INACG

International
Nutritional Anemia
Consultative
Group

Chairman:
Dr. E.M. DeMaeyer, WHO

Secretary
Dr. Samuel G. Kahn, AID

Secretariat:
The Nutrition Foundation
1126 16th St. NW, 3rd Floor
Washington, D.C. 20036
Cable: NUTRITION WASHINGTONDC
Telex: "NUFOUND" 6814107
Phone: (202) 659-9024

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INTERNATIONAL NUTRITIONAL ANEMIA CONSULTATIVE GROUP®
ANNUAL REPORT

54954

1 January, 1987 to 31 December, 1987

DAN-0227-G-SS-1029-00

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. The initial grant was for the period from 16 February through 31 December, 1977. An additional grant was provided for the two-year period of 1 January 1978 through 31 December, 1979. The grant period was extended by means of amendments through 31 December, 1980. The current grant, DAN-0227-G-SS-1029-00, initially covered the period 1 January, 1981 through 31 December, 1982. In July 1982 the grant was amended to include the three-year period of 1 January, 1983 through 31 December, 1985. The grant was again amended in 1986 to increase the obligated amount to $629,920 with an expiration date of 28 February, 1987.

   In May 1987 the grant was amended to increase the obligated amount to $701,324 with an expiration date of 30 June, 1987. In August 1987, the Nutrition Foundation, Inc. received a no-cost extension of the obligated funds with an expiration date of 30 September, 1987. On September 25, 1987, an agreement was made that allowed the Nutrition Foundation, Inc. to use remaining obligated funds from this grant until 31 December, 1987 for continuation of INACG projects nearing completion.

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 in nutritional anemia through the development of strategies for the particular region and by furnishing consultants to these programs.

II. Activities

A. Continuation of research project on "Comparison of iron bioavailability from three iron compounds in man, animals, and in vitro"

1. A study was conducted comparing bioavailability from ferric orthophosphate and electrolytically reduced iron of two particle sizes (20 mu and 45 mu) with that of ferrous sulfate in human, animal, and in vitro systems. The study was funded in total by U.S. food industries, the U.S. Food and Drug Administration (FDA), and United States Department of Agriculture (USDA). This funding was approximately $250,000. International chemical companies, Nestlé (of Switzerland), FDA, USDA, and the University of Kansas Medical Center all contributed laboratory time or consultation time. It is estimated that these in-kind contributions totalled $300,000.

   Data for the clinical studies, the animal experiments, and in vitro characteristics were collected in 1986. Because of discrepancies in data additional in vitro experiments were performed late in 1986.

2. Manuscripts for publication were prepared and received by the INACG secretariat in early 1987. The final meeting of investigators occurred 26 and 27 March, 1987. The investigators discussed all the data and, after considerable deliberation, reached consensus on research conclusions. The group discussed plans for publication of the research as a single paper in the American Journal of Clinical Nutrition.

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3. The results of the research were presented at the FASEB meeting in Washington, D.C. Dr. Catherine Adams (INACG secretariat) presented a poster presentation on the in vitro experiments and the animal experiments for hemoglobin depletion/repletion on 31 March. On the same day Dr. Eugene Morris (USDA) presented a poster on the animal iron experiment. On 1 April Dr. Paul Whittaker (University of Kansas and FDA) presented an oral paper on the clinical trials. During the discussion after Dr. Whittaker's paper, Dr. Richard Hurrell (Nestlé) gave a summary of results and conclusions for the overall study.

4. In the October 1987 the final manuscript was submitted to the American Journal of Clinical Nutrition for peer review and publication. The task force anticipates publication in 1988 with the title "Comparison of In Vitro, Animal, and Clinical Determinations of Iron Bioavailability: International Nutritional Anemia Consultative Group Task Force Report on Iron Bioavailability." The October draft of the manuscript is included in this report as appendix 1.

B. Initiation of research project for determination of iron bioavailability in weaning foods

1. On 22 April, 1987, representatives from Gerber, H.J. Heinz, Beech-Nut, General Mills, AID, University of California San Francisco Medical School, ILSI-NF, and the INACG secretariat met to discuss tentative plans to conduct a clinical study in Chile which would assess bioavailability of electrolytic iron from cereals and the iron's effectiveness in preventing iron deficiency for infants. The tentative plans stemmed from some negative allegations regarding the bioavailability of iron from cereals, particularly in respect to foods fed to infants.

   A proposed protocol for implementation in Santiago, Chile with Dr. Tomas Walter (Instituto De Nutricion Y Tecnologia De Los Alimentos) as primary investigator was discussed. At the close of the meeting several of the representatives felt that their companies could be financially supportive of the study. The minutes for this meeting are included in this report as appendix 2.

2. It was decided that the INACG secretariat would serve as the administrator for this study. During the remainder of the year, the secretariat made inquiries regarding firm financial support for the study. The secretariat also conferred with Dr. Walter regarding necessary equipment and reagents.

3. Dr. Walter expects to initiate the study during the first half of 1988.

C. Task force meeting to review Joint Nutrition Support Program document "Preventing and controlling iron deficiency anemia: the contribution of primary health care"

1. JNSP asked Dr. Edouard DeMaeyer to develop a document addressing the issue of the contribution of primary health care in the control of iron deficiency anemia. Further, JNSP requested that INACG review the draft of the document prior to its submission for publication by WHO/UNICEF.

2. An INACG task force met in May 1987 in conjunction with the X INACG meeting in Quito, Ecuador to discuss the draft position paper. Revisions were made as necessary to yield a paper more consistent with current INACG recommendations.

3. Dr. DeMaeyer delivered the revised paper to the WHO publication offices and circulated this paper to the task force in October 1987. A copy of the revised paper is included in this report as appendix 3.

4. WHO publication of the position paper is expected during the first half of 1988.

D. Social marketing of programs to control iron deficiency anemia

1. In 1986 a workshop, funded largely by U.S. food industry, was held to develop a strategy for the social marketing of programs to control iron deficiency anemia. The resulting strategy is targeted to policy makers who determine the priority given to social programs in countries.

2. During 1987 the secretariat initiated fund raising efforts to enable implementation of the strategy. A financial contribution was received from Gerber Products. Foster Parents Plan agreed to consider future collaboration on the strategy.

3. A summary report of the 1986 workshop was presented at the X INACG meeting.

4. Until sufficient funds are available, it is unlikely that implementation of the strategy will proceed. As funds become available, the work of this task force will continue in 1988.

III. International Meeting

A. X INACG meeting

1. Quito, Ecuador was the site of the X INACG meeting, 12-15 May, 1987. 32 Ecuadorians and 22 international participants from 9 other countries attended the meeting. Ecuador's National Development Council (CONADE) co-hosted the meeting with INACG; Pan American Health Organization (PAHO), Nestlé, and Latinreco were additional sponsors. Dr. Edouard DeMaeyer chaired the meeting.

2. Several presentations at the beginning of the meeting provided background in preparation for the working group sessions later in the week. These preliminary sessions included summaries of the INACG workshop on social marketing of iron deficiency anemia and the INACG iron bioavailability research project, and reports on iron deficiency in relation to assessment of infants and children, infant and child development, school performance, and pregnancy.

   Country reports from Chile, Egypt, India, Philippines, Caribbean, and China exposed current strategies and programs in progress. Suggestions were made that might be helpful particularly for the development of a strategy to combat iron deficiency in Ecuador. See item 4 below.

3. A major portion of the program was devoted to presentation and discussion of the Ecuador National Nutrition Survey design, analysis, and results. Iron status and its implications were given special attention.

4. During the meeting the participants divided into three working groups to develop a strategy for Andean countries to control iron deficiency anemia. The participants hoped that the resulting stragegy could be integrated into programs aimed toward maternal health and child well-being. Consensus statements for technical, marketing, and operational groups resulted from the discussions. After the meeting, these statements were submitted to CONADE for consideration in Ecuadorian national planning.

5. Several non-governmental organizations and industries with interest in nutritional anemia gave brief updates of their activities. Local media coverage highlighted meeting events relevant to the Eduadorian situation.

6. Appendix 4 contains the agenda, a list of participants, minutes, and working group reports from the X INACG meeting in Quito.

7. Events in conjunction with the meeting included a meeting of the task force to review the JNSP document "Preventing and controlling iron deficiency anemia: the contribution of primary health care" and a tour of the Nestlé research facility Latinreco.

B. XI INACG meeting

1. A tentative invitation was issued by the Chinese ministry of health to hold the XI INACG meeting in Beijing in September or October 1988. Several individuals in China also offered to facilitate arrangements for the meeting.

2. H.J. Heinz Company of Canada, Ltd. offered to cosponsor this meeting if the meeting was planned for China in September or October 1988.

3. Inquiries regarding AID relations with China were initiated during the summer of 1987. The secretariat was informed that AID does not have a policy permitting use of AID funds for projects in China.

4. Alternative plans for the XI meeting will be determined during January and February 1988.

IV. Publications

A. Combating Iron Deficiency in Chile: A Case Study

1. This case study of national programs to control iron deficiency in Chile was published in 1986. The monograph contains information concerning the prevalence of the anemia problem and the variety of programs instituted to reduce the severity of the public health problem. The Chilean innovations are intended to serve as a model for other effective country programs.

2. During 1987 this monograph was translated into both French and Spanish. A simply produced Spanish edition was available for distribution at the X INACG meeting. Publication of both editions as monographs under the auspices of International Life Sciences Institute-Nutrition Foundation was initiated. Copies of both editions will be available from the secretariat in 1988.

V. Calendar of 1987 INACG Activities

Activity/Task Force	Status
1. Iron bioavailability research project	- meeting to coordinate manuscript held 26-27 March - 3 presentations made at 1987 FASEB meetings - paper submitted to AJCN for peer review and publication
2. Iron bioavailability in weaning foods	- initial meeting held 22 April - protocol drafted and submitted to potential funding organizations - equipment purchased on assurance of Gerber funding - initiation of study anticipated first half of 1988
3. 1987 International meeting	- 3 March meeting with representatives of AID, PAHO, WHO, FAO regarding agenda and funding - 12-15 May in Quito, Ecuador - meeting minutes mailed to participants
4. Task force to review JNSP position paper "Preventing and controlling iron deficiency anemia: the contribution of primary health care"	- 16-17 May in conjunction with international meeting - paper submitted to WHO for publication and circulated to task force in October 1987

5.	Combating Iron Deficiency in Chile: A Case Study	- translation to French and Spanish completed - publication of translations initiated - publications available from secretariat in 1988
6.	Social marketing of programs to control iron deficiency anemia	- initial efforts to implement strategy proposed in August 1986 instituted
7.	1988 International meeting	- tentative invitation issued from Chinese ministry of health - offer of cooperation from H.J. Heinz if meeting is held in China
8.	The relationship between immune response, infections and iron deficiency	- no action during 1987
9.	Safety of sodium-EDTA and ferric-EDTA	- no action during 1987

VI. 1987 Expenditures and Funding

A. AID vouchered expenditures

Activity	Expense
International travel	$23,159.77
Domestic travel	1,006.61
Domestic per diem	721.51
International per diem	11,384.03
Communications	7,887.78
Reports, publications	38,375.98
Editorial services	151.19
Supplies	601.35
Consulting services	295.13
Meeting room rental	109.38
Administration	9,433.39
Credit to AID for previous years	(13,432.15)
TOTAL	79,693.97

B. Additional sources of funding

1. Many groups provided support for the X INACG meeting in Quito, Ecuador. AID contributions to the meeting were approximately $32,500. Support from other organizations was in excess of $32,000 and represented money allocated for travel, per diem, secretarial support, and expenditures not reimbursable by AID. Supporting organizations included:

   Abbott Laboratories del Ecuador
   The Coca-Cola Company
   CONADE
   Doremus Porter Novelli
   Instituto De Nutricion Y Tecnologia De Los Alimentos (INTA)
   Brazilian National Academy of Nutrition (INAN)
   Indian Council of Medical Research
   Joint WHO/UNICEF Nutrition Support Program (JNSP)
   Latinreco S.A.
   Manoff International, Inc.
   Nestlé Research Department
   Pan American Health Organization (PAHO)
   U.S. Food and Drug Administration
   World Health Organization (WHO)

2. Many groups provided support for task force meetings and other INACG activities during 1987. This support was in excess of $11,000 and represented money allocated for travel, per diem, and other expenditures. Supporting organizations included:

General Mills, Inc.
Gerber Products
Heinz U.S.A.
INTA
JNSP
Nestec S.A.
Nestlé Research Department
PAHO
WHO

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XD-MAX-951-A
151-54955

APPENDIX 1

International Nutritional Anemia Consultative Group

Task Force Report on Iron Bioavailability

DRAFT

NOT FOR CIRCULATION

Report of the International Nutritional Anemia Consultative Group Task Force on Iron Bioavailability[7]

Allan L Forbes, MD, (Chairman)[1]; Catherine E Adams, PhD[2]; Maurice J Arnaud, PhD[3]; C O Chichester, PhD[2]; James D Cook, MD[4]; Bertha N Harrison, PhD[1]; Richard F Hurrell, PhD[3]; Samuel G Kahn, PhD[5]; Eugene R Morris, PhD[6]; James T Tanner, PhD[1]; Paul Whittaker, PhD[1,4]

[1]Food and Drug Administration, Washington, DC 20204

[2]International Life Sciences Institute - Nutrition Foundation, Washington, DC 20036

[3]Nestle Research Laboratories, Lausanne, Switzerland

[4]University of Kansas Medical Center, Kansas City, KS 66103

[5]Agency for International Development, Washington, DC 20523

[6]US Department of Agriculture, Beltsville, MD 20705

[7]Address reprint requests to: Paul Whittaker, PhD, Food and Drug Administration, 200 C St SW, Washington, DC 20204

Please address correspondence and galley proofs to:

Technical Editor
Food and Drug Administration
Center for Food Safety and
Applied Nutrition
200 C Street, S.W., HFF-40
Washington, D.C. 20204

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Participants

The Task Force gratefully acknowledges and expresses its sincere appreciation to the following who contributed to various aspects of this study.

Mattie R Spivey Fox, PhD, Ann O Lee Jones, Martin P Bueno, Donna Waldrop-Scott and Kathleen K Cook from the Food and Drug Administration, Division of Nutrition, Washington, DC;

A David Hill from the US Department of Agriculture, Beltsville Human Nutrition Research Center, Beltsville, MD;

Paul-Andre Finot, PhD and Anne Klein from the Nestle Research Center, Lausanne, Switzerland;

Paul D Maugle, PhD from the Nutrition Foundation.

Acknowledgements of Sponsors

The Task Force gratefully acknowledges the financial support of the following companies and government agencies.

Companies:

Nestle Food Co Purchase, NY	General Mills, Inc Minneapolis, MN
Kellogg Co Battle Creek, MI	HJ Heinz Co Pittsburgh, PA
The Quaker Oats Co Barrington, IL	Kraft, Inc Glenview, IL
Proctor and Gamble Co Cincinnati, OH	The Glidden Co St. Louis, MO
Gerber Products Co Fremont, MI	Roman Meal Tacoma, WA
Hoffmann-LaRoche, Inc Nutley, NJ	Carnation Co Los Angeles, CA
Nabisco Brands East Hanover, NJ	General Foods White Plains, NY
Ross Laboratories Columbus, OH	Bristol Meyers Co Evansville, IN

Acknowledgements of Sponsors (cont'd)

Government Agencies:

Food and Drug Administration
Washington, DC

US Department of Agriculture
Beltsville, MD

World Health Organization
Geneva, Switzerland

Agency for International Dev.
Washington, DC

Summary of iron bioavailability study participants and experiments

Participating institutions	Clinical tests	Rat Hb repletion tests	Rat iron absorption tests	In vitro tests
FDA Washington, DC		*		*
Nestle Research Lausanne, Switzerland		*	*	*
U of KS Med Ctr Kansas City, KS	*	*	*	
USDA Beltsville, MD			*	*

Introduction

The present series of studies is a result of objectives established since 1978 by the International Nutritional Anemia Consultative Group (INACG)[1] and the protocols which followed to compare iron bioavailability methods (Fig 1).

Though iron bioavailability has long been measured by the rat bioassay, no comparative studies adequately compare iron bioavailability utilizing the same test iron compounds in clinical, animal and *in vitro* experiments which establish a viable comparison of results of the three approaches. The ultimate goal was to establish a laboratory method for accurately predicting bioavailability of iron compounds commonly used in fortification. Electrolytic iron (average particle size 20 um) and ferric orthophosphate with ferrous sulfate as a reference were tested concomitantly. The absorption ratio relative to ferrous sulfate in the clinical studies produced an index which was used to compare the relative biological values obtained in animal studies and *in vitro* experiments. Research protocols were developed and the project was designed as a collaborative effort involving industrial, academic and governmental agency laboratories.

The importance of iron in nutrition has been a topic of concern in the third world countries as well as in maternal and child health

[1]INACG was organized to promote international efforts aimed at reducing nutritional anemia.

care in developed countries. The practical regulation of iron fortification compounds would be aided by the establishment of reliable methods for predicting iron compound bioavailability in humans.

The following discusses the production of iron compounds as well as details of the various experiments using in vitro tests. Animal and clinical studies will follow with which a feasible method for predicting bioavailability may be developed.

Production procedures

Iron compounds for use in the study were produced by New England Nuclear (NEN, North Bellerica, MA). All products were manufactured approximately 2 years prior to their use in the experiments. Iron compounds were produced according to specifications given to NEN by the Nutrition Foundation. The non-radioactive and radioactive compounds were manufactured in a similar manner with the exception of scale. The non-radioactive compounds were manufactured and sized in batches that were 4000 times greater than that for the radioactive compounds.

