	Relative availability*			
			Electrolytic			
			^{55}Fe	Fe	$^{55}FePO_4$	$FePO_4$
Human	Double isotope, extrinsic tag	3	0.75		0.25	
Rat	Hemoglobin depletion-repletion					
	AOAC	1		0.66		0.25
	AOAC	4		0.77		0.33
	HRE (35 mg Fe/kg diet)	3		0.78		0.58
	HRF (20 mg Fe/kg diet)	4	0.80	0.86	0.60	0.61
Rat	Double isotope, extrinsic tag	1	0.48		0.26	
		2	0.69		0.26	
		3	0.47		0.22	
In vitro	Solubility	1		0.75		0.04
		4		0.60		0.03
In vitro	Dialysis	1		0.75		0.28
		2		0.58		0.27
		4		0.71		0.46

* Relative availability = fortification $Fe/FeSO_4$.

nounced differences in dialyzable Fe. With further experience and refinement, this method will probably produce results comparable to those obtained with Hb-repletion techniques. Dialysis measurements can be performed at a small fraction of the cost of animal studies and can be undertaken by laboratories without animal facilities. Further evaluation of this technique using different meals and a greater range in Fe-fortification compounds might lead to a simplified approach for evaluating Fe bioavailability.

A summary of results obtained by all methods is presented in **Table 10.**

Conclusion summary

1) Effectiveness of several methods for predicting bioavailability of various Fe sources in humans, with ferrous sulfate as the reference material: AOAC rat model—good predictor for all compounds; HRE rat model—good predictor for high bioavailability compounds, underestimates for low bioavailability compounds; rat isotopic model—good predictor for low bioavailability compounds, underestimates for high bioavailability compounds; in vitro solubility—good predictor for high bioavailability compounds, underestimates for low bioavailability compounds; and in vitro dialysis—promising but needs precise standardization.

2) Recommended method of choice for predicting human Fe bioavailability: preliminary screening with in vitro dialysis followed by evaluation by modified AOAC method, slope ratio technique.

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Guidelines for the Control of Maternal Nutritional Anemia

A Report of the International Nutritional Anemia Consultative Group (INACG)®

I. Introduction

Anemia is a major and pressing problem. Recent World Health Organization (WHO) statistics indicate a worldwide prevalence of about 30%, with even higher figures in developing countries. Young children and pregnant women are the most affected groups, with an estimated global prevalence of about 40% and 50%, respectively. Although many causes of anemia have been identified, nutritional deficiency due primarily to a lack of bioavailable dietary iron accounts for well over half the total number of cases. Deficiency of the vitamin folate is a contributing factor in many areas. Nutritional anemia is most prevalent in Africa and South Asia, but even in industrialized countries a significant number of women have insufficient nutrient reserves to meet the increased requirements of pregnancy. These guidelines outline short- and long-range strategies for controlling prenatal nutritional anemia.

II. Consequences of Iron and Folate Deficiency

Consequences to the Mother

Iron needs increase significantly during pregnancy because of growth of the fetus and placenta and expansion of the mother's blood volume. Women frequently enter pregnancy with inadequate iron stores, and thus the increased demands associated with pregnancy result in anemia—i.e., a decline in hemoglobin concentration to well below the normal range for healthy pregnant women. Adult women living at sea level are anemic when hemoglobin levels fall below 12.0 g/dL in nonpregnant women or below 11.0 g/dL in pregnant women.

Women frequently enter pregnancy with inadequate iron stores, and thus the increased demands associated with pregnancy result in anemia.

Anemia with hemoglobin concentrations below 11.0 g/dL is associated with decreased work capacity and mental performance and probably with impaired resistance to infection. Anemic women are less tolerant of blood loss at delivery, particularly when hemoglobin levels fall below 8.0 g/dL. When malaria and/or hookworm complicate iron deficiency, anemia can be more severe, with hemoglobin levels below 6.0 or even 4.0 g/dL. At 6.0 g/dL, evidence of circulatory decompensation becomes apparent. Women experience breathlessness and increased cardiac output at rest. At this stage, added stress from labor, sponta-

neous abortion, or other major complications can result in maternal death.

Without effective treatment, maternal death from anemic heart failure and the effects of severe hypoxia is likely with a hemoglobin concentration of 4.0 g/dL. Even a blood loss of 100 mL can cause circulatory shock and death. Moreover, malaria and pregnancy both increase folate needs, and folate deficiency compounds anemia.

Identify and reinforce those local dietary and health practices that improve iron nutriture.

Consequences to the Newborn

Low birth weight and increased perinatal mortality are associated with maternal anemia. Low birth weight is a major factor in subsequent infant morbidity and mortality. Any intervention that decreases the prevalence of low birth weight reduces infant mortality. Maternal iron and folate deficiencies have relatively little effect on hemoglobin concentrations in the newborn. Nevertheless, a major benefit of preventing these deficiencies in the mother is a healthier baby.

Benefits of Preventing Iron and Folate Deficiencies

To the mother:

- **Improved work capacity**
- **Improved mental performance**
- **Improved resistance to infection**
- **Safer pregnancy and delivery**

To the newborn:

- **Higher birth weight**
- **Improved health**

III. Guidelines for Short-Range Intervention

Iron Supplementation

Programs for the control of maternal nutritional anemia aim to provide sufficient absorbable iron to women before and during pregnancy. As a short-range measure, give all pregnant women an oral iron preparation at the first prenatal visit. This should occur by the fifth month of pregnancy. Continue treatment throughout the remainder of pregnancy and during the first months of lactation. Ferrous sulfate is inexpensive and is usually the preparation of choice. A daily dose of 120 mg elemental iron in two tablets containing 60 mg each (300 mg hydrated ferrous sulfate contains 60 mg iron) taken on an empty stomach is preferred. However, in the presence of undesirable side effects, the dose may be given with or immediately after a meal. Some women experience minor side effects, such as a change in stool character or abdominal distress, especially during the first week. Assure them that these mild symptoms are temporary. If the side effects are debilitating, minimize them by dividing the dose between two meals, or start with smaller daily doses of 30 mg or 60 mg iron. In women with relatively mild iron deficiency, a single tablet providing 60 mg per day is probably adequate.

Folate requirements also increase during pregnancy. Some prenatal supplements contain a combination of 60 mg iron and 250 μg folate, providing 500 μg folate daily when two tablets are taken. The cost of iron tablets is not increased significantly when folate is added. Folate deficiency is not a problem in all populations, but routine addition of folate to prenatal iron supplements will maintain adequate folate status in individuals whose diets supply insufficient folate.

Importance of the Delivery System

The delivery system is critical to a supplementation program. Many iron-supplementation programs have failed because the tablets did not reach the mothers or the mothers did not take them.

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Schedule of Iron Supplementation for Pregnant Women

Pregnant women from 4th or 5th month of pregnancy through early lactation:

Two tablets, each containing 60 mg elemental iron* and 250 μg folic acid, taken once daily**

- **300 mg ferrous sulfate (hydrated) is equivalent to 60 mg elemental iron**
- ** **Usual administration is one tablet, two times daily, without food. In the presence of undesirable side effects, take dose with or immediately after a meal, or decrease dose to 30–60 mg daily for a week before taking full dose.**

Exert all efforts to achieve reasonable program efficiency.

Generally, iron tablets can be distributed through existing channels of the country's health care delivery system. The program must be considered integral to (and not added on to) the system. Iron supplementation is usually integrated into maternal and child health (MCH) programs within the context of primary health care. Attendance at prenatal clinics is often poor in developing countries, especially in rural areas. Use community health care workers to reach mothers and increase distribution. Alternatively, use traditional care givers such as midwives or traditional birth attendants.