Ferric orthophosphate

Non-radioactive: Four kg of ferrous sulfate ($FeSO_4$, heptahydrate) were dissolved in 1 L of warm water. The solution was filtered through glass wool into a 4 L beaker containing a

stirring bar. Concentrated phosphoric acid was added with continuous stirring until the solution was blue-colored and an excess of 0.05 had been attained. Sodium hypochorite was added until the solution turned green with a 0.05 excess. The addition of sodium hypochlorite was slow with constant and vigorous stirring to avoid precipitating iron as ferrous phosphate. Total yield was 800 g.

Trisodium phosphate dodecahydrate dissolved in water to saturation was slowly added to the 4 L beaker containing iron with constant stirring. Trisodium phosphate dodecahydrate precipitated the iron as ferric phosphate. The solution was covered and allowed to stand overnight. The supernatant was removed and water was added with stirring. Iron phosphate was again allowed to settle and the water was removed. The iron was repeatedly washed until the supernatant was pH 7 for 3 consecutive washings. The resulting precipitate was then filtered through a coarse frit and washed twice using water with continuous suction. Suction was maintained until the precipitate cake cracked. The ferric phosphate cake was then transferred to crystalizing dishes and dried in a 90°C oven, after which it was crushed with mortar and pestle until it was a powder. The product was then weighed and transferred to shipping vials, which were sealed under nitrogen.

Radioactive: Radioactive ferrous sulfate (1.0 g) was produced in a 50 mL centrifuge cone using iron-55 chloride ($^{55}FeCl_3$) (specific

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activity = 3.7×10^6 Bq/mg Fe; total activity = 3.7×10^7 Bq) as the starting material. The $^{55}Fe\ Cl_3$ was evaporated to dryness and dissolved in dilute sulfuric acid. Sulfur dioxide gas (SO_2) was bubbled through the solution to cause the reduction of iron from ferric to ferrous state. The solution was then heated to expel all SO_2 and the solution was cooled. $FeSO_4$ was added as the carrier iron source. Total yield was 200 g of radioactive ferrous sulfate with a specific activity of approximately 1.5×10^5 Bq/mg iron.

This solution was then used as the starting material in the production of radioactive ferric phosphate following the process outlined above for the non-radioactive compound.

Electrolytic iron compounds

Non-radioactive: A plexiglass plating cell (2.4 x 2.0 x 2.8 cm) was immersed in a water bath (50°C). $FeSO_4$ (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 iron 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 throughout the plating process. Each plating cycle was 4 - 8 h, at the end of which plated iron was removed from the cathode. The plated iron was then pulverized

using a hand grinder to approximately 0.6 cm pieces and passed through a ball mill purged with nitrogen. Iron was removed from the ball mill at 4 - 8 h intervals and was passed through brass sieves (sizes 30 and 10 um) to identify particles with an average size of 20 um. (Twenty um average size particles were those that passed through the 30 um sieve, but were caught in the 10 um sieve.) The sized product, total yield of 1000 g, was then weighed and dispensed into shipping vials sealed with nitrogen.

Radioactive: Radioactive $^{55}Fe\ Cl_3$ (specific activity = 3.7×10^6 Bq/mg Fe; total activity = 7.4×10^5 Bq) was converted to the sulfate form as described in the process for radioactive ferric phosphate. After the non-radioactive iron had been plated out, $^{55}FeSO_4$ was added to the plating bath. The power was turned on and the system allowed to run for 4 h. Plated iron was removed from the cathode, was pulverized and passed through the ball mill under nitrogen. Total yield was 1.5 g iron. Iron particles with 20 um average size were separated, weighed and dispensed into shipping vials sealed under nitrogen.

Iron bioavailability by *in vitro* tests

James T Tanner, PhD, Richard F Hurrell, PhD, Eugene R Morris, PhD, and Paul Whittaker, PhD

ABSTRACT The feasibility and reliability of predicting iron bioavailability in humans using solubility and dialyzability in simulated digestion was evaluated using iron fortification compounds commonly added for human consumption. Electrolytic iron-55 and non-radioactive electrolytic iron, radioactive and non-radioactive ferric orthophosphate were tested for solubility and non-radioactive iron sources were compared to ferrous sulfate for dialyzability. It was found that radioactive sources were relatively more soluble as compared to the non-radioactive counterparts. Three laboratories then compared dialyzability of the identical non-radioactive compounds combined in a farina based meal in a simulated *in vitro* gastrointestinal digestion system using ferrous sulfate as reference. Relative dialyzability among the three participating laboratories ranged from 0.58 to 0.75 for electrolytic iron and from 0.28 to 0.46 for ferric orthophosphate.

KEY WORDS Iron, *in vitro*, iron bioavailability

MATERIALS AND METHODS

Non-radioactive and radioactive iron sources were investigated for their solubility in dilute HCl and non-radioactive iron sources for their dialyzability in an in vitro simulated gastrointestinal digestion system (1). Laboratory 1 compared the non-radioactive and the radioactive sources by solubility; laboratory 4 made the solubility measurements only with non-radioactive sources. Laboratories 1, 2 and 4 made the dialyzable iron determination with non-radioactive sources.

Solubility in dilute HCl

The method used for non-radioactive iron 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 0.2 g /100 g HCl (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 for 3 h. Two aliquots of 2 mL each were taken at 30 min and centrifuged for 5 min at 2000 r/min. One mL supernatant was transferred to a 25 mL volumetric flask and filled to volume with 0.2 g /100 g HCl. Iron content was measured by atomic absorption spectroscopy (3).

For the radioactive iron, the above method was scaled down to approximately one-fourth (micro method) due to the limited quantities of material available. Five mg of radioactive iron was shaken for 30 min in 65 ml 0.2 g /100 g HCl and an aliquot of 1 mL

was centrifuged. The non-radioactive iron was also tested by the scaled-down method for comparison.

Dialyzable iron

The method was that of Miller et al (1), as modified by Hurrell et al (4), in which dialyzable iron passes from an in vitro enzymatic digestion of a test meal into the interior of a dialysis bag containing 25 mL of 0.5 mol/L $NaHCO_3$. The test meal was a scaled-down version of the farina meal fed in the human study. The non-radioactive test iron sources and $FeSO_4$ were premixed with powdered sucrose in a no. 45 sieve or a coffee mill to a concentration of 0.6 mg Fe/g.

The test meal was prepared in the following manner: fifty mL distilled water containing 0.1 g NaCl were brought to a boil in a 250 mL erlenmeyer flask or 150 mL wide-necked conical flask. Farina (8 g) was added and the mixture boiled for 1 min with constant stirring. Whole milk (24.0 g), 2.8 g butter, 3.33 g sucrose-iron premix (containing 2 mg iron) and 1.47 g powdered sucrose were added and mixed thoroughly. When cool, the pH was adjusted to 2 with 6 mol/L HCl and the final weight of the test meal adjusted to 100.0 g with 0.01 mol/L HCl. Twenty gram aliquots were measured in triplicate for dialyzable iron as described previously by Hurrell et al (4), using bathophenanthroline to measure iron in the dialysate.

RESULTS

Solubility in dilute HCl

Table 1 illustrates the solubility of the non-radioactive iron sources in 250 mL 0.2 g /100 g HCl. The electrolytic iron was much more soluble than the $FePO_4$. In laboratory 1, after 30 min shaking, 0.75 of the electrolytic iron had been dissolved and 0.60 in laboratory 4. $FePO_4$ was very insoluble with a range of 0.03 to 0.04 solubility after 30 min (Table 1).

The results for the micro-method (65 mL 0.2 g /100 g HCl) are given in Table 2. Under these conditions, solubility of the non-radioactive iron was similar to values obtained with the macro-method in laboratory 4 but slightly less than the values obtained in laboratory 1 (Table 1). Within the same laboratory, however, and using the same method, radioactive sources were slightly more soluble in dilute HCl than corresponding non-radioactive compounds. After 30 min shaking, the radioactive electrolytic ^{55}Fe source was 0.87 soluble compared to 0.60 for the non-radioactive electrolytic iron, and the solubility of $^{55}FePO_4$ was 0.10 compared to 0.03 for $FePO_4$.

Dialyzable iron

Dialyzable iron was less in all laboratories. The amount dialyzed from the <u>in vitro</u> enzymatic digestion of the ferrous sulfate-fortified farina meal ranged from 1.39-3.4 % in the

three participating laboratories. Dialyzability relative to $FeSO_4$ was 0.58-0.75 for electrolytic iron and 0.28-0.46 for $FePO_4$.

DISCUSSION

Solubility in dilute HCl

Solubility in HCl was measured by two methods: the macro method, based on the procedure of Shah et al (2) and the scaled-down micro method. Although the solubility of an iron source naturally varies with time, a single value was necessary in order 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 iron and its relative bioavailability as measured in the hemoglobin repletion studies. The micro method was used specifically to compare the radioactive and non-radioactive iron sources. Both radioactive iron sources, but especially the electrolytic iron were more soluble than the corresponding non-radioactive sources. Although the $^{55}FePO_4$ had a greater solubility than the non-radioactive $FePO_4$, it was a relatively insoluble iron source compared to $FeSO_4$.

Dialyzable iron

The dialyzable iron values were low and slightly variable. The low values are presumably due to possible inhibitory substances present in cereals which bind iron 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 iron sources. The longer an iron source remains in a liquid system at low pH, the more it will dissolve and be dialyzed. If sufficient time is allowed so that the iron source is dissolved, it will be dialyzed similarly to $FeSO_4$ or other soluble iron sources. In the present study, each laboratory used an identical method but was not required to adhere to a strict time schedule. Nevertheless, $FePO_4$ was predicted as a relatively poorly bioavailable iron source by all laboratories and the electrolytic iron 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 and it seems that the product of <u>in vitro</u> digestion in some way chelated iron soluble forms able to pass through the dialysis membrane.

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TABLE 1

Solubility of non-radioactive iron sources in 0.2 g/100 g HCl by the macro method*

	Laboratory 1		Laboratory 4	
Time (min)	Electrolytic Fe	$FePO_4$	Electrolytic Fe	$FePO_4$
30	0.75	0.04	0.60	0.03

*Solubility ratio of dissolved iron to 20 mg iron in 250 mL 0.2 g/100g HCl at 37°C, duplicate analysis.

TABLE 2

Solubility of radioactive and non-radioactive iron sources in 0.2g/100g HCl by the micro method*

	Radioactive (iron-55)		Non-radioactive	
Time (min)	Electrolytic ^{55}Fe	$^{55}FePO_4$	Electrolytic Fe	$^{55}FePO_4$
30	0.87	0.10	0.60	0.03

*Solubility ratio of dissolved iron to 5 mg iron in 65 mL 0.2g /100g HCl at 37°C, single analysis.

TABLE 3

Dialyzable iron from farina meals fortified with non-radioactive iron.

Iron source	Laboratory 1		Laboratory 2		Laboratory 4	
	Dialyzable iron (% of dose)	Relative dialyzability*	Dialyzable iron (% of dose)	Relative dialyzability	Dialyzable iron (% 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

*Relative dialyzability = $\frac{\text{Dialyzable test iron (\% of dose)}}{\text{Dialyzable iron of } FeSO_4 \text{ (\% of dose)}}$

+Mean ± SEM

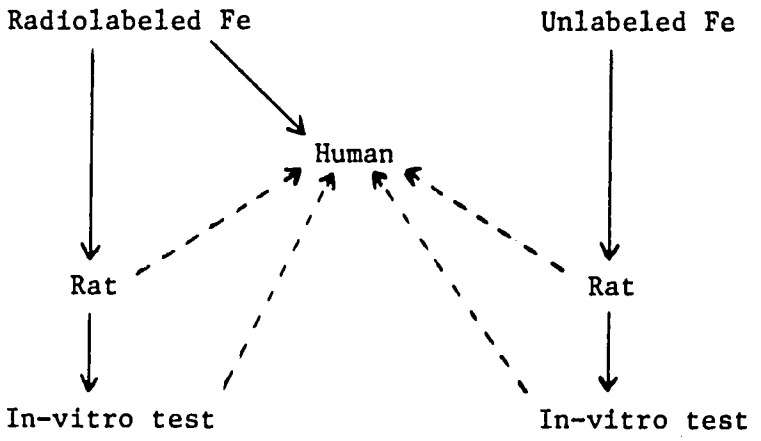

Fig 1. Basic study design for comparing different methods for the prediction of iron bioavailability in the human.

Assessment of bioavailability of fortification iron compounds in the rat model

Richard F Hurrell PhD, James D Cook, MD, Bertha N Harrison PhD, Eugene R Morris PhD, and Paul Whittaker PhD

ABSTRACT In order to evaluate the reliability and usefulness of the rat model in predicting human iron bioavailability, two series of experiments were designed using common iron fortification compounds: 1) Evaluation of the two most feasible hemoglobin repletion test methods and 2) Evaluation of iron absorption tests using radiolabeled compounds in an attempt to approximate human absorption studies. The two repletion tests evaluated were the Hemoglobin Regeneration Efficiency (HRE) method in which ordinary dietary depletion was supplemented by phlebotomy and the AOAC method. Radiolabeled and non-radiolabeled iron sources were compared using a modified HRE method. Weanling Sprague Dawley rats were first depleted, then repleted on a standard diet fortified with ferric orthophosphate, electrolytic iron and ferrous sulfate as reference. Laboratories using the AOAC method found the bioavailability of electrolytic iron to be 0.66 and 0.77 and that of ferric orthophosphate to be 0.25 and 0.33 relative to the reference. The HRE method found Relative Biological Value (RBV) of electrolytic iron to be 0.78 and ferric orthophosphate to be 0.58. The RBV of radiolabeled and non-radiolabeled sources were similar with electrolytic iron 0.60 and 0.61 respectively and ferric

orthophosphate values 0.80 and 0.78. Three laboratories participated in iron absorption tests using the same radiolabeled fortification compounds offered to animals on an iron replete diet in order to more closely resemble human studies. Relative absorption of the electrolytic iron source was 0.48, 0.69 and 0.47 and that of ferric orthophosphate 0.26, 0.26 and 0.22 in the three laboratories.

KEY WORDS: Iron, iron bioavailability, rat

Evaluation of the hemoglobin repletion tests in rats

MATERIALS AND METHODS

Bioavailability tests of the non-radioactive iron sources were based on hemoglobin repletion in anemic rats, as measured by two different methodologies. Laboratories 1 and 4 used a method similar to that recommended by the Association of Official Analytical Chemists (AOAC) (1,2), whereas Laboratory 3 used a technique similar to that of Mahoney (3), the Hemoglobin Regeneration Efficiency (HRE). Laboratory 4 used the HRE method to test the radioactive iron sources.

Modified AOAC Method - Laboratories 1 and 4

One hundred and forty male, weanling Sprague-Dawley rats individually housed in stainless steel cages, were fed a low-iron diet and deionized, distilled water ad libitum for 24 days. The low-iron diet contained (g/kg): glucose · H_2O 684.8, vitamin-free casein 200, corn oil 50, monosodium phosphate 20, calcium carbonate 20, potassium chloride 5, sodium chloride 5, trace element premix 2.7, choline chloride 1.5, vitamins (AIN 1976) 10, DL-methionine 1. The trace element premix contained (g/kg): $MgSO_4$, anhydrous 738.2; $ZnSO_4$, heptahydrate 196.6; $MnSO_4$, heptahydrate 57.3; $CuSO_4$, pentahydrate 7.3; KIO_3 0.6. After the depletion period, the rats were weighed and approximately 100 uL of blood was drawn from the orbital venous plexus (5) for hemoglobin analysis using a

commercial kit (Hoffman-La Roche Diagnostica, Basel, Switzerland) based on the cyanmethemoglobin method (5). One hundred and ten rats with hemoglobin values between 30 and 60 g/L were selected for the repletion test. Eleven groups of 10 rats, housed in a randomized block design, each received one of 11 experimental diets, which were prepared by fortifying the low-iron diet with $FeSO_4$ or with one of the test iron sources. $FeSO_4$ was added at 0,6,12,18 and 24 mg Fe/kg of diet; electrolytic iron and $FePO_4$ were added at 12, 24 and 36 mg Fe/kg (6).

The animals were fed the iron supplemented diets ad libitum for 14 days, after which they were weighed, blood was drawn, and hemoglobin level was measured as before. Food intake was recorded throughout the repletion period. The bioavailability of each test iron source relative to $FeSO_4$ was calculated by comparing gain in hemoglobin with the iron level in the diet by the slope ratio procedure (7,8).

HRE Method - Laboratories 3 and 4.

Laboratory 3

Thirty male weanling Sprague-Dawley rats, individually housed in stainless steel cages, were fed ad libitum a low iron diet (9 mg

Fe/kg, US Biochemical Co, Cleveland, OH) (6) and deionized, distilled water for 8 days. Approximately 1 mL of blood was drawn from the orbital venus plexus of each animal into a heparinized capillary tube on days 4 and 6 (4). After 8 days of depletion, the animals were weighed and blood was again drawn for hemoglobin determination by the cyanmethemoglobin method (5). The animals were randomized into 3 groups of 10 rats each with approximately equal average body weight and hemoglobin concentration. Each group was fed one of 3 experimental diets over a 10 day repletion period. The low-iron diet contained (g/kg of diet): corn starch 560, hydrogenated vegetable oil 140, vitamin-free casein 270, salt mixture (Hubell, Mendel and Wakeman) (9) without $FePO_4$ 30 and total vitamin supplement 4 (US Biochemical Co, Cleveland, OH).