To ensure efficient delivery of supplements and a high level of adherence, implement the following before and throughout the course of the program:

1. Obtain the full commitment of health policy makers and planners at the top level and managers at the middle level through intensive and periodic orientation in the program's benefits and its place in the health care delivery system.
2. Motivate traditional care givers and supervise them closely.
3. Educate, train, and retrain all personnel adequately.
4. Ensure a continuous supply of supplements and provide other logistic requirements (e.g., storage areas and monitoring forms).
5. Educate mothers and the community (including husbands, local leaders, adolescent girls, and young women) about the program's importance, citing benefits they will derive from the program within the context of their own health priorities. Use traditional community and women's organizations (including female initiation societies), social clubs, and schools to maximize community involvement, cooperation, and support.
6. Assure mothers that the side effects of iron supplements are harmless and temporary. Provide for dosage changes if side effects persist.
7. Monitor program implementation continuously and evaluate program effectiveness periodically.

IV. Guidelines for Long-Range Intervention

The success of interventions depends on the participants' perception that the program addresses their own health interests and that the project is supported by local and national leaders.

Dietary modification and fortification are the most effective long-range strategies for alleviating nutritional anemia. Fortification has the advantage that it increases the iron content of the diet without a change in dietary habits. However, there are problems in identifying foods suitable for fortification and absorbable iron compounds compatible with those foods. An alternative is to improve iron nutrition by increasing the bioavailability of dietary iron and by modifying dietary habits (e.g., by increasing the intake of ascorbic acid-containing foods and reducing the intake of foods that are known to inhibit iron absorption). This may

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be difficult, especially in populations with very limited financial and food resources.

Iron Fortification

The effectiveness of any fortification program is enhanced by determining the prevalence of and reasons for iron deficiency in the population. First, establish the relative importance of total dietary iron content, decreased bioavailability, and increased blood losses from, for example, hookworm infection. Failure to eliminate hookworm may negate the effects of an otherwise effective fortification program.

Elements for Successful Iron Supplementation Programs

- **Commitment from top-level policy makers and planners down to village-level implementers**
- **Motivated and closely supervised primary-level workers**
- **Adequately educated and trained personnel at all levels**
- **Adequate logistics**
- **Well-informed, motivated mothers and involved, supportive communities**
- **Continuous monitoring and periodic evaluation**

Next, identify a suitable food or foods to fortify. Choose foods with the following characteristics:

- Commonly and widely consumed and preferred by the population at risk
- Centrally processed
- Technologically and economically possible to fortify

Successful pilot fortification programs have used whole wheat flour, salt, sugar, 26%-fat milk powder, cookies, curry powder, and fish sauce as vehicles and various iron compounds as fortificants. Fortification should produce minimal changes in the taste, texture, and appearance of foods. Targeted groups may reduce their consumption of fortified staples or condiments if the foods are radically changed, thus countering the aims of the program.

Identifying foods suitable for fortification may be difficult in developing countries, where few foods are centrally processed. Although salt and sugar may be suitable for national and regional fortification programs, other foods may be better for programs targeted at specific population groups. For example, in a successful program for schoolchildren in Chile, cookies containing dried hemoglobin were provided with school lunches. Similarly, infants in that country were fed formula prepared from full-fat (26%) milk powder fortified with both iron and ascorbic acid.

Another problem is the poor bioavailability of most dietary iron. Consequently, any fortification iron added to the diet also is poorly absorbed. Consider these two strategies to overcome the problem: Use an iron compound that is less susceptible to dietary inhibitors; add ascorbic acid, a potent promoter of iron absorption, to the iron fortificant.

Regarding dietary fortification with folate, data indicate that a cereal such as maize can be fortified successfully with folate and that the consumption of folate-fortified maize prevents prenatal folate deficiency.

Fortification programs are urgently needed because they offer the best way to ensure that women enter pregnancy with normal hemoglobin concentrations and sufficient nutrient reserves to cope with the extra requirements of pregnancy. *A successful fortification program requires cooperation among scientists, the food industry, and government agencies.*

Dietary Modification

Where opportunities for fortification are limited, increase iron intake and improve iron and folate bioavailability by modifying the population's methods of selecting, processing, and consuming food. It is rarely desirable or easy to change people's eating habits radically. Therefore, be familiar with existing dietary conditions and the availability and cost of foods, and base dietary modifications on that knowledge. Be aware of food taboos (especially before, during, and just

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after pregnancy), intentional caloric restriction during pregnancy, the symbolic value of particular foods, the use of traditional medicines, and preferences for foods that affect the absorption of iron or folate. Local conversion from iron pots to aluminum cookware also may reduce the amount of iron available. These factors will influence the method and extent of dietary modification.

> *The success of interventions depends on the participants' perception that the program addresses their own health interests and that the project is supported by local and national leaders.*

Dietary variety also depends on economic and cultural factors. Nevertheless, in addition to increased intake of foods rich in bioavailable iron, encourage increased eating of vegetables and fruits high in vitamin C with the main meal. Identify and reinforce those local dietary and health practices that improve iron nutriture. Discourage consumption of tea and other tannin-containing products with meals, because these products can reduce iron absorption.

Complicating Conditions

In communities in which iron deficiency anemia is complicated by malaria, hookworm infection, and schistosomiasis, treat these conditions as well. Where malaria is endemic, administer antimalarial drugs at the mother's first prenatal visit and provide them throughout pregnancy.

Bear in mind the importance of clean water. A safe water supply decreases enteric infections and increases nutrient absorption.

Suggested Reading

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International Nutritional Anemia Consultative Group (INACG)
Work Plan 1 October 1989 through 31 September 1990

The secretariat, in consultation with the Office of Nutrition, Bureau of Science and Technology, Agency for International Development, plans to undertake the following activities in the area of nutritional anemia during this fiscal year:

1. Organize and convene two INACG Advisory Committee meetings

2. Develop agenda for implementation of a social marketing program to increase the awareness of iron deficiency anemia

3. Select members, organize and convene a workshop on operational systems to combat iron deficiency anemia

4. Meet with ICCIDD regarding salt fortification with iodine and iron

5. Conclude the work of the Task force on the relationship of nutritional anemia and mental development by publishing a monograph

6. Identify members, organize and convene a task force on FeEDTA as a fortificant

7. Continue to support, monitor, and review progress of research project determining the bioavailability of elemental iron in weaning foods

8. Provide Information on nutritional anemia to teaching scientists, government ministries, and leading researchers

9. Provide INACG news to various newsletters and information centers to increase exposure and understanding of the program

10. Provide annual, quarterly, financial and trip reports as requested in the cooperative agreement

These activities are described in more detail on the following pages, with "Q1, Q2,..." referring to the quarter during which the activity is planned to occur. Letters in brackets following each section refer to applicable paragraphs in the cooperative agreement.

INACG Advisory Committee Meetings (Q1, Q3)

The secretariat will organize two meetings of the INACG Advisory Committee to develop action programs to stimulate field programs for the reduction of nutritional anemia. The meetings are planned to be held during the first and third quarters of FY 1990. Alternatively, the second advisory committee meeting may be held at the end of the second quarter of FY 1990 in conjunction with the proposed workshop on operational systems to combat iron deficiency anemia. [D.2(c), D.2(h), D.3(f)]

Social Marketing of Programs to Increase the Awareness of Iron Deficiency Anemia (Q2-Q4)

The secretariat will initiate the first phase of a strategy for social marketing of programs to control iron deficiency anemia. This strategy was the outcome of a workshop held in 1986 and is designed to convince policy makers, government officials, and development agencies about the importance of supporting efforts to control iron deficiency anemia. The first phase of this marketing strategy consolidates the image of iron deficiency anemia. This phase begins with a graphic identity program that includes the following elements:

- Review and analyze all existing materials on the iron deficiency anemia issue used by groups that are potential participants in the program.

- Develop an umbrella theme line and graphic identity program for communications efforts on the iron deficiency anemia issue. The theme line would be used on all marketing and public relations materials at national and international levels. These materials could include press releases, flyers, brochures, posters, media kit covers, television and radio public service advertising, newsletters, slides, exhibits, signs, and videotapes.

- Concepts will be tested to ensure that standards will apply to all developing countries involved in the program.