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

$$\text{mg Hb iron} = \text{kg body wt} \times \frac{0.075 \text{ L blood}}{\text{kg body wt}} \times \frac{\text{g Hb}}{\text{L blood}} \times \frac{3.35 \text{ mg Fe}}{\text{g Hb}}$$

HRE values were then calculated for each animal as follows (3):

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

Laboratory 4

The HRE method was also used in laboratory 4, although modified due to a limited quantity of fortification iron. Fifty weanling male rats were randomized into five groups of 10 animals each so that mean body weight and hemoglobin concentration were approximately equal among the groups. The procedure was identical to that of laboratory 3 except that animals were depleted for 7 days and repleted for 9 days and five diets were prepared for the repletion period containing 20 mg fortification Fe /kg of diet (6). Each diet was fed during the nine day repletion period and supplemented with one of the non-radiolabeled or radiolabeled iron compounds as follows: 1) $FeSO_4$, heptahydrate; 2) Electrolytic Fe; 3) $FePO_4$, dihydrate; 4) Electrolytic ^{55}Fe and 5) $^{55}FePO_4$, dihydrate.

Dietary consumption was recorded daily, with correction for spillage. The HRE values were calculated as in laboratory 3.

RESULTS AND DISCUSSION

Using the modified AOAC technique, laboratories 1 and 4 reported similar Relative Bioavailability Values (RBV) for the test iron sources (Table 1). The RBVs of electrolytic Fe were 0.66 and 0.77, and $FePO_4$ were 0.25 and 0.33 in laboratories 1 and 4 respectively.

Laboratory 3 used the HRE technique to calculate RBV (Table 2). HRE values in this laboratory were found to average 0.75, 0.59 and 0.44 in anemic rats fed $FeSO_4$, electrolytic Fe, and $FePO_4$, respectively, yielding RBVs of 1.00, 0.78 and 0.58. Hemoglobin gain in the same rats averaged 86g/L in those fed $FeSO_4$, 64 g/L for those fed electrolytic Fe, and 44 g/L for those receiving $FePO_4$. Using hemoglobin gain to calculate RBV, values of 0.75 and 0.51 resulted for the electrolytic Fe and $FePO_4$, which were slightly lower, but of the same order, as the values calculated from HRE values. In this laboratory the RBV for $FePO_4$ was higher than that

reported by the AOAC method, although the RBV for electrolytic Fe was similar.

In laboratory 4, both the radiolabeled and non-radiolabeled iron compounds were tested by the HRE method. Results were similar to those of laboratory 3 for the non-radiolabeled iron compounds: HRE values for $FePO_4$ were 0.46 and 0.44, electrolytic Fe 0.65 and 0.59, $FeSO_4$ 0.76 and 0.75 for laboratories 4 and 3, respectively. In laboratory 4 similar HRE results were also obtained for the radiolabeled and non-radiolabeled electrolytic Fe (0.61 and 0.65, respectively). Values for radiolabeled and non-radiolabeled $FePO_4$ were both 0.46.

The reason for the higher RBV of $FePO_4$ in the HRE method (table 2) than in the AOAC method (table 1) is most likely due to the different depletion/repletion methodologies used. In laboratories using the modified AOAC method, weanling rats were depleted to a hemoglobin level of 30 to 60 g/L over 24 days solely by dietary means. In the HRE method, weanling rats were depleted within 7 or 8 days by a combination of phlebotomy and low-iron diet. The anemic rats were then fed diets containing a single level of iron (20 or 35 mg Fe/kg diet) for 9 or 10 days.

Using the HRE method iron stores are not as severely depleted (11) and anemic rats were approximately two weeks younger when repleted with the test iron sources. The HRE method also takes the body weight gain factor into account.

Evaluation of the iron absorption test in rats

MATERIALS AND METHODS

This assay was developed to resemble human iron absorption studies as closely as possible. Rats seven to eight weeks of age were fed a single meal containing both $^{59}FeSO_4$ and one of the ^{55}Fe labeled test iron sources. The level of incorporated red cell activity was measured 2 weeks later by scintillation counting and absorption of the test iron sources relative to $FeSO_4$ was calculated (12,13). Three laboratories participated in the iron-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 idential aliquots of 5 mg of electrolytic ^{55}Fe (6.1 x 10^5 Bq 8 March 1985), and 25 mg $^{55}FePO_4$ [$^{55}FePO_4$, dihydrate (9.3 x 10^5 Bq 8 March 1985)] but used its own $^{59}FeSO_4$. The latter was produced by dissolving non-radioactive $FeSO_4$ in water and adding sufficient $^{59}FeSO_4$ to yield a specific activity of 1.4-1.9 x 10^5 Bq/mg Fe. The solution was freeze-dried to a grey powder of approximately 0.33 iron, indicating that it was predominantly $FeSO_4$, monohydrate (6).

Laboratories 1 and 2: Twenty weanling male Sprague-Dawley rats were housed individually in stainless steel cages and fed <u>ad libitum</u> an iron-adequate diet containing 35 mg Fe/kg as $FeSO_4$ for 4 to 5 weeks. The diet was identical to that used in the hemoglobin

repletion study but with a higher iron content. Deionized, distilled water was provided throughout. After this preliminary period, the rats were divided into 2 groups of 10 rats and were trained to eat by meal. Each rat was offered 20 g of diet between 6:00 and 8:00 pm in the evening and any remaining diet, between 7:00 and 9:00 am the following morning. At 6:00 pm on the third day, rats weighing an average of about 250 g were fed one meal consisting of 10 g of diet containing both $^{59}FeSO_4$ and one of the labeled test iron sources. Each group of 10 rats received a different ^{55}Fe labeled test iron source. The meal was removed after 2-2.5 h, by which time most 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 (14,15). The iron-adequate diet was continued the following morning and was fed ad libitum with deionized, distilled water for 14 days. On day 14, following 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 radio labeled meal contained about 400 ug 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 iron sources with 9 g of the low iron basal diet (as used in the

hemoglobin repletion test) from which an equivalent quantity of glucose had been omitted. The glucose-iron premixes were prepared in a coffee mill and the homogeneity and iron content confirmed by atomic absorption measurements (6).

Laboratory 2 prepared and fed the radioactively-labeled meals similar to that in laboratory 1. The meals were prepared by thoroughly mixing 2 g of a glucose-iron premix with 8 g of low-iron basal diet. The glucose-iron premixes were produced by mixing and pulverizing until homogeneity was assured. Each 10 g meal contained approximately 230 ug Fe as $^{59}FeSO_4$ plus one of the following: 370 ug Fe as electrolytic ^{55}Fe or 650 ug Fe as $^{55}FePO_4$ (6).

In Laboratory 1, simultaneous measurement of ^{59}Fe and ^{55}Fe activity in 100 uL blood was made by liquid scintillation counting after digesting in 1.5 mL Protosol/ethanol (1:2) and decolorization with 0.5 mL 0.30 H_2O_2 for 30-60 min in a shaking water bath at 60°C (13). Background counts were determined in identical quantities of non-radioactive 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 diluted with deionized, distilled water to 100 mL. One hundred uL of digested premix and 100 uL of non-radioactive blood were digested, decolorized, and counted as above. Laboratory 2 used the same method with 200 uL of blood. The glucose-iron premixes (0.5 g) were ashed, dissolved in HCl and diluted to 25 mL.

Absorption of each isotope was calculated by dividing cpm into total blood volume [Body weight (kg) x 0.075 L/kg] (10) by cpm ingested. No adjustment was made for the portion of absorbed dietary iron not present in the blood. The retention of ^{59}Fe was also determined by whole body counter measurements.

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

Two diets were prepared for the iron-adequate test each containing 33 ug Fe/g of fortification iron (6). Each animal received 20 g of diet containing 330 ug of Fe (2.6×10^4 Bq ^{55}Fe) from the ^{55}Fe labeled compound ($FePO_4$ or electrolytic Fe) and 330 ug of Fe (1.9×10^4 Bq ^{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 (14,15). Rodent Laboratory Chow # 5001 (Ralston Purina Company, St. Louis, MO) was then fed <u>ad libitum</u> with deionized, distilled water for 14 days. 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 venus plexus (4) into a heparinized capillary tube for blood parameters and radioactivity measurements.

Simultaneous determination of ^{59}Fe and ^{55}Fe activity in 100 uL blood was accomplished by liquid scintillation counting (13). A mixture of Soluene-350/2- propanol 1:2 (V/V) was added and the vial was swirled gently; within half an hour, the blood was solubilized. Five tenths mL of 0.30 hydrogen peroxide was then added dropwise to increase the bleaching effect. To dissociate the peroxides, vials were placed in a water bath at 40°C for 30 min. After cooling to room temperature, a mixture of 15 mL Insta-Gel:0.5 mol/L HCl (10:1 V/V) was added to the vial, vortexed and counted. Background counts were determined using the same quantity of non-radioactive rat blood. To determine the amount of ^{59}Fe and ^{55}Fe consumed, 20 g samples of each diet were first wet-digested and counted using the above procedure.

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

RESULTS

The absorption values of $^{59}FeSO_4$ and electrolytic ^{55}Fe in the rat were reported by the three participating laboratories, are shown in table 3. The $^{59}FeSO_4$ percent absorption was 32.7 and 19.7, in laboratories 1 and 2 respectively, compared to 63.5 in the mildly anemic rats of laboratory 3. The elemental ^{55}Fe source was not as well absorbed as $^{59}FeSO_4$ with an average percent absorption of 16.0 in laboratory 1, 13.7 in laboratory 2 and 30.1 in laboratory 3. 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. Absorption of the elemental iron source relative to $FeSO_4$ was 0.48, 0.69 and 0.47, respectively, in the three laboratories.

Iron absorption values for rats fed $^{59}FeSO_4$ and $^{55}FePO_4$ simultaneously are shown in table 4. Absorption of ^{59}Fe from $FeSO_4$ 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. Absorption of $^{55}FePO_4$ relative to $^{59}FeSO_4$ was 0.26, 0.26 and 0.22 respectively in the participating laboratories.

DISCUSSION

Mean percent absorption of iron from $FeSO_4$ by rats in the different laboratories varies from 20 to almost 70, although within the same laboratory, mean absorption of $FeSO_4$ from the two different meals fed was almost identical. The high absorption of iron by rats in Laboratory 3 is due to their being slightly anemic (mean Hb 117 g/L). The ^{55}Fe labeled test iron sources were also better absorbed by rats in Laboratory 3 and consequently the absorption of these iron sources relative to the absorption of $FeSO_4$ was similar among the laboratories. This is especially true for $FePO_4$ (approximately 0.25 that 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 Laboratory 2 (a value which was confirmed by whole body counter). Laboratories 1 and 4 fed identical amounts of ^{59}Fe and ^{55}Fe (approximately 400 ug) whereas Laboratory 2 fed 230 ug ^{59}Fe, 370 ug ^{55}Fe as electrolytic Fe and 650 ug ^{55}Fe as $FePO_4$. This explanation does not appear valid since a high level of iron in the test meal would be expected to lower the absorption and thus yield a lower absorption ratio.

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ɜLE 1

ɜBV of test iron sources as measured by the AOAC method.

Iron Source	Supplemental iron (mg Fe/kg diet)	Hemoglobin Gain (g / L)	RBV ratio*
Laboratory 1			
$FeSO_4$	0, 6, 12, 18, 24	-10, 5, 15, 32, 42	1.00
Electrolytic Fe	12, 24, 36	8, 26, 42	0.66 ± 0.02+
$FePO_4$	12, 24, 36	-3, 2, 12	0.25 ± 0.02
Laboratory 4			
$FeSO_4$	0, 6, 12, 18, 24	-10, 7, 20, 42, 49	1.00
Electrolytic Fe	12, 24, 36	17, 42, 58	0.77 ± 0.04
$FePO_4$	12, 24, 36	-6, 13, 21	0.33 ± 0.03

*Slope of test iron source (Hb gain vs level of supplementation) relative to slope of $FeSO_4$.

+Mean ± SEM

TABLE 2

RBV of test iron sources as measured by the HRE method.

Iron Source	Supplemental iron (mg Fe/kg diet)	Hb Gain (g/L)	HRE ratio	RBV ratio*
Laboratory 3				
$FeSO_4$	35	86 ± 3	0.75 ± 0.02+	1.00 ± 0.03
Electrolytic Fe	35	52 ± 3	0.59 ± 0.03	0.78 ± 0.04
$FePO_4$	35	44 ± 3	0.44 ± 0.02	0.58 ± 0.03
Laboratory 4				
$FeSO_4$	20	41 ± 2	0.76 ± 0.03	1.00 ± 0.05
Electrolytic ^{55}Fe	20	27 ± 3	0.61 ± 0.03	0.80 ± 0.05
Electrolytic Fe	20	35 ± 2	0.65 ± 0.05	0.86 ± 0.06
$^{55}FePO_4$	20	14 ± 2	0.46 ± 0.02	0.60 ± 0.03
$FePO_4$	20	19 ± 3	0.46 ± 0.03	0.61 ± 0.04

* Mean hemoglobin regeneration efficiency of test iron source divided by mean hemoglobin regeneration efficiency of $FeSO_4$.

+ Mean ± SEM

TABLE 3

Absorption of electrolytic ^{55}Fe

Rat	Laboratory 1 absorption $^{59}FeSO_4$ (A) (% of dose)	Laboratory 1 absorption ^{55}Fe (B) (% of dose)	Laboratory 1 Ratio (B/A)	Laboratory 2 absorption $^{59}FeSO_4$ (A) (% of dose)	Laboratory 2 absorption ^{55}Fe (B) (% of dose)	Laboratory 2 Ratio (B/A)	Laboratory 3 absorption $^{59}FeSO_4$ (A) (% of dose)	Laboratory 3 absorption ^{55}Fe (B) (% of dose)	Laboratory 3 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

TABLE 4
Absorption of $^{55}FePO_4$, dihydrate

Rat	Laboratory 1 absorption $^{59}FeSO_4$ (A) (% of dose)	Laboratory 1 absorption $^{55}FePO_4$ (B) (% of dose)	Laboratory 1 Ratio (B/A)	Laboratory 2 absorption $^{59}FeSO_4$ (A) (% of dose)	Laboratory 2 absorption $^{55}FePO_4$ (B) (% of dose)	Laboratory 2 Ratio (B/A)	Laboratory 3 absorption $^{59}FeSO_4$ (A) (% of dose)	Laboratory 3 absorption $^{55}FePO_4$ (B) (% of dose)	Laboratory 3 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

Assessment of bioavailability of fortification iron compounds in human subjects

Paul Whittaker, PhD and James D Cook, MD

ABSTRACT Iron compounds commonly used for the fortification of cereal foods were radiolabeled to permit studies of their bioavailability in human subjects. When compared directly with ferrous sulfate in a farina-based meal, relative absorption of ferric orthophosphate was only 0.25 as compared to 0.75 with electrolytically-reduced iron of small (20 um) particle size. The addition of 100 mg ascorbic acid to the meals produced a brisk parallel increase in absorption of all labeled iron forms but did not alter the absorption ratio between less available forms of iron and ferrous sulfate. The use of doubly-tagged meals provides a highly sensitive method for assessing bioavailability of fortification compounds in humans and the results are independent of meal composition.

KEY WORDS: Iron, iron bioavailability, human

This work was supported by AID Cooperative Agreement DAN-0227-A-00-2104-00.

INTRODUCTION

There is a continuing need for reliable methods to assess the bioavailability of iron fortification compounds, especially in countries where fortification is extensively practiced. Ferrous sulfate is the most bioavailable iron source but produces an organoleptic effect in many food vehicles. This has resulted in the use of more inert forms of iron such as phosphate compounds or elemental iron powders. While undesirable changes in color and taste can often be avoided by using these forms of iron, 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 iron sources. A variety of physical chemical methods have been used to evaluate elemental iron powders including particle size, solubility in hydrochloric acid and reactive surface area (1). The most widely used animal method is to compare the hemoglobin response in anemic rats given graded quantities of the fortification iron source with that obtained from ferrous sulfate (2). The degree to which these animal measurements predict bioavailability in human subjects is uncertain since 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 iron

compounds. $FePO_4$ and electrolytic Fe 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.

MATERIALS AND METHODS

Subjects

Absorption measurements were performed in 25 normal volunteers, 12 men and 13 women, ranging in age from 18-38 yr. None of the subjects gave a history of disorders which might affect the absorption of iron from the gastrointestinal tract. All subjects were in good health although two had depleted iron stores as reflected in a serum ferritin level under 12 ug/L. All the 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

Two iron sources were labeled with ^{55}Fe under conditions believed to produce a product comparable to commercially available sources. These consisted of $FePO_4$, and electrolytic Fe of small (20 um) particle size. With each of these iron compounds, a limited amount of ^{55}Fe labeled material was prepared together with a larger quantity of unlabeled material under identical conditions. The radiolabeled and cold forms of iron 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 iron was evaluated in two separate studies by comparison with $FeSO_4$. An identical farina-based meal was employed in both studies. The first test meal contained 3 mg iron as $^{59}FeSO_4$ and the same quantity of iron from the alternate iron 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 days later from meals containing added ascorbic acid. Four separate radioiron absorption tests were performed in each subject from two separate doubly-labeled meals.

Absorption Tests

All test meals were served between 7:00 and 9:00 am following an overnight fast. Only water was allowed for the subsequent 3 h. After obtaining blood for measurement of background radioactivity and serum ferritin (3), the first test meal containing 3.7×10^4 Bq ^{59}Fe and 1.1×10^5 Bq ^{55}Fe was administered. Blood was obtained 14 days later to assay incorporated red cell radioactivity. The second test meal containing the same quantity of ^{55}Fe and ^{59}Fe was then eaten and 14 days later a final blood samples was drawn. Absorption was determined from the radioactivity incorporated into circulating red cells for the first pair of tests and from the

the increase in radioactivity from day 14 to day 28 for the second test meal. All measurements of ^{55}Fe and ^{59}Fe were performed on duplicate 10 mL samples of whole blood (4). Absorption of iron was calculated on the basis of the blood volume estimated from weight and height of the subject (5,6). Red cell incorporation of absorbed radioactivity was assumed to be 0.80 (7).

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 iron and the same quantity of the alternate iron compound tagged with ^{55}Fe was thoroughly mixed with the meal immediately prior to serving. $FeSO_4$, heptahydrate, analytical grade, was used. The second meal given 2 weeks 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 iron absorption measurements in humans (8). 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 ln 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% with a range of 0.12 to 7.07 (Table 1). The absorption ratio of $FePO_4$ to $FeSO_4$ was relatively constant, ranging from 0.12 to 0.44 (Fig. 1). The mean ratio of 0.25 (± 1SE, 0.23-0.28) indicated that the absorption of $FePO_4$ was only 0.25 that of $FeSO_4$. It is of interest that although the addition of 100 mg ascorbic acid resulted in a striking increase in iron absorption of about four-fold, the absorption ratio was unchanged, averaging 0.25 (ratio C/D, Table 1). This indicates that the facilitating effect of ascorbic acid on these two forms of fortification iron was identical.

The absorption of electrolytic Fe relative to $FeSO_4$ was much higher than $FePO_4$. The geometric mean absorption averaged 3.37% as compared to 4.52% with $FeSO_4$ (Table 2). Absorption averaged 0.75 relative to $FeSO_4$ (ratio A/B ±1 SE, 0.72-0.78). Ascorbic acid produced a sharp increase in absorption of both iron forms although the relative increase was slightly less with electrolytic Fe; the mean ratio with/without ascorbic acid for $FeSO_4$ was 2.83 as

compared to only 2.37 with electrolytic Fe. This difference was statistically significant ($p = 0.007$). As a result, relative availability decreased from 0.75 to 0.62 (Fig 1).

DISCUSSION

One of the earliest clinical studies of the availability of fortification iron compounds using radioisotopic techniques was that of Steinkamp, Dubach, and Moore (9) who could detect no obvious differences among the several preparations 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 iron 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 iron with $FeSO_4$ by adding both isotopes to the same meal. It is theoretically possible that some interaction occurs between two iron sources present in the same meal. For example, $FeSO_4$ which served as the reference iron compound may promote the solubility of less available forms of iron or perhaps the absorption of $FeSO_4$ is reduced by adsorption to the surface of less soluble particulate forms of iron. This possibility was carefully exclused in previous

studies showing 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 (1,10).

The absorption of nonheme dietary iron in humans varies widely depending on the nature of the meal. This could pose a problem when evaluating bioavailability. Highly soluble forms of iron such as $FeSO_4$ undergo complete isotopic exchange with the common nonheme iron pool (11,12), but the degree of exchange of less soluble iron sources may vary with different meals. To examine this possibility, the various iron compounds in the present study were evaluated using test meals with and without added ascorbic acid to obtain a wide difference in nonheme iron absorption. With $FePO_4$, relative bioavailability was identical despite a dramatic three-fold increase in absorption when ascorbic acid was added to the farina meal. This indicates that at least with this form of fortification iron, the nature of the meal does not influence the estimation of relative bioavailability. On the other hand, the increase in absorption of electrolytic Fe with ascorbic acid was somewhat less than with $FeSO_4$ (Fig 2). This is not too surprising because all of the iron in powdered $FeSO_4$ is available to react with ascorbic acid, whereas with elemental iron ascorbic acid cannot react with iron that is contained within the core of an iron particle. Nevertheless, the differences in estimates of bioavail-

ability at the extremes of nonheme iron absorption were minor and it can be concluded that the nature of the meal has little influence on bioavailability measurements in humans.

There is a remarkable similarity in the bioavailability of $FePO_4$ determined in the present study and that observed in a previous study in which this form of iron was baked into wheat rolls (12). The mean absorption ratio for $FePO_4$ relative to $FeSO_4$ in the earlier report was 0.31 as compared to a value of 0.25 in the present study. These estimates are consistent with rat studies in which bioavailability relative to $FeSO_4$, referred to as relative biological value (RBV), ranged between 0.07 and 0.32 (13,14). The difference in reported RBV for $FePO_4$ is similar to that observed by Harrison and coworkers (15) who obtained values between 0.06 and 0.46. It is not clear whether the wide range of bioavailability values in animal studies is a reflection of differences in the sources of $FePO_4$ or due to methodologic variability.

The bioavailability of elemental iron which is used extensively for cereal and flour fortification has been evaluated more extensively. Elemental iron powders are preferred because of a lesser tendency than $FeSO_4$ to produce off-flavors and discoloration. The commonest forms used by the food industry are electrolytic Fe which is produced by the electrolytic deposition of iron onto stainless steel cathodes and reduced iron which is prepared by reducing

ground iron oxide with either hydrogen or carbon monoxide. A variety of physical chemical methods such as particle size, solubility in dilute acid and reactive surface area have been used to predict bioavailability in human subjects. While these physical chemical methods are helpful in characterizing elemental iron powders, the rat hemoglobin repletion method remains the basis for predicting bioavailability in humans. In general, a much lower bioavailability for the type of electrolytic Fe evaluated in the present study has been reported in animal studies. For example, Shah and coworkers (16) reported an RBV of 0.32 for electrolytic Fe with a mean particle size of 4 um. Fritz and colleagues (13) reported an RBV of 0.75 for electrolytic Fe with a particle size between 10-20 um and only 0.48 with a particle size of 20-40 um. In the present study, a value of 0.75 was observed for the small particle size. However, the value is consistent with relative bioavailability of 0.95 observed previously for reduced iron with a particle size between 5 and 10 um (10). Humans may be more efficient in solubilizing particulate forms of fortification iron than small laboratory animals.

There is ample reason for concern about existing methods to characterize the bioavailability of fortification iron compounds. Studies based both on physical chemical methods and hemoglobin response in anemic rats have consistently shown that carbonyl iron is a highly bioavailable form of elemental iron. For example, Shah and coworkers (16) reported that carbonyl iron has the smallest

particle size, the greatest solubility, and the highest bioavailability (RBV 0.61). On the basis of these and other studies, carbonyl iron has been ranked superior to both electrolytic and reduced iron which has led to its extensive use in Sweden for flour fortification. However, when radiolabeled carbonyl iron was prepared by neutron activation and used to fortify wheat flour, the relative bioavailability in human volunteers varied between only 0.05 and 0.20 relative to $FeSO_4$ (17). It is conceivable that the process of neutron irradiation may have altered the bioavailability of the product used for human studies although the rate of dissolution of the labeled material was similar to commercial sources of carbonyl iron. Surprisingly, the absorption of this batch of elemental iron was not influenced by the addition of ascorbic acid (17).

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TABLE 1. Bioavailability of Ferric Orthophosphate

Subject	Sex Age	Packed Cell Volume	Serum Ferritin (ug/L)	IRON ABSORPTION -Ascorbic Acid $FePO_4$ (A)	-Ascorbic Acid $FeSO_4$ (B)	+Ascorbic Acid $FePO_4$ (C)	+Ascorbic Acid $FeSO_4$ (D)	ABSORPTION RATIO A/B	C/D	C/A	D/B
				(% of dose)							
1.	M32	0.43	109	0.22	0.75	1.32	4.72	0.29	0.27	5.99	6.29
2.	M23	0.47	76	1.98	4.47	3.20	10.28	0.44	0.31	1.61	2.29
3.	F23	0.42	50	0.51	1.70	3.92	12.87	0.29	0.30	7.68	7.57
4.	F21	0.36	42	0.58	3.11	1.57	5.91	0.18	0.26	2.70	1.90
5.	M26	0.44	40	1.12	3.46	1.10	7.01	0.32	0.15	0.98	2.02
6.	F24	0.39	33	0.12	0.98	1.20	9.28	0.12	0.21	10.00	9.46
7.	F24	0.40	28	0.26	1.03	0.82	4.78	0.25	0.17	3.15	4.64
8.	F21	0.40	23	0.46	2.76	4.23	12.45	0.16	0.33	9.19	4.51
9.	M21	0.44	19	0.71	3.15	4.76	14.07	0.22	0.33	6.70	4.46
10.	F22	0.41	14	7.07	24.88	21.11	49.33	0.28	0.42	2.98	1.98
11.	F25	0.41	13	1.67	7.13	7.50	30.78	0.23	0.24	4.49	4.31
12.	F23	0.38	5	6.33	17.36	17.72	76.56	0.36	0.23	2.79	4.41
MEAN*	24	0.41	28	0.83	3.30	3.29	13.00	0.25	0.25	3.95	3.94
			-1SE	0.58	2.41	2.41	10.03	0.23	0.23	3.21	3.37
			+1SE	1.20	4.52	4.48	16.85	0.28	0.28	4.86	4.61

*Geometric means except age and packed cell volume

TABLE 2. Bioavailability of Small Particle (20 um) Electrolytic Iron

Subject	Sex Age	Packed Cell Volume	Serum Ferritin (ug/L)	IRON ABSORPTION -Ascorbic Acid Fe°(20) (A)	-Ascorbic Acid $FeSO_4$ (B)	+Ascorbic Acid Fe°(20) (C)	+Ascorbic Acid $FeSO_4$ (D)	ABSORPTION RATIO A/B	C/D	C/A	D/B
				(% of dose)							
1.	M19	0.49	79	2.38	2.83	3.41	5.20	0.84	0.65	1.43	1.83
2.	M18	0.47	72	1.20	2.38	3.71	5.47	0.50	0.67	3.09	2.29
3.	M19	0.48	69	2.98	3.78	6.30	8.68	0.78	0.72	2.11	2.29
4.	M22	0.43	62	1.01	1.35	2.35	3.60	0.74	0.65	2.32	2.66
5.	M19	0.48	51	6.68	8.41	9.96	15.48	0.79	0.64	1.49	1.84
6.	M18	0.46	50	1.21	1.63	13.73	20.35	0.74	0.67	11.34	12.48
7.	F20	0.36	25	1.20	1.41	4.12	6.21	0.85	0.66	3.43	4.40
8.	F21	0.43	24	12.45	16.15	12.72	18.98	0.77	0.67	1.02	1.17
9.	M20	0.48	20	6.66	7.85	16.61	33.71	0.84	0.49	2.49	4.29
10.	M18	0.46	18	3.76	5.88	11.61	13.77	0.63	0.46	3.08	4.21
11.	F22	0.37	15	4.57	5.86	13.68	15.81	0.77	0.86	2.99	2.69
12.	F29	0.41	14	15.67	21.72	15.88	35.57	0.72	0.44	1.01	1.63
13.	F23	0.40	11	3.82	5.16	9.31	15.40	0.74	0.60	2.43	2.98
MEAN*	21	0.44	28	3.37	4.52	7.97	12.76	0.75	0.62	2.37	2.83
				2.62	3.53	6.63	10.37	0.72	0.59	1.99	2.40
				4.33	5.78	9.59	15.71	0.78	0.66	2.82	3.34

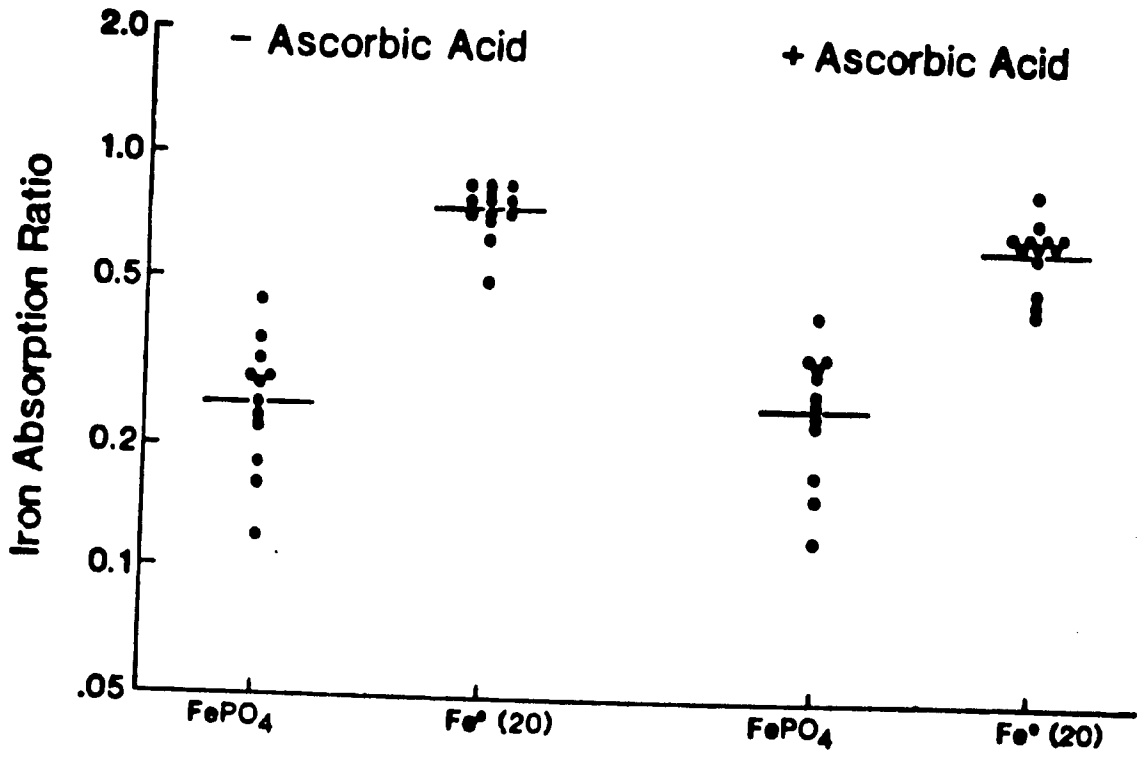

Fig 1. Absorption of fortification iron compounds expressed as a ratio of ferrous sulfate absorption from a farina meal with and without ascorbic acid.

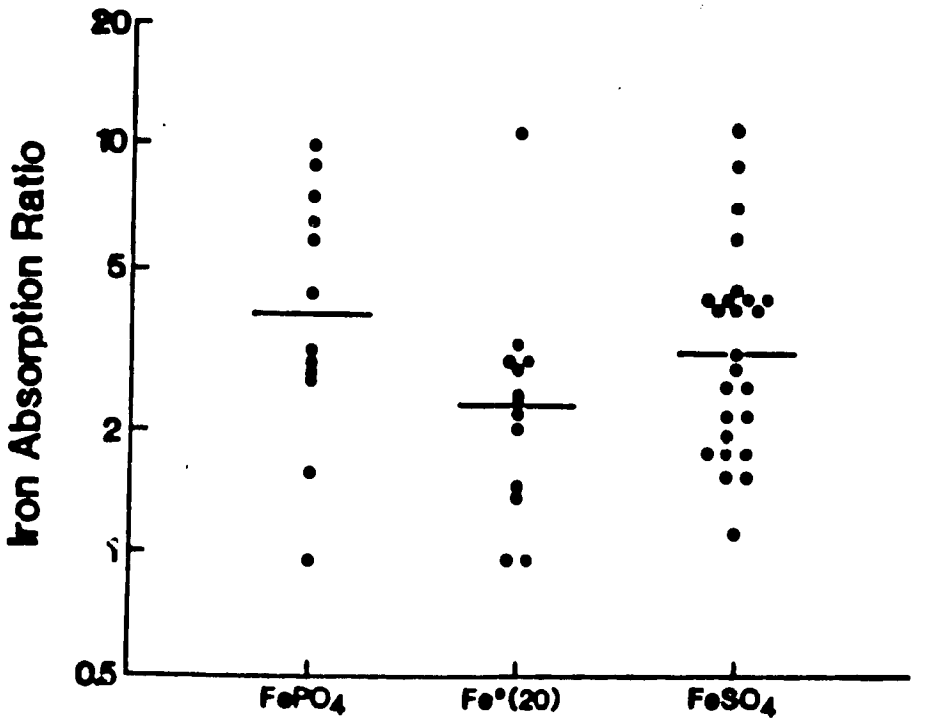

Fig 2. Increase in absorption of various iron compounds following the addition of 100 mg ascorbic acid to the meal.

Summary of the iron bioavailability study

Clinical Study

The clinical measurements of iron absorption in this study serve as the reference point against which other animal and physical chemical methods for assessing iron bioavailability are compared. It is important therefore to consider any potential pitfalls in these measurements. The reliability of absorption tests in human subjects has improved dramatically in recent years because of progressive refinements in methodology. In earlier clinical studies of iron availability from fortification compounds each labeled iron compound was studied in a separate group of subjects. The drawback of this approach is that the characteristically wide subject-to-subject variation in absorption obscures any differences related to the form of administered iron. In the present study, all iron compounds were assessed within the same subject by calculationg absorption ratios of either $FePO_4$ or electrolytic Fe to $FeSO_4$.