- Develop graphic standard guidelines for use of logo and theme line/graphic identity. Set standards for typestyle, colors, and formats that will give iron deficiency anemia a consistent, quality look.

Carrying out the first phase of this strategy will span the second, third, and fourth quarters of FY 1990. [D.2(e), D.2(f), D.2(h)]

Workshop on Operational Systems to Combat Iron Deficiency Anemia (Q2)

During the second quarter of FY 1990 the secretariat will organize and host a workshop on operational systems to combat iron deficiency anemia. This workshop will be held in Washington, D.C. or New York. The purpose will be to develop recommendations for practical ways to control iron deficiency anemia by dietary fortification with iron. The approach will be to set forth policies and procedures that can be broadly applied in various country settings. Representatives will be invited from major bilateral and

multilateral donor agencies and selected international experts in the area of iron deficiency anemia, major industry representatives, and interested PVO's.

Meeting with ICCIDD Regarding Salt Fortification with Iodine and Iron (Q2)

Women in developing countries often suffer from both iron and iodine deficiency. Because salt can be effectively fortified with iodine, the International Council for Control of Iodine Deficiency Disorders (ICCIDD) approached INACG to consider the feasibility of dual fortification of salt with iodine and iron. Problems of efficacy and availability of both nutrients will be considered in initial discussions between INACG and ICCIDD. This meeting should be scheduled during the second quarter of FY 1990. [D.2(c), D.2(f), D.2(h), D.2(j)]

Task Force on the Relationship of Nutritional Anemia and Mental Development (Q1-Q3)

Due to the long-standing interest in the relationship of nutritional anemia and mental development, a new task force on this topic began its work during FY 1989. This task force will continue work on a monograph during the first quarter FY 1990, complete the monograph during the second quarter of FY 1990, and publish the monograph in the third quarter of FY 1990. [D.2(c), D.2(d), D.2(f), D.2(j), D.3(a)]

Task Force on EDTA (Q3-Q4)

One marketable approach to combatting the high incidence of iron deficiency is to fortify commonly used foods with iron-EDTA (FeEDTA). This promises to be a marketable delivery system for improving the iron intake by populations at risk, but needs to be better presented to authorities in target countries. The INACG secretariat will organize a task force to prepare a statement on EDTA. This statement should encourage the initiation of programs for using FeEDTA or NA_2EDTA plus ferrous sulfate as a fortificant for local foods. Field studies in South Africa, Guatemala, Thailand and Egypt could be used as examples of successful programs. The work of this task force is planned to begin during the third quarter of FY 1990, continue its work during the final quarter of FY 1990 and complete its work during the first quarter of FY 1991. [D.2(c), D.2(d), D.2(f), D.2(h), D.2(i), D.2(j), D.3(a), D.3(e)]

Research Project Determining the Bioavailability of Elemental Iron in Weaning Foods (Q1-Q4)

In collaboration with Instituto De Nutricion Y Tecnologia De Los Alimentos, the secretariat will continue to support, monitor, and review the progress of a research project in Chile. The project is a three year activity which will determine the bioavailability of elemental iron in weaning foods. Funding is provided by the private sector, with technical assistance provided through the INACG network of experts. [D.2(h)]

Provide Information on Nutritional Anemia to Teaching Scientists, Government Ministries, and Leading Researchers (Q1-Q4)

In the past fiscal year the secretariat responded to more than 225 requests for information. Ninety percent of these requests were from developing countries. During this fiscal year the secretariat will continue to provide information regarding nutritional anemia when this information is requested by government ministries, policy-makers, scientists, and managers of field programs. [D.2(g)]

The secretariat will promote information concerning nutritional anemia and increase liaisons with other organizations through exhibiting at local, national, and international conferences and meetings. Additionally, the secretariat will provide INACG information to members of the Advisory Committee for their use when travelling on behalf of INACG. These efforts facilitate the work of private voluntary organizations and nongovernmental organizations in developing countries. [D.2(f), D.2(i), D.2(j)]

Provide INACG Information to Newsletters and Information Centers (Q1-Q4)