Even with comparisons in the same subject, day-to-day variations in absorption typically range between 20-40%. These variations may still mask differences in iron availability unless the number of subjects is quite large. This second component of variation was eliminated in the present study by adding both forms of radioiron to the same test meal. These methodologic refinements result in narrow confidence bands for estimates of bioavailability. For

example, in the case of $FePO_4$, the 0.95 confidence limits for the relative availability value of .25 are .21-.31 (Table 1).

One theoretical concern when both labeled compounds are added to the same meal is that some interaction might occur between the two iron sources during the digestion process. For example, $FeSO_4$ which served as the reference iron compound might promote the solubility of less available forms of iron or perhaps the absorption of $FeSO_4$ is reduced by the presence of less soluble particulate forms of iron. However, this theoretical possibility was carefully excluded in previous studies which showed that the bioavailability of fortification iron compounds relative to $FeSO_4$ is the same whether the two radiolabeled sources are administered on different days or added to the same meal.

Another consideration in clinical studies is whether estimates of iron bioavailability are influenced by the nature of the test meal. It is known that highly soluble forms of iron such as $FeSO_4$ undergo complete isotopic exchange with the common nonheme iron pool, but it is conceivable that the degree of exchange with less soluble iron forms depends on the level of food iron absorption. To explore this possibility, we decided n the present study to examine two levels of food iron 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 13.0% in the study with $FePO_4$ (Table 1) and from 4.5 to 12.8% in the study with electrolytic Fe (Table 2). With ferric orthophosphate, the estimate of bioavailability remained identical despite a three-fold increase in absorption. Obviously, the bioavailability of this form of fortification iron is not influenced by the level of absorption from the test meal. In the case of electrolytic Fe, the increase in absorption with ascorbic acid was less than with $FeSO_4$; as a result, relative availability was reduced from .75 to .62. These findings suggest that the facilitating effect of ascorbic acid may be somewhat diminished with elemental iron powders as compared to iron salts although the difference is minor. Nevertheless, it seems reasonable to conclude from the clinical studies that the nature of the test meal has little or no effect on estimates of iron bioavailability. For comparison with the animal and in vitro measurements of availability, we have chosen the value of .75 obtained with the farina test meal containing no added ascorbic acid.

A final consideration sometimes discussed in clinical studies is whether estimates of relative bioavailability are influenced by the iron status of the volunteer subjects. Although all of the volunteers in this study were hematologically normal, serum ferritin levels indicated a wide range in iron status in both study

groups. This variation is also reflected in differences in iron absorption from $FeSO_4$, which ranged from 0.8 to 24.9% in the study with $FePO_4$ and from 1.4 to 21.7% in the study with electrolytic Fe. However, there was no obvious difference in bioavailability ratio between subjects with low and high serum ferritin levels. For example, in measurements with $FePO_4$, absorption ratio of 0.29 and 0.44 were observed in subjects with the highest ferritin levels and 0.23 and 0.36 in the subjects with the lowest serum ferritin values. These observations indicate that estimates of bioavailability in human subjects are not affected significantly by either the nature of the meal or the iron status of the volunteers.

Comparison of Methods

Though animal studies may serve as useful predictors, it must be considered that in practical terms, human diet is mixed, containing components which potentially enhance or inhibit iron absorption whereas the experimental animal diet is more homogeneous in nature.

The most widely accepted method for predicting iron bioavailability in human subjects is hemoglobin repletion. This technique measures the hemoglobin response to graded amounts of iron in rats with induced iron 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 0.77 were observed in the rat method as compared to a value of 0.75

in humans. Excellent agreement was also obtained with $FePO_4$ which gave RBV values of 0.25 and 0.33 as compared with a value in clinical studies of 0.25.

A modified hemoglobin repletion technique, the hemoglobin regeneration efficiency (HRE) method, was also evaluated in the present study. 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 with this method is that only one level of dietary iron is tested and calculations are based on the efficiency of converting dietary iron into hemoglobin. The method is more economical because only one quarter of the number of animals are required to test an iron compound. This was a major advantage in the present study because it permitted a direct comparison of bioavailability from the cold and radiolabeled iron sources; there was not a sufficient amount of labeled material to perform the standard AOAC method. The depletion period is only 7 days for the HRE method as compared to 28 days or longer for the AOAC method and the repletion period is 10 days for the HRE method as opposed to 14 days for the AOAC method. The results obtained with the radiolabeled sources were virtually identical to those obtained with the cold iron sources. Thus, with electrolytic Fe, the radiolabeled material gave an RBV of 0.80 as compared with a value of 0.86 using the unlabeled material. With $FePO_4$, a value of 0.60

was obtained as compared to a value of 0.61 using the unlabeled source.

The bioavailability of radiolabeled iron sources was also evaluated in iron 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 to a clinical RBV of 0.75. We could find no explanation for this interlaboratory variation. It is interesting however, that in one of the laboratories (laboratory 3), the rats were mildly iron deficient when studied. Nevertheless, the RBV value of 0.47 for electrolytic Fe was nearly identical with the value of 0.48 obtained in laboratory 1 in iron replete rats. This indicates that as in human studies, iron status does not influence estimates of bioavailability. It should also be noted that a radioactive animal method is of little practical value in the assessment of fortification iron compounds because of the difficulty in preparing radiolabeled iron sources.

<u>In vitro</u> tests of solubility in dilute acid showed acceptable agreement with other measures of bioavailability. With electrolytic Fe, relative solubility values ranged between 0.60 to 0.87; the mean of 0.72 compares well with the clinical value of 0.75. With a more insoluble source such as $FePO_4$, <u>in vitro</u>

solubility appeared to underestimate bioavailability as measured in human subjects. Values ranged between 0.03 and 0.10 with a mean of 0.05. These tests of in vitro solubility again showed no consistent difference between the labeled and unlabeled iron sources. As suggested in previous studies, solubility in dilute acid provides a valuable screening method for assessing iron fortification compounds.

Studies were also performed with an in vitro technique that has not previously been used to assess the bioavailability of fortification iron. The major differences is that availability is measured in the presence of food by determining the proportion of dialyzable iron following 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 ranging from 0.58 to 0.75 and for $FePO_4$, values varied between 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 pronounced differences in dialyzable iron. It seems likely that with further experience and refinement, this method may give results comparable to those obtained with the hemoglobin repletion technique. Dialysis measurements can be performed at a small fraction of the cost of animal studies and can be undertaken

by laboratories that do not have animal facilities. Further evaluation of this technique using different meals and a greater range of iron fortification compounds might lead to a simplified approach for evaluating iron bioavailability.

Conclusions

1. Equivalency of radioactive and non-radioactive sources: confirmed as equal by HRE method.
2. Effectiveness of several methods for predicting bioavailability of various iron sources in humans, with ferrous sulfate as the reference material:

   AOAC (modified) 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

   In vitro dialysis: promising, but needs precise standardization
3. Recommended present method of choice in predicting human iron bioavailability: preliminary screening using in vitro dialysis followed by evaluation using modified AOAC method, slope ratio technique.

APPENDIX 2

Minutes of the INACG Meeting on the Chilean Clinical Study of Electrolytic Iron Bioavailability from Cereals in Infants

Minutes of the INACG Meeting on the Chilean
Clinical Study of Electrolytic Iron Bioavailability
from Cereals in Infants
22 April, 1987
ILSI-NF Office, Washington, D.C.

Present: C.O. Chichester, Chairman; Catherine Adams; Peter Dallman; Jack Filer; Thad Jackson; Guy Johnson; Samuel Kahn; Ida Laquatra and Kathy Weimer

The meeting began at 09:30 and was chaired by C.O. Chichester. Dr. Chichester described the purpose of convening the meeting and initiating the clinical trial of electrolytic iron bioavailability from cereals in infants. The data from the recently completed INACG research study of iron bioavailability indicates that the bioavailability of electrolytic iron in humans is about 75% of that for ferrous sulfate and about 50% for iron depleted and iron adequate animals. Most animals study report that electrolytic iron bioavailability is from 55-75% that of ferrous sulfate. Dr. Samuel Fomon has recently raised the question of electrolytically-reduced iron bioavailability in infants.

Three points were identified by the committee that refute Dr. Fomon's allegations regarding the poor bioavailability of electrolytic iron. These include that:

(1) There is evidence that the contribution of iron to infant's diets from cereals is considerably greater than is implied by Dr. Fomon.

(2) Animal studies with electrolytic iron have demonstrated it to be reasonably bioavailable.

(3) A research study at the University of Illinois demonstrated that consumption of electrolytic iron from commercial cereal established adequate iron status in infants.

A clinical trial was proposed by Dr. Tomas Walter and Dr. Peter Dallman. The purpose of the study is to demonstrate the bioavailability of electrolytic iron from cereals and the iron's effectiveness in preventing iron deficiency for infants. The project would be conducted in Santiago, Chile with Dr. Walter as primary investigator.

The following protocol was proposed:

Group	Cereal source	Duration of Breastfeeding
1	Unfortified rice	Brief**
2	Fortified rice*	Brief**
3	Unfortified rice	Long***
4	Fortified rice*	Long***

* Fortification with electrolytic iron at 15 mg Fe/day consuming 30 g cereal/day
** Brief duration of breastfeeding = 3 months
*** Long duration of breastfeeding = 3 months

- Vitamin C fortification at 100 mg/liter milk for all groups
- Blood drawn for analysis at 3 (baseline), 9 and 12 months of chronological age.
- Solubility tests for iron compounds in cereal tentatively to be conducted at FDA laboratories
- Compliance evaluated by analyzing fecal iron content
- Data to be collected for anthropometrics, morbidity and weekly dietary intake
- If it proves to be impractical to fortify dried milk with ascorbate, then another means will be utilized to ensure adequate and equivalent intake of ascorbate for all groups. Gerber and Nestle will work on the technological aspects of ascorbate fortification of dried milk.

The meeting participants talked with Dr. Tomas Walter by telephone regarding several questions about typical infant feeding practices and feasibility of the project in Chile. A brief summary of conclusions from this discussion is given below.

- Typical infant feeding practices:
  - 60-70% breastfed for 3 months
  - 30-40% breastfed for 6 months
  - cereals usually introduced at 3-4 months
  - meat and vegetable soup puree fed at 4 months
  - dilute fruit juices fed at 2-3 months with small amounts fed

- Malnutrition is not a typical condition for Chilean infants.

- Dried cereal is usually consumed with milk at 10% solution (after 3 months) as part of bottle feeding.

- Mothers and infants are seen once/month at the clinic, but nurses visit children in homes once/week. Morbidity is evaluated weekly during nurse visitations.

- A good rapport exists between the clinic personnel and patients. This rapport facilitates that suggested dietary modifications fitting experimental protocol are followed (e.g., the inclusion of heme-fortified cookies in children's diets during a recent iron supplementation program).

- The inclusion of low-birth-weight infants in the study is not recommended.

There was a commitment for participation in the clinical study by Gerber Products, Nestle Foods Company and H.J. Heinz Company.

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APPENDIX 3

Preventing and Controlling Iron Deficiency Anemia: The Contribution of Primary Health Care

JEA023/17.07.87

PREVENTING AND CONTROLLING IRON DEFICIENCY ANAEMIA: THE CONTRIBUTION OF PRIMARY HEALTH CARE

A Guide for Health Administrators and Programme Managers

by

E.M. DeMaeyer

with the collaboration of

P. Dallman, M. Gurney, L. Hallberg, K. Sood and S.G. Srikantia

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PREFACE

The understanding of the epidemiology of iron deficiency anaemia and the technical means for preventing and controlling it has increased greatly in recent years. The International Anaemia Consultative Group, by providing a framework within which scientists interact and disseminate their findings, has contributed to the expansion of knowledge about this major threat to the well-being and productivity of so many millions of people the world over.

There are as yet relatively few examples, however, of the application of this knowledge in the development and implementation of comprehensive anaemia prevention and control strategies. On the other hand, the primary health care approach has been adopted by many countries in widely differing cultural, socioeconomic and environmental settings. A considerable body of evidence is accumulating based on these many national experiences indicating that primary health care may well offer the practical means for translating this new knowledge and understanding into lower anaemia prevalences.

The Joint WHO/UNICEF Nutrition Support Programme (JNSP) is active in eighteen countries in applying the primary health care approach, the general objective being to improve the nutritional status of children and their mothers. JNSP has generated a body of knowledge regarding the practical aspects of integrating primary health care at various levels of organisation to achieve nutritional goals. It is in this context that JNSP has sponsored this document, which is written for the use of health administrators and programme managers to help them develop suitable strategies for preventing and controlling iron deficiency anaemia through primary health care.

Grateful acknowledgement is made, through its Government, to the people of Italy, who made the publication of this document, and the JNSP itself, possible.

The help of Mr J. Akré in the preparation of the text is also gratefully acknowledged.

I. INTRODUCTION

Iron deficiency anaemia (IDA) is a problem of serious public health significance given its implications for both social behaviour and physical performance. It is the most common nutritional problem in the world today, affecting more than 700 million persons (1). Simply stated, a deficiency of iron occurs when the amount of iron absorbed is insufficient to meet the body's requirements. This is due either to inadequate iron intake, insufficient bioavailability of dietary iron, or chronic blood loss. Prolonged iron deficiency leads to iron deficiency anaemia.

Iron is by far the most common nutritional deficiency resulting in anaemia; a folate deficiency may be associated, especially during pregnancy. Other nutrient deficiencies such as vitamin B12, pyridoxine and copper are of little public health significance given their infrequency. Infants, preschool-age children, adolescents and women of child-bearing age, particularly pregnant women, are at greatest risk of developing iron deficiency anaemia. Adult males may also be at risk, however, especially where iron intake is low because of inadequate food intake or parasite infection frequent.

Treating IDA is technically relatively simple and requires only the administration of an iron supplement. Yet, for a variety of reasons, many people (particularly in developing countries) fail to obtain relief. Preventing it is somewhat more complex and may require food fortification, which is not yet practicable in many countries. Fortunately there are a number of simple prevention and control measures, which lend themselves to application through primary health care. In addition to reviewing these measures, this document discusses in some detail the scope of the problem, its causes and consequences, methods for assessing iron status, and the cost/benefit implications of prevention and control programmes.

II. THE NATURE OF THE PROBLEM AND ITS PREVALENCE AND CONSEQUENCES

Iron deficiency anaemia is said to exist when the haemoglobin concentration is lower than that which is considered normal for a given individual. In such cases increasing the intake of absorbable iron will raise the haemoglobin concentration (2). The use of haemoglobin concentration to determine the prevalence of anaemia, tends to underestimate the real extent of the problem, since many individuals with seemingly normal levels respond to iron administration. This implies that such individuals, were in fact in need of iron (3,4).

A homeostatic mechanism in the body regulates normal haemoglobin level. The distribution of normal values is generally similar the world over, after due allowance is made for such factors as age, sex, pregnancy, and altitude (5).

Based on information from published and unpublished sources, and using the cut-off points for haemoglobin levels recommended by a WHO scientific group (5) it is estimated (1) that some 30% of the world's population of 5 000 million (1987) are anaemic.

Young children and pregnant women are the most affected groups, with an estimated global prevalance of anaemia of 43% and 51% respectively. They are followed by school-age children with 37%, non-pregnant women with 35%, and adult males with 18% (see Table 1). Although a paucity of data concerning anaemia in adolescents and the elderly precludes any precise prevalence estimates for these two groups, it is thought that the rate is near that for adult females for the first and slightly higher than that of adult males for the second.

IDA is considerably more prevalent in developing than industrialized countries (36%, or about 1 400 million persons, out of an estimated population of 3 800 million in the former vs. 8%, or just under 100 million persons, out of an estimated population of 1 200 million in the latter). Africa and South Asia have the highest overall regional prevalence of anaemia. With the exception of adult males, the estimated prevalence of anaemia in all groups is over 40% in both regions and reaches 65% in pregnant women in South Asia. In Latin America, the prevalence of anaemia is lower, ranging from 13% in adult males to 30% in pregnant women. East Asia follows with an estimated range from 11% in adult males to 22% in schoolage children.

Table 1. Estimated prevalence of anaemia by region, age and sex in 1980 (in %)

Region	Percentage of anaemic individuals				
	Children 0-4 years	Children 5-12 years	Men	Women 15-49 years	
				Pregnant	All
Developed regions	12	7	3	14	11
Developing regions	51	46	26	59	47
World	43	37	18	51	35

Anaemia may be caused by several factors, not all of which are nutritional in origin. Infections, for example malaria, hookworm and bilharzia in tropical climates, play an important role in this regard. Congenital haemolytic diseases such as sickle cell anaemia and thalassemia are also observed in some races, particularly in Africa, Asia and some Pacific islands, but rarely constitute a significant public health problem. In some Asian countries, however, such as Viet Nam, Thailand, Burma and Laos the occurance of thalassaemia may warrant special consideration when iron supplementation programmes are being considered.

Taking all these factors into consideration, it is estimated that roughly 700-800 million people are affected by IDA in the world today. It should be noted that this is a very conservative estimate, however; the real figure is probably higher. Likewise, since IDA is the result of a relatively long process of deteriorating haemoglobin blood levels, many more persons are suffering from iron deficiency, with all that this implies in terms of general levels of health and physical stamina, than those who have actually reached the stage of anaemia.

The consequences of IDA are many and include:

In infants and children: (6,7,8,9)

- impaired motor development and coordination;
- impaired language development and scholastic achievement;
- behavioural effects (inattention, fatigue, insecurity, etc.);
- decreased physical activity;

In adults of both sexes (10,11)

- decreased physical work and earning capacity;
- decreased resistance to fatigue.