To increase awareness of the availability of INACG information and to increase dissemination of this information, the secretariat plans to continue placing INACG news in various newsletters published by other organizations such as the Clearinghouse on Infant Feeding and Maternal Nutrition of the American Public Health Association and International Life Sciences Institute-Nutrition Foundation. Additional opportunities to convey INACG information will be sought. This will be an activity throughout FY 1990. [D2(f), D.2(j)]

Provide annual, quarterly, financial and trip reports as requested in the cooperative agreement (Q1-Q4)

Throughout the year the secretariat will submit required annual, quarterly, financial and trip reports as specified in the cooperative agreement to indicate progress made toward achieving overall and specific objectives of INACG. [E., E.1, E.2(a), E.2(b), E.2(c)]

During the first quarter of FY 1990, the secretariat will submit the INACG Annual Report for FY 1989 to the AID Office of Nutrition. Ten copies of this report will be submitted to the Office of Nutrition. The secretariat will also provide copies of the annual report to the INACG Advisory Committee. The report will include a description of the year's activities including technical, scientific, managerial, and fiscal information. It will also include a copy of the FY 1990 work plan and budget. [E.2(b)]

The secretariat will prepare quarterly progress reports for the AID Office of Nutrition. These progress reports will contain status reports of proposed INACG activities noting achievements made during the quarter and activities planned for the following quarter. The quarterly reports will also contain financial status reports with an analysis of the quarterly expenditure in relation to the FY budget. [E.2(a)]

Annual workplans for FY 1991 will be developed during the fourth quarter in accordance with the amendment to the cooperative agreement cited as "E.2.(e) Annual Workplans" in the 4 April 1989 letter from Mr. Jay Bergman, Agreement Officer.

INACG Meetings

Advisory Committee Meetings (Q1 & Q3)
These one-day meetings will be held in Washington, D.C. at the ILSI-Nutrition Foundation offices. The participants in addition to the secretariat will be:

Dr. Richard Theuer
Dr. Fatimah Linda Collier Jackson
Dr. Peter Greaves
Dr. Samuel Fomon
Dr. Ray Yip
Dr. Alberto Pradilla, INACG Senior Advisor
Dr. Samuel G. Kahn, INACG Secretary

Workshop on Operational Systems to Combat Iron Deficiency Anemia (Q2)
This two-day workshop will be held in Washington, D.C. at the ILSI-Nutrition Foundation offices. An illustrative list of potential participants in addition to the secretariat:

Donor Agencies
- AID
- WHO
- UNICEF
- FAO
- World Bank
- PAHO
- SIDA
- CIDA

Industry Representatives

Scientific Experts

Advisory Committee

EDTA Task Force initial meeting (Q3)
This two-day meeting will be held in Washington, D.C. at the ILSI-Nutrition Foundation offices. The participants in addition to the secretariat will be:

Dr. James Cook
Dr. Samuel Kahn
Dr. John Vanderveen
Dr. Fernando Viteri
Industry Representatives from W.R. Grace and BASF

INACG Work Plan by Quarters

Q1 (10/1/89-12/31/89)	Q2 (1/1/90-3/31/90)	Q3 (4/1/90-6/30/90)	Q4 (7/1/90-9/30/90)
Mental Dev. manuscr. circ.	Mental dev. finish	Mental dev. publish	
	Soc. Mktg. Planning	Soc. Mktg. get subcontract	Soc. Mktg. proceeds
Adv. Comm. mtg		Adv. Comm. mtg.	
	Operational Systs. Workshop		
	Discussion with ICCIDD		
Chilean study cont.	Chilean study cont.	Chilean annual funding	Chilean study cont.
		EDTA TF initial mtg.	EDTA TF wkg.
Info requests	Info requests	Info requests	Info requests
Newsl: Adv. Comm, new pubs		Newsl: new pubs, new TF	
Quarterly report	Quarterly report	Quarterly report	Quarterly report
Annual Report FY 1989			Annual Report FY 1990
			Annual Workplan FY 1991

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