In pregnant women (12,13,14,15)

- increased maternal and fetal morbidity and mortality;
- increased risk of low birth weight.

The key role that haemoglobin plays in transporting oxygen to tissues explains the diminished work capacity and physical performance demonstrated by persons suffering from IDA. Iron deficiency not only causes a decreased concentration of haemoglobin but also leads to a number of functional changes at the cellular level in some of the above-mentioned impairments.

There is a growing body of evidence to suggest that iron deficiency affects the body's immune response. Defects in cell-mediated immunity and bacterial killing have been well demonstrated, although it is not definite whether morbidity resulting from infections is greater in iron-deficient persons. Early studies of the effects of IDA on infection frequency (16,17) appeared to show that the administration of iron to anaemic children, either as a medication or as a food fortificant, resulted in a decreased rate of respiratory and gastrointestinal diseases when compared to untreated children.

A more recent study has shown that correcting anaemia had little effect on the frequency of respiratory or gastrointestinal infections (18). It needs to be pointed out, however, that other factors such as associated PEM and the unfavorable environmental sanitation may have contributed to the lack of a positive effect.

In recent years it has also been proposed that iron administration might be harmful by predisposing a person to infection. There is some experimental evidence to suggest that iron-binding proteins protect animals from infection by withholding iron from the invading organisms that require it for growth (19). This phenomenon would explain why the administration of large doses of iron by injection could predispose to infection. However, an increased risk of infection has been demonstrated primarily when transferrin is nearly saturated with iron. Under ordinary circumstances transferrin is less than 35% saturated and only minimal changes occur in the percentage with iron consumption. Although iron deficiency may in fact protect the host against certain specific organisms under laboratory conditions, there is no evidence that such a possibility outweighs the many more tangible and persistent handicaps imposed by iron deficiency.

An investigation of the effects of iron deficiency on work performance indicates an obvious impairment with a number of health and economic implications. Its biochemical basis is relatively well understood. The biochemical basis for altered behaviour remains uncertain. Where the immune function is concerned, abnormalities that have been detected in the laboratory are impressive but their health implications are still not completely understood (20).

III. THE ETIOLOGY AND EPIDEMIOLOGY OF IRON DEFICIENCY ANAEMIA

A thorough discussion of iron requirements, intake and bioavailability is the basis for understanding why some individuals, for example women in their reproductive years, particularly pregnant women, infants and young children, are at greater risk of developing IDA than others.

3.1 Iron requirements

A dietary intake of iron is required to cover losses incurred through the stools, the urine and the skin. These basal losses represent approximately 14 ug/kg/day, or 0.9 mg for an adult male and 0.8 mg for an adult female (21). Account must also be taken of iron losses due to menstruation among women of reproductive age (see Table 2).

Table 2. Median iron requirements in terms of absorbed iron*, by age group and sex (28)

Age/sex	in ug/kg/day	in mg/day**
4-12 months	96	0.77
13-24 months	45	0.49
2-5 years	35	0.56
6-11 years	32	0.94
12-16 years (girls)	32	1.62
12-16 years (boys)	28	1.46
Pregnant women***		
Menstruating women	23	1.25
Post-menopausal women	14	0.77
Adult males	14	0.91
Lactating	19	1.05

* Absorbed iron is that fraction of iron which passes the gastro-intestinal tract into the body for further use.

** Calculated on basis of median weight for age.

*** Requirements during pregnancy depend on the iron status of the mother prior to pregnancy. See text for further explanations.

While the amount of menstrual blood lost is relatively constant for a given woman from month to month, it varies greatly from one woman to the next. Several studies have shown that the median blood loss during menstruation ranges between 25-30 ml/month, which represents an iron loss of 12.5-15 mg/month or 0.4-0.5 mg/day over 28 days. Added to basal losses this represents a total iron loss for menstruating women of 1.25 mg/day. The iron requirements of 50%

of all women are thus in excess of 1.25 mg/day. Taking into account the skew of the frequency distribution of menstrual blood loss, one can calculate that 10% of all women have iron losses in excess of 2.1 mg/day and 5% in excess of 2.4 mg/day.

While menstruation-related iron losses are reduced to nil during pregnancy, additional iron is still required for the fetus, the placenta and the increase in maternal blood volume. This amounts to approximately 1000 mg of iron over the entire pregnancy. Requirements during the first trimester are relatively small (0.8 mg/day), but increase considerably during the second and third (to a high of 6.3 mg/day) (see Fig. 1). Part of these requirements can be met by iron stores and by an adaptive increase in the percentage of iron absorbed, but when stores are low or non-existant and dietary iron is poorly absorbed, as is often the case in developing countries, iron supplementation is required. During lactation the absence of menstrual blood loss is partially offset by the secretion of about 0.3 mg/day of iron in breast milk. When added to basal losses a woman's mean iron requirement during the first six months of lactation is estimated at about 1 mg/day (see Table 2).

Infants, children and adolescents also require iron for expanding red cell mass and growing body tissue. The amount of iron in a normal infant at birth is about 75 mg/kg, two thirds of it in red blood cells. During the first two months of life there is a marked decrease in haemoglobin concentration with a consequent increase in iron stores. These stores are subsequently mobilised to supply needs for growth and replace iron losses; during this period there is a minimal requirement for dietary iron. By four to six months, however, iron stores have decreased significantly and the infant needs a generous dietary source of iron. During the first year of life a child triples its body weight and doubles its iron stores. Changes in the concentration of serum ferritin according to age parallel changes in iron stores (see Fig. 2).

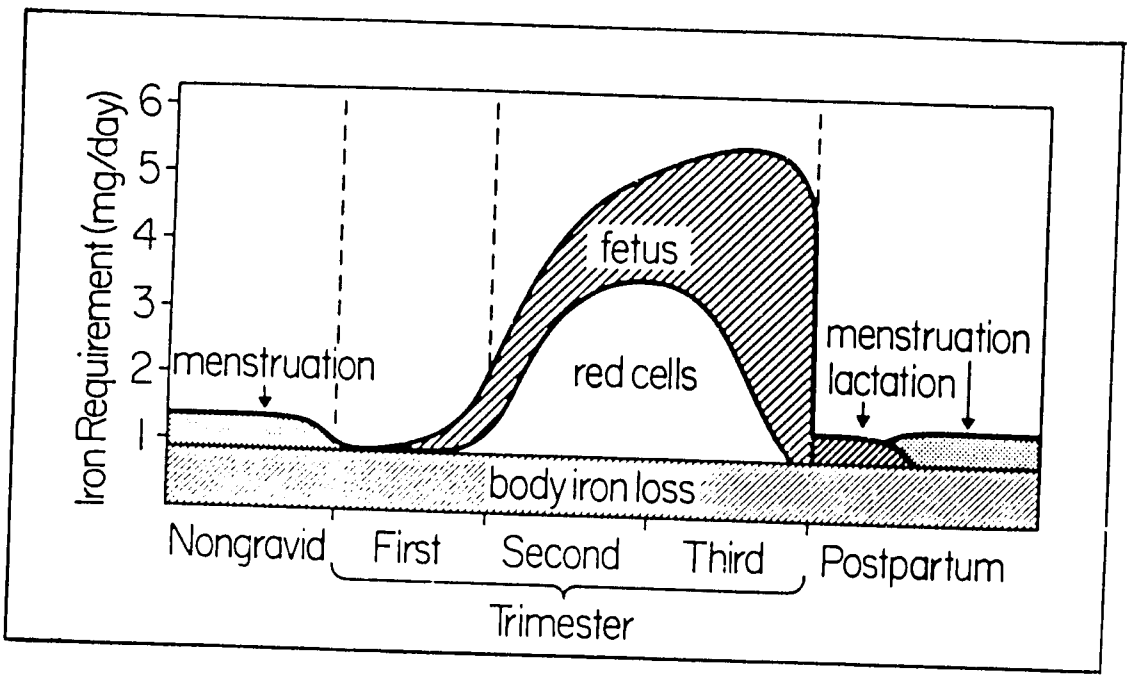

Fig. 1 Daily iron requirements during pregnancy

These requirements are needed to meet the normal losses from the body and to provide iron for the enlarging red cell mass, the fetus and for lactation after the birth of the child (21a).

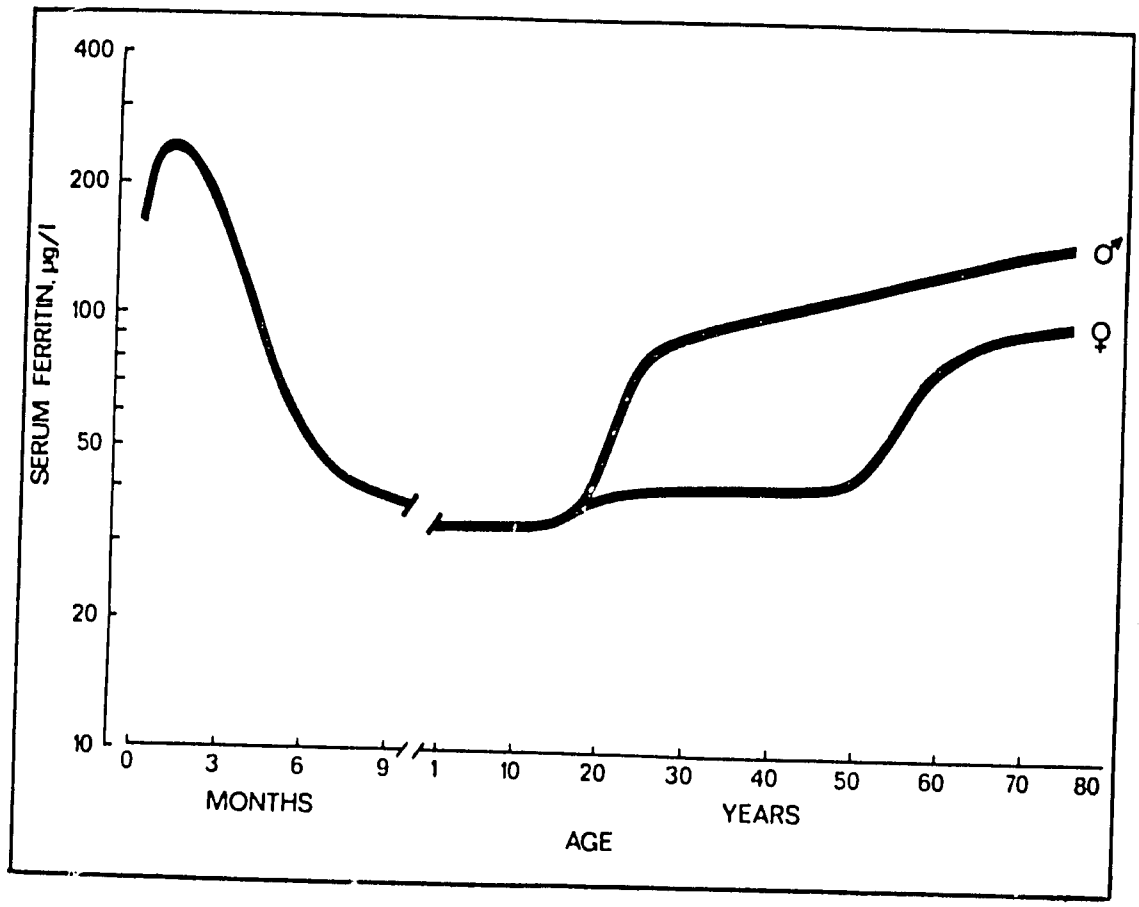

Fig. 2 Developmental changes in concentration of serum ferritin

Mean values (geometric means) in healthy populations are shown. At all ages, a serum ferritin concentration below 10 or 12 ug per litre is considered indicative of depleted iron stores (24,25,26,27).

Iron requirements are substantially higher in infants and children than in adults when expressed per kg of body weight (see Table 2). Since this group's total energy requirements are lower than adults', they eat less and if their diets have low bioavailability of iron, they are at greater risk of developing deficiency.

Iron requirements increase in cases of chronic bleeding caused by such parasites as hookworm, Bilharzia and possibly Trichuris trichiura, which are frequent infections in countries with hot and humid climates and poor sanitation. In the case of hookworm, blood loss varies from 2-100 ml/day according to the severity of infection (22). Part of the iron contained in the blood shed by the worm in the intestine will be reabsorbed lower down, but the remainder will be lost via the stools. It is estimated that the iron loss per thousand eggs per gram of faeces is 0.8 mg/day in the case of Necator americanus and 1.2 mg/day for Ankylostoma duodenale (23).

Infections interfere with food intake and the absorption, storage and use of many nutrients including iron. In many rural communities where environmental sanitation is poor, morbidity is high due to gastrointestinal infections. Respiratory infections and viral fevers are also common. It is in these same communities that diets are most often energy deficient. In situations where iron balance is precarious, repeated episodes of infection may lead to the development of anaemia, particularly in young children whose morbidity burden is much higher than that of adults. This situation explains in part the high prevalence of anaemia among infants and preschool children. By implication, the control of infection may have the greatest impact on the problem of anaemia and iron deficiency in this segment of the population.

3.2 Iron intake

There are two distinct types of dietary iron - haem iron and non-haem. Haem iron is present in haemoglobin and myoglobin and therefore in meat, fish, and poultry as also in blood products. It forms a relatively small fraction of total iron intake - usually less than 1-2 mg/day, or approximately 10-15% of dietary iron consumed in industrialized countries. In many developing countries haem iron intake is low or even negligible. The remaining iron is non-haem iron and forms the predominant part of dietary iron; it is found in all foods of plant origin. The absorption of these two types of iron is quite different.

Besides food iron the diet may also contain exogenous iron originating from the soil, dust, water or cooking vessels. This form of iron is more frequently found in diets of developing countries where the amount of contaminating iron in a meal may be several times higher than the content of food iron. The cooking of foods in iron pots may increase the iron content of a meal several fold. This is especially true of soups with vegetables having a low pH that are cooked for a long time. Frying in iron pans does not usually increase the iron content of a meal. Iron released during cooking is integrated into the non-haem iron pool and is available for absorption. The quantity of iron absorbed is determined by the overall properties of the meal and the iron status of those who are consuming it. Foods may also be fortified with iron in the form of reduced iron or iron salts added to flour, sugar, salt, etc. in order to increase iron intake. Contamination and fortification are the two major sources of exogenous iron (see Table 3).

Table 3. Iron in food

Chemical form and type of iron	Food source
Haem iron	In meat, fish, poultry and blood products. Between 0-15% of iron intake in industrialized countries. Usually less than 10% of total iron intake, often negligible amounts, in developing countries. Bioavailability high: absorption 20-30%
Non-haem iron:	
- food iron	Mainly in cereals, tubers, vegetables, pulses. Bioavailability determined by the presence of enhancing and inhibiting factors in the diet.
- contamination iron	In soil, dust, water, from iron pots etc. Can be present in large amounts. Potential availability usually low but its significance cannot be overlooked.
- fortification iron*	The iron compounds used vary in potential bioavailability. Bioavailability of soluble fraction determined by composition of meal.

* Fortification is used to describe the process whereby nutrients are added to foods to maintain or improve the quality of the diet of a group, a community, or a population (29).

The amount of iron present in habitual diets is relatively constant when expressed in terms of 1000 calories (iron density). The actual amount, however, varies considerably between countries. For example, in developed countries, whose diets contain large amounts of meat and fish as also refined cereals, iron density is around 5.5 mg/1000 calories, while in most developing countries, whose diets are based predominantly on foods of plant origin, it can be twice as high, as in India.

Dietary iron intake is thus influenced by levels of energy consumed. The energy needs of children and women are relatively low and they, therefore, consume less food than do other segments of the population. This makes it more difficult for them to have their iron needs met and places them at increased risk of developing deficiency. This is also true of persons, who for any reason subsist on low energy diets.

The consumption of a high iron-density diet does not necessarily imply that it will help to meet iron requirements, since the amount available after absorption, is as important as the total content. This availability varies considerably depending upon the types of food which constitute the total diet.

3.3 Absorption of dietary iron

Several factors influence the absorption of dietary iron, for example the amount and chemical form of iron, the presence in the diet of factors enhancing and/or inhibiting iron absorption, and the iron status of the individual (see Tables 3 and 4).

Table 4. Major determinants of iron absorption

DIETARY FACTORS

- factors enhancing non-haem iron absorption
  - ascorbic acid
  - meat, poultry, fish and other seafood
  - low pH
    (e.g. lactic acid)

- factors inhibiting non-haem iron absorption
  - phytates
  - tannins and polyphenols

NON DIETARY FACTORS:

- iron status of the individual
- infections and malabsorption

The absorption mechanisms for haem and non-haem iron are different. Haem iron is readily available (20-30% absorption) and may account for as much as one-fourth of the iron absorbed from high-meat diets. It is little affected by the nature and composition of a given meal. The absorption of non-haem iron is highly variable and depends on the nature of the meal, especially the balance between those factors that promote or inhibit iron availability.

The determinants of iron absorption are summarized in Table 4. Ascorbic acid is the most important single factor promoting iron absorption in developing countries where meat intake is usually low. As little as 50 mg of ascorbic acid in pure form, or in vegetables or fruits (for example 100 g of cabbage or 200 g of amaranth or an orange or a lemon), added to a meal will increase iron absorption by 100% (See Fig. 3).

Many compounds such as polyphenols, tannins, phytates, certain forms of protein, and some dietary fibres are known to impair iron absorption. Phytates are present in all cereals and in nuts and certain vegetables. The dietary content of phytates is often high in developing countries due to a high intake of cereals and the use of unrefined flour. This is probably one of the main reasons for the low bioavailability of iron in many diets in this environment since even very small amounts of phytates markedly reduce iron absorption. Ascorbic acid counteracts this effect.

Tannins, which are present in certain vegetables and in tea, and to a lesser extent in coffee, also inhibit iron absorption (see Fig. 4). Once again ascorbic acid counteracts their inhibitory effect. Soy protein products can reduce the percentage of iron absorption under certain circumstances, especially when used as a meat substitute. The net effect of an addition of soy protein to a meal, however, is increased iron absorption due to the high iron content of soy protein products.

Fortification iron. Easily soluble iron compounds, for example ferrous sulfate, are readily available but often cause technical problems such as discoloration and rancidity of the food vehicle. Other compounds such as metallic iron powders are only partially available but usually cause fewer technical problems (32).

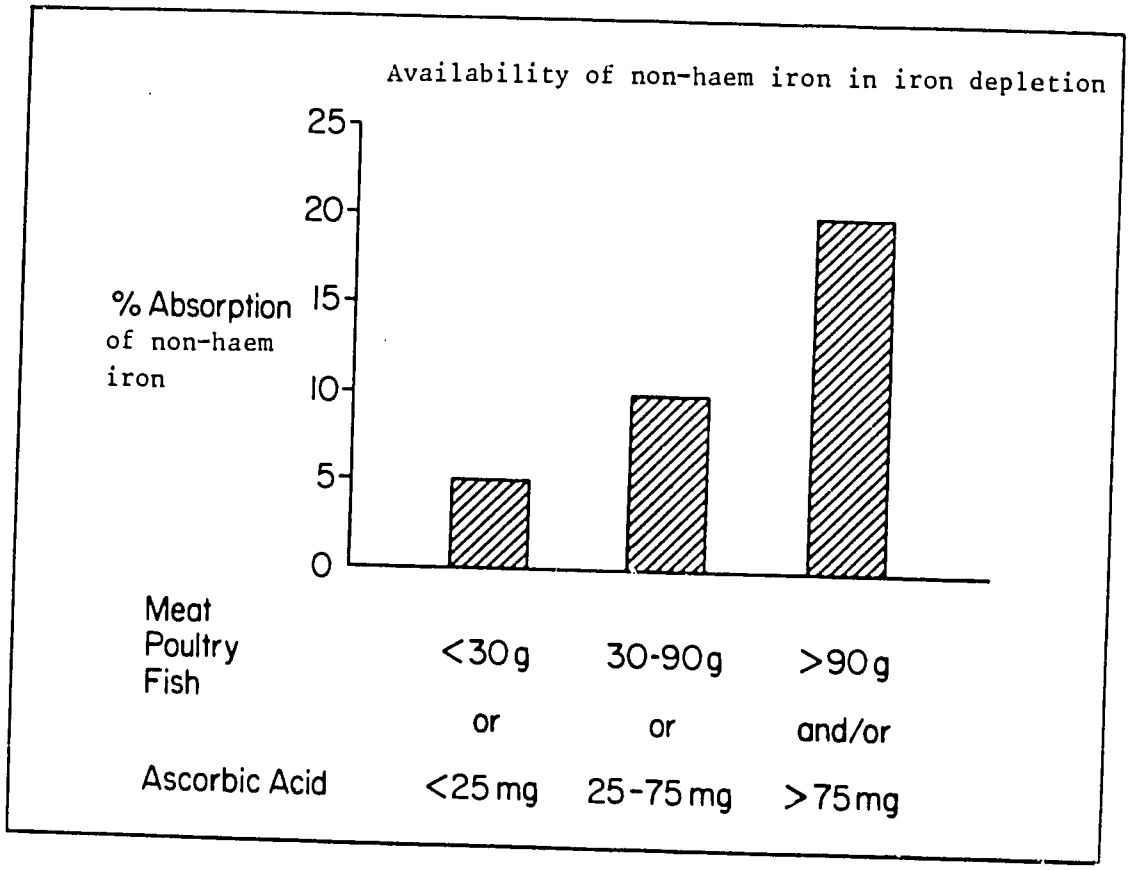

Fig. 3 Effect of meat and ascorbic acid on the availability of non-haem iron

The percentage absorption of non-haem iron by individuals with no body iron stores from three different types of diet. A daily diet of low availability is one containing less than 30 g of meat, poultry or fish (lean, raw weight) or less than 25 mg ascorbic acid. The comparable figures for a diet of medium availability are 30 to 90 g meat, poultry or fish or 25 to 75 mg ascorbic acid, while a diet of high availability is one containing more than 90 g meat, poultry or fish or more than 75 mg ascorbic acid. Alternatively it is one containing 30 to 90 g meat, poultry or fish plus 25 to 75 mg ascorbic acid. (Data from Monsen et al. (30)).

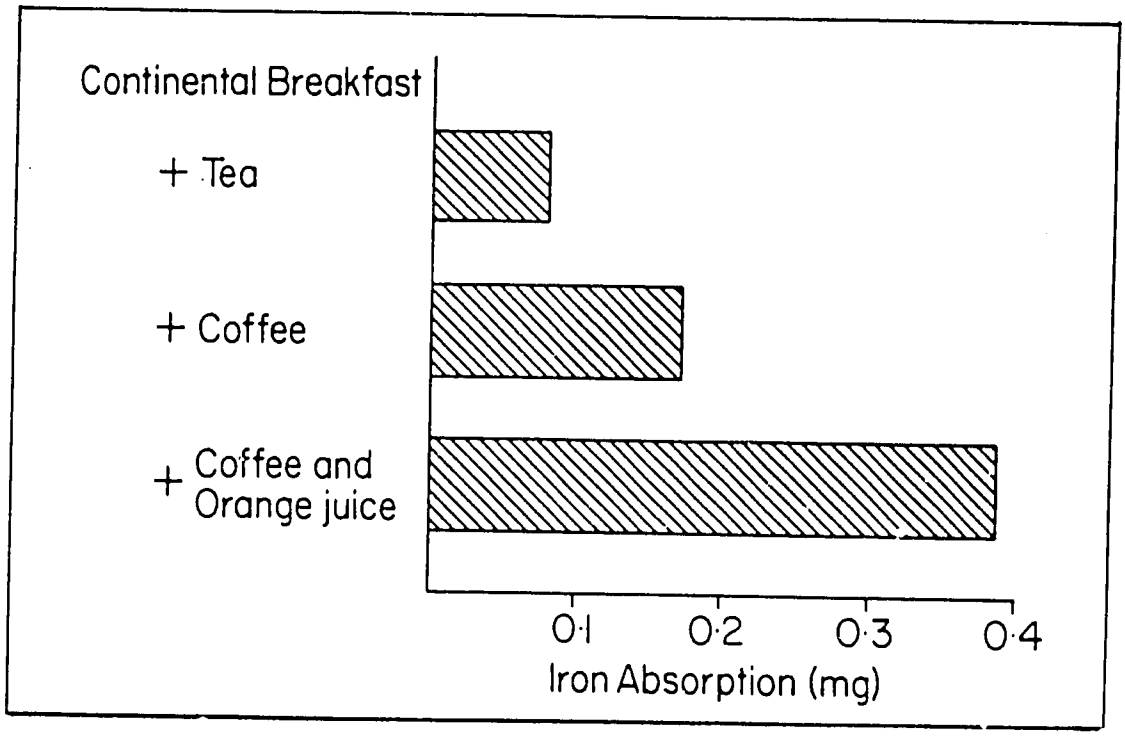

Fig. 4 Effect of different beverages on dietary iron absorption.

Effects of different beverages on the absorption of iron from a continental breakfast. (Data from Rossander et al. (31)).

Contamination iron. Contamination iron usually has a very low bioavailability. There are exceptions, however, such as iron derived from cooking utensils.

Iron absorption and individual iron status. Iron absorption is related to iron status: more iron is absorbed by iron-deficient persons and less by those who are iron-replete. The regulating mechanism for iron absorption is not understood; however, it is known that the adaptive increase in iron absorption is inadequate to prevent a deficiency given the type of diets consumed by many people in developing countries today.

Iron absorption is also influenced by the combination of foods in a given meal, as illustrated by the following. In Thailand the amount of iron absorbed from a simple meal composed of rice, vegetables and spices is 0.16 mg, but increases two and a half times (0.40 mg) with the addition of some fish. The presence of ascorbic acid (50 mg) or cauliflower (125 g) increases the iron absorbed from a meal composed of maize, rice and black beans from 0.17 to 0.41 mg (two and half times) and to 0.58 mg (three and a half times) respectively. These examples show the effect of meal composition on iron absorption and the urgent need for more information on the effect of diets on iron absorption in developing countries.

A low bioavailability of dietary iron is probably the most important factor governing iron deficiency, although low iron intake may also be responsible in some instances.

3.4 Recommended iron intakes

Recommended intakes are influenced by two factors: physiological requirements and bioavailability of dietary iron. Recommended intakes by age and sex for diets having different iron availability are presented in Table 5. Recommended intakes for women of reproductive age are relatively high in order to cover the requirements of women with large menstrual losses. At the same time, it is clear that not every individual woman requires such a high intake. In pregnancy, requirements during the second and third trimesters cannot be satisfied by dietary iron alone, even if it is of high bioavailability. Iron supplementation is indicated unless stores of about 500 mg are present at the beginning of pregnancy. Bioavailability has a marked effect on recommended iron intakes, increasing them, for example, from 10 mg/day for menstruating adult females enjoying diets with high bioavailability to 29 mg/day or even more for those on the low side.

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Table 5. Examples of recommended iron intakes (mg/day) for diets with different bioavailability*

Age/sex	Bioavailability of dietary iron (% of iron absorbed)		
	low (7.5%)	intermediate (15%)	high (22.5%)
4 - 12 months (both sexes)	14	7	5
13 - 24 months "	8	4	3
2 - 5 years "	9	5	3
6 - 11 years "	16	8	5
12 - 16 years (girls)	27	13	9
12 - 16 years (boys)	24	12	8
Adult males	15	8	5
Adult females			
- pregnant**	-	-	-
- menstruating	29	14	10
- lactating	17	9	6
- menopausal	13	6	4

* Source: FAO/WHO (28).

** See Table 2 and text.

The three broad categories of iron bioavailability indicated in Table 5 ma be characterized as follows:

Low bioavailability diet (iron absorption about 7.5%)

This is a simple, monotonous diet containing cereals, roots and tubers with negligible quantities of meat, fish or ascorbic acid. This type of diet contains a preponderance of foods that inhibit iron absorption such as maize, rice, beans, whole-wheat flour, and sorghum, and is typical for many developing countries, particularly among lower socioeconomic groups. There are, however, some diets from which iron absorption is even lower. It may be as low as 2-3% for diets composed almost entirely of cereals, as reported in India (33).

Intermediate bioavailability diet (iron absorption about 15%)

Diets in this category consist mainly of cereals, roots, or tubers, but include some foods of animal origin and/or ascorbic acid, both of which promote iron bioavailability. A low bioavailability diet can be converted to this level by increasing the intake of foods that enhance iron absorption such as those containing ascorbic acid, meat or fish. Similarly, a high bioavailability diet can be reduced to an intermediate level by the regular consumption of iron absorption inhibitors such as tea or coffee with meals.

High bioavailability diet (iron absorption about 22.5%)

This is a diversified diet containing generous quantities of meat, poultry, fish, or foods with high amounts of ascorbic acid. Such a diet would be typical for most segments of populations in industrialized countries. When large quantities of meat, fish and poultry are included, absorption of dietary iron may approach 25% or more.

It is obvious that, when the bioavailability of dietary iron is low, it will not be possible for persons in some age groups to meet their iron requirements at a consumption level that is sufficient to meet their energy requirements. This is especially true for young children, adolescent girls, menstruating adult females, and pregnant women. Nor is it surprising that the prevalence of IDA is so high in developing countries given the low bioavailability of dietary iron due to the absence of meat products and the small amount of ascorbic acid-rich fruits and vegetables consumed.

The iron content of human breast milk is low - about 0.5 mg per litre - but so is the term infant's iron requirement for the first 4-6 months of life. On the basis of a daily breast milk intake of 600-650 ml, this represents approximately 0.3 mg of iron per day. The bioavailability of this iron is quite high, however, and it is possible that as much as 0.15 mg/day are absorbed. From about six months of age a infant's iron requirements increase markedly and the supply from breast milk alone is no longer sufficient. Low-birth-weight infants exhaust their iron stores at an earlier age and require additional exogenous iron before the term infant.

Cow's milk has about the same iron content as human milk, although iron absorption from the former is lower than from the latter because of differences in chemical composition. The bioavailability of iron from infant cereal products depends on the type of cereal used and, especially, its iron and phytate content. Commercial products are often fortified with an iron compound in addition to containing ascorbic acid to facilitate iron absorption. In choosing fruits and vegetables it is important to ensure that ascorbic-acid intake is high. While iron requirements during the first years of life are not very different from those of an adult male, energy requirements, and thus food intake, are much lower in infants and children. This means that the density and bioavailability of dietary iron must be particularly high for this group.

IV. ASSESSING THE PREVALENCE OF IRON DEFICIENCY ANAEMIA

Because the clinical manifestations of anaemia are few and difficult to detect, diagnosis must be made by laboratory tests on a routine basis in groups at high risk. This principle also applies to iron deficiency without anaemia, which cannot be diagnosed in the absence of laboratory tests. Indeed, clinical detection of anaemia is influenced by so many variables such as skin thickness and pigmentation, that it becomes unreliable, unless the anaemia is very severe. Laboratory tests are, therefore, used to detect anaemia and to assess the iron status of individuals or of population groups.

Screening tests of anaemia

The measurement of the packed volume of red cells (hematocrit) and of the concentration of haemoglobin in circulating blood are the best means of detecting the anaemia that is associated with severe iron deficiency.

Haemoglobin concentration and haematocrit can be determined by using either capillary or venous blood specimens. The latter are more accurate but the former easier to perform under field conditions. The use of skin puncture blood substantially decreases the diagnostic reliability. In the case of haemoglobin, sequential venous values in the same individual usually remain within 0.6 g/dl. In contrast the discrepancy between skin puncture and venous values ranges between 0.5 and 1.0 g/dl (34).

In PHC routine practice, an error of 0.5 g/dl is of relatively little or no consequence in terms of the action to be taken. It may be a more serious drawback when following the effect of iron therapy in an anaemic individual. To obtain the best possible skin puncture sample, it is important to warm the extremity in order to facilitate blood flow and to avoid any squeezing so as to minimize the contamination of blood with tissue fluid. Venous samples are difficult to obtain in some developing countries, however, and carefully collected capillary samples generally provide acceptable results.

Capillary blood

A specimen for the collection of capillary blood is obtained from the finger tip (heel for infant). After sterilization of the area, a clean puncture is made with a sterile lancet to obtain a free flow of blood without squeezing. (The use of unsterilised needles is dangerous). Depending on the procedure to be followed the blood is taken into a pipette, heparinized tube, glass cell or dropped onto filter paper.

Venous blood

Venous blood is generally taken from the antecubital vein with a dry sterile syringe. Twenty or 21 SWG needles are generally preferred since smaller gauge needles are not suitable for obtaining free blood flow. Alternatively vacuum tubes (for example Vacutainers) may be used. In persons whose veins are not easily visible or felt, the skin area may be warmed and a tourniquet or sphygmomanometer cuff tied to maintain diastolic pressure. The area of venipuncture is cleaned with 70% alcohol and allowed to dry before the needle is inserted into the vein. Blood is drawn into the syringe, the tourniquet or sphygmomanometer cuff is removed, and the needle is withdrawn while the swab is kept in place for a few minutes to ensure stoppage of leaking.

Haemoglobin determination

There are several laboratory techniques for measuring haemoglobin concentrations. Most of those that have been and are still used in PHC are inaccurate in routine practice and should be discarded (35,36,37,38). The procedures by which Hb is converted to one of its compounds and its

concentration determined by matching the colour with a known standard in a photoelectric colorimeter or by measuring absorption in a spectrophotometer can be recommended as reliable and accurate. The three commonly used techniques are the cyanmethaemoglobin (HbCN), the oxyhaemoglobin (Hb O2), and the alkaline haematin methods (39). The HbCN method (40) has become the most popular of the three because it measures practically all haemoglobins except sulfhaemoglobin. Another major advantage over other procedures is that HbCN standards remain stable for a long time. In the HbCN method blood is mixed with Drabkin's solution in order to convert haemoglobin into cyanmethaemoglobin, the absorbency of which is then measured at 540 mu in a photoelectric colorimeter or a spectrophotometer.

An absolute prerequisite for using cyanmethaemoglobin to determine haemoglobin concentration is an accurate amount of blood delivered into an accurate amount of diluent. This is simple to accomplish for an established laboratory where measurements can be made on the same day. However, if blood is collected in the field where there is no laboratory, it will have to be transported to one where the determination can be made.

Transporting blood samples presents a serious problem in developing countries. In warm, humid climates, unrefrigerated blood quickly becomes unsuitable for Hb determination due to evaporation or contamination. A possible alternative is to carry an accurately measured 5 ml volume of diluent (Drabkin's solution) in a quantity of stoppered test tubes. The blood is collected and delivered into the diluent, the tubes are once again stoppered, and the blood-reagent mixture is transported to a laboratory where measurements are possible. Leakage from the stoppered tubes is not infrequent, however, and this seriously compromises the accuracy of the results obtained.

There are two ways to overcome this problem:

(a) five ml of Drabkin's solution are pipetted into the test tube at the time of blood collection. Automatic pipettes or seripettors may be used for this purpose. Once an accurate dilution has been made, a small spillage of the sample would not interfere with results since the concentration does not change. Evaporation from open vials will affect concentration, however.

(b) an accurate amount of blood can be delivered on No. 1 Whatman filter paper cut into 1.5 x 1.5 cm sizes. The blood is allowed to dry and the paper is labelled with a pencil. The papers with blood specimens delivered onto them are placed in small envelopes and sent to a laboratory There the filter papers with their blood specimens are dropped into accurately measured amounts of diluent (Drabkin's solution), and the blood is allowed to diffuse out of the filter paper and into the diluent for two hours. The solution is then mixed by shaking and read in a photoelectric colorimeter or a spectrophotometer. This method is relatively easy to reproduce and reasonably suitable for those situations where a laboratory is located at some distance from blood collection points. Battery-operated small colorimeters are now available that allow the determination to be made also in the field.

Packed cell volume (haematocrit)

Microhaematocrit can be measured in place of determining haemoglobin concentration (40). This is done by centrifugation of a minute quantity of blood that has been collected in a heparinized capillary tube. One of this method's advantages is its technical simplicity, particularly when applied to blood specimens obtained by skin puncture. Another advantage is that it can be performed in the field by using battery-operated microcentrifuges. On average, haematocrit is roughly equivalent to haemoglobin concentration multiplied by three.

With both the haemoglobin and haematocrit determination, it is essential to interpret results in relation to age-specific and sex-specific reference standards (5). Table 6 shows reference values for haemoglobin concentration after six months of age (laboratory testing rarely being done before this age).

Data from population surveys show a slight, gradual decrease in haemoglobin concentration in elderly men (41). It remains to be shown whether this is a normal concommitant of aging (possibly related to decreased androgen levels) or, alternatively, whether it represents an increasing prevalence of anaemia. For this reason haemoglobin levels for this group have not been tabulated. The haemoglobin concentration in women remains much more stable, however, and the lower limit of normal is considered to be 12 g/dl from six years of age onwards. An exception occurs during pregnancy when haemoglobin concentration decreases concurrently with a large expansion in blood volume.

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Table 6. Haemoglobin values below which anaemia is likely to be present in populations living at sea level as proposed by WHO*

Subject/age	level
Children 6 months-6 years	11 g/dl
Children 6-14 years	12 g/dl
Adult males	13 g/dl
Adult females (non-pregnant)	12 g/dl
Adult females (pregnant)	11 g/dl

*WHO (5).

Although anaemia is often graded as "mild", "moderate" and "severe", the haemoglobin values at which the division into these three categories is made vary and are arbitrary. For the purposes of this document anaemia is considered to be mild, moderate or severe when Hb concentrations are less than 3 g/dl, between 3 and 6 g/dl, or more than 6 g/dl below cut-off points for anaemia respectively. It should be remembered that local circumstances must be taken into consideration when setting these limits.

Testing for iron deficiency

Several laboratory tests are available to confirm the presence of iron deficiency (40). These should not be used routinely for diagnostic purposes in PHC. They are, however, useful in assessing and monitoring the iron status of population groups. The most commonly used are the serum ferritin, the transferrin saturation and the erythrocyte protoporphyrin concentration.

Serum ferritin

Ferritin is normally present in serum but in such small quantities that it remained undetected until recently. It is measured by radioimmunoassay or by enzyme-linked immunoassay (40). An advantage of measuring the serum ferritin level is that it allows an evaluation of iron status in conditions of iron deficiency or excess - information that cannot be provided by any other means. At all ages a serum ferritin value of less than 10-12 ug/l (or ng/ml) indicates a depletion of iron stores. The assay which until recently was expensive and time-consuming has been simplified and can now be used as a laboratory routine procedure (42).

Transferrin saturation

Transferrin saturation is calculated by dividing serum iron concentration by total iron-binding capacity and multiplying by 100 to express the result as a percent. Almost all of the iron in the serum is bound to the iron-binding protein transferrin. Serum iron and iron-binding capacity are generally measured by spectrophotometric techniques (40). Values below 16% in adults are considered indicative of iron deficiency. The corresponding values for infants and children are 12 and 14% respectively.

Erythrocyte protoporphyrin

Protoporphyrin accumulates in red blood cells when there is insufficient iron to combine with protoporphyrin to form haem. Erythrocyte protoporphyrin (EP) can be measured rapidly by a simple fluorescence assay (40). This can be done directly on a thin film of blood, using an instrument specifically designed for this purpose. The latter requires little technician time or training. EP is elevated (higher than 80 ug/dl RBC below 4 years and 70 ug above that age) in cases of iron deficiency; values are also high where there is lead poisoning (43) and they are therefore used to screen infants and young children in urban, low-income areas where both conditions are common.

Combined nutritional deficiencies

Iron deficiency is often combined with other nutrient deficiencies, particularly in developing countries. Iron and either protein-calorie or folate deficiency are common examples.

Protein-calorie malnutrition: Anaemia is common in children suffering from severe protein-calorie malnutrition, even where there is no evidence of a concurrent iron deficiency. However, the increased growth due to nutritional rehabilitation may provoke an iron deficiency unless iron is included in the diet from about 2 weeks after the initiation of treatment. Laboratory evaluation of iron status is more reliable after general malnutrition has been treated for about two weeks than would be the case initially when infection, dehydration, and other acute and life-threatening problems are likely to be confounding factors.

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Folate deficiency: Folate deficiency combined with iron deficiency may occur in preterm infants who are fed unfortified evaporated milk-based formula and in infants who get goat's milk. Infants suffering from diarrhoea, malnutrition, infection and haemolytic anaemia are a increased risk of developing a folate deficiency. Combined folate and iron deficiencies are especially common in women during the last half of pregnancy.

Other nutrient deficiencies: A deficiency of vitamin B_{12} in strict vegetarians (taking no food of animal origin including milk and eggs) and in patients suffering of malabsorption of this vitamin (pernicious anaemia) may result in megaloblastic anaemia. The interaction of vitamin B_{12} deficiency with iron deficiency is similar to that of folic acid in that the milder of the two deficiencies becomes evident only after the more severe one has been treated.

V. TREATMENT AND PREVENTION OF IRON DEFICIENCY ANAEMIA

In current medical practice, patients in whom anaemia is diagnosed are treated. In large scale public health programmes, especially in developing countries, it may be difficult if not impossible to screen every individual of groups at risk, such as pregnant women, for anaemia; as a result all of them should receive an iron supplement. It should also be stressed that prevention is always cheaper than treatment in terms of cost/benefit. It is difficult under these circumstance to draw a sharp distinction between treatment and prevention.

5.1 Treating iron deficiency anaemia

Iron deficiency anaemia, especially severe cases, cannot be corrected by manipulating the diet only; therapy with medicinal iron is the most efficient means of controlling this condition. Oral administration of ferrous sulfate fumarate or gluconate is the treatment of choice for almost all cases of iron deficiency. It is very seldom and only in the most severe cases (for example, Hb less than 3 g/dl) that a transfusion may be necessary.

5.1.1 Oral iron therapy

Iron tablets: In 1832 Blaud introduced iron therapy in the form of a tablet containing ferric carbonate as its main constituent (Blaud's pill). It was effective in correcting IDA and remained the mainstay of treatment until other iron preparations were introduced (44). It soon became obvious, however, that ferrous iron was better absorbed than ferric iron and to this day practically

all medicinal iron preparations contain ferrous compounds. Ferrous forms of sulfate, fumarate and gluconate are commonly used. Other ferrous compounds that have been, or are still being, used include ferrous succinate, lactate, glycine sulfate, glutamate, citrate, tartrate and pyrophosphate. Although ferrous succinate is probably more completely absorbed, these compounds, in addition to being more expensive, offer no advantages over sulfate, fumarate or gluconate iron compounds.

Iron tablets contain a percentage of elemental iron that varies with the molecular weight of the iron compound. Some examples are given in Table 7.

Table 7. Percentages and amounts of iron in some iron tablets

Preparation	Weight of the iron compound in usual tablet (mg)	Amount of elemental iron in the tablet (mg)	% of iron
Ferrous sulfate ($7H_2O$)	300	60	20
Ferrous sulfate, anhydrous	200	74	37
Ferrous sulfate, exsiccated ($1H_2O$)	200	60	30
Ferrous fumarate	200	66	33
Ferrous gluconate	300	36	12

Several forms of iron tablets/capsules are in common use: uncoated (compressed) iron preparations, coated iron tablets, and slow-release preparations. The uncoated tablets containing a ferrous salt become oxidized over time, especially in humid climates, and are therefore less effective. Some coatings, especially enteric-coated tablets, disintegrate only partly in the stomach when exposed to gastric juices. All coated preparations and batches should be subjected to an in vitro HCl disintegration test and those that do not disintegrate in 0.1 M, HCl within two hours should be rejected (45).

Slow-release iron preparations were introduced based on an understanding that iron absorption is inversely related to the amount of iron present in the duodenum-jejunum and that the frequency of gastrointestinal side-effects is directly proportional to that amount. Slow-release preparations allow only a small amount of iron at any given moment to come into contact with the duodenal mucosa from a single source thus improving absorption and decreasing side-effects. As a consequence the same results can be obtained with smaller doses of iron, while the decrease in side-effects improves compliance with treatment. However, many coated and slow-release tablets tend to pass the duodenum and proximal jejunum and release iron in the ileum where absorption is poor. New preparations, however, are developed where the iron is slowly released in the stomach, thus avoiding this problem. The cost of slow-release iron preparations is currently high, although bulk purchases by governmental and international agencies could result in a significant reduction in price. The greater effectiveness of this form of iron supplementation has also to be taken into consideration.

Liquid preparations: There are many syrups and liquid preparations (drops) available that contain iron. They are usually expensive, tend to deteriorate in storage, and often contain minerals and vitamins that are unnecessary for most patients. Liquid preparations are useful for paediatric purposes, however, since infants and young children tend to reject solid preparations or are unable to swallow them.

Combination with other nutrients: During pregnancy, iron and folate deficiency are usually combined. It is therefore interesting to combine both haematinics in the same tablet. The addition of folate (250 mcg) to iron sulfate (60 mg iron) increases only very slightly the cost of the iron tablet.

Because ascorbic acid is known to promote iron absorption, it has been incorporated into many iron preparations. When added in sufficient amounts (200 mg or more), ascorbic acid increases iron absorption by about 30% (46). Unfortunately, however, not only is ascorbic acid relatively expensive, but its addition also results in a greater frequency of side-effects and therefore poor compliance (47,48).

Dosage of iron and side-effects by iron therapy: Erythropoietic activity following iron administration is directly related to the severity of anaemia and thus the rise in Hb concencentration is inversely proportional to the initial Hb level. Therapeutic iron is best absorbed during the first month of treatment. For example, adults given 100 mg of iron in the form of iron sulfate twice a day with meals absorbed an average of 14% during the first week of therapy compared with 7% after three weeks and 2% after four months (47). The first month of therapy would thus appear to be the most important for determining the success of treatment. A positive response to treatment can be defined as a daily increase in haemoglobin concentration of 0.1 g/dl from the fourth day onwards. Although the response in haemoglobin is virtually complete after two months, iron therapy should continue for another 2-3 months to build up storage iron to a level of about 250-300 mg, or serum ferritin level to 30 ug/l.

The normal dose routinely given to adolescents and adults (including pregnant women) is 60 mg of elemental iron twice a day, in cases of moderate to severe anaemia and once a day in cases of mild anaemia.

Three mg of iron/kg of body weight per day is the recommended dose for infants and children. Higher doses have been used, for example twice this amount, although they are probably unnecessary. A major problem where children are concerned is the possibility of an accidental overdose, especially among children between one and five years of age.

For pregnant women the administration of folate with iron will be beneficial since the anaemia is usually caused by a deficiency of both nutrients. A suitable combination tablet may contain 250 ug of folate and 60 mg of iron. On the basis of two tablets a day, a pregnant woman will thus receive a daily supplement of 500 ug of folate, which is sufficient to cover her requirements.

The oral administration of iron can cause gastrointestinal signs and symptoms in some individuals such as epigastric discomfort, nausea, vomiting, constipation and diarrhoea. The frequency of side-effects is directly related to the dose of iron administered and is independent of the type of iron compound used; none appears to have an advantage over the others in terms of tolerance. Iron consumed with a meal is better tolerated than that taken on an empty stomach (49) but the amount of iron absorbed becomes reduced. While there is no doubt that iron administration can produce a number of side-effects, there is also good evidence that these are psychological in origin in some individuals.

Therapy should not be discontinued in cases of gastrointestinal intolerance; rather the dose should be reduced and then gradually increased until the full amount has been reached and is well tolerated. Frequency of side-effects can also be reduced by the administration of iron with meals, thus increasing compliance and the probability of successful treatment.

Failure of oral therapy: The major reason for the failure of iron therapy is non-compliance, which presents a serious problem. This is often due to the presence of side effects as a result of the administration of too high doses of iron. Therapy is followed for a few days but is too often stopped soon after an increased sense of well-being has been achieved, which is long before Hb has reached the normal level.

5.1.2 Parenteral iron therapy

The most commonly used preparation is Imferon R (iron dextran). A 2 ml dose contains 100 mg of iron and can be administered either intramuscularly or intravenously. The intravenous method offers the advantage that the complete iron requirement can be given in a single dose (total dose infusion or TDI). This has been used especially in obstetric practice (50). It solves the problem of non-compliance and permits the requirement during pregnancy to be met in full. The recommended intravenous dose for an adult is 500 mg of iron in a 10 ml saline solution given over a period of ten minutes following a test dose of 1-2 drops. The administration should be done in a hospital setting. Intramuscular administration should be used only when adequate facilities for intravenous dosing are not available.

There is little justification for giving parenteral iron when oral therapy is possible. The parenteral route is indicated only when there is evidence of intolerance to oral iron and attempts to circumvent these with low iron doses fail, gastrointestinal malabsorption, and non-compliance.

5.1.3 Cost of treatment

Treating iron deficiency anaemia is inexpensive and within the reach of most people. Several pharmaceutical preparations containing 60-100 mg of iron per dose cost between US$ 2 and $ 5 per 500 tablets. Tablets containing 60 mg of elemental iron plus 250 ug of folate are supplied by UNICEF for approximately US$ 1.00 per 1000, FOB, UNIPAC, Copenhagen. Two hundred and fifty tablets containing 60 mg of elemental iron, at a cost of approximately US$ 0.25 per individual treated, are sufficient to correct a deficiency state in most people.

On the basis of estimates made recently (1), about 40% of the populations of Africa and South-East Asia are anaemic. On the assumption that anaemia is due to iron deficiency in two-thirds of the cases, 26% of the total population in these regions would require iron therapy. The cost of iron tablets per 1000 persons of the total population would be approximately US$ 65, not including distribution (26% of 1000 persons = 260 persons requiring iron). When weighed against other public health priorities, this may represent, in many cases, an excessive burden justifying the setting up of programmes of prevention such as iron food fortification and education of the public.

5.2 PREVENTING IRON DEFICIENCY ANAEMIA

5.2.1 Dietary changes

Dietary iron intake can be increased in poor communities by two methods. One has to do with ensuring that people consume larger amounts of their existing diet so that their energy needs are fully met. The additional amount of iron ingested will depend on the extent to which the existing calorie gap is bridged. Since no qualitative changes in the diet are needed this approach may appear to be simple but since it involves raising purchasing power of households it is more complex than it appears to be. This is beyond the capabilities of the primary health care system only.

The bioavailability of dietary iron is believed to be of greater relevance in the etiology of anaemia than total iron ingested. From a practical point of view, however, the importance of the amount of iron should not be underestimated, particularly in situations where it may be difficult to improve bioavailability. In parts of rural India, for example, total iron consumption increases by about 25-30% by correcting the calorie shortage (51,52). The result has some impact on iron economy despite bioavailability remaining unchanged.

The bioavailability of iron can be enhanced by reducing the concentration of iron absorption inhibitors, increasing the concentration of iron absorption promotors, or increasing the proportion of haem to non-haem iron.

Improving the bioavailability of iron from predominantly vegetable-based diets by decreasing the concentration of inhibitors calls for major changes in food practices. A campaign to promote the inclusion of meat in such diets is unlikely to succeed because of cost considerations in addition to the lack of animal foods in many developing countries. Philosophical considerations may also constitute an obstacle. Even though efforts are being made to increase the production and availabity of meat, it would be unrealistic to assume that its consumption by poor rural populations, among whom anaemia is most widespread, will significantly increase in the near future. A recommendation that meat should be increased in habitual diets in order to combat IDA should be made with caution and only in situations where the recommendation could be successfully implemented and where it would not lead to health problems of a different nature.

In contrast promoting iron absorption by increasing the ascorbic acid content of the diet appears to have a greater chance for success. The effect of ascorbic acid on the absorption of non-haem iron is truly striking, and the level of increase is similar with both crystalline and native ascorbic acid present in foods. It is dose-dependent and the presence of 25, 50, 100, 250 and 500 mg of vitamin C in the diet is associated with a near 2, 3, 4, 5 and 6-fold enhancement (53,54) (See Figure 5). The content in ascorbic acid of some vitamin C rich foods is given in Table 8.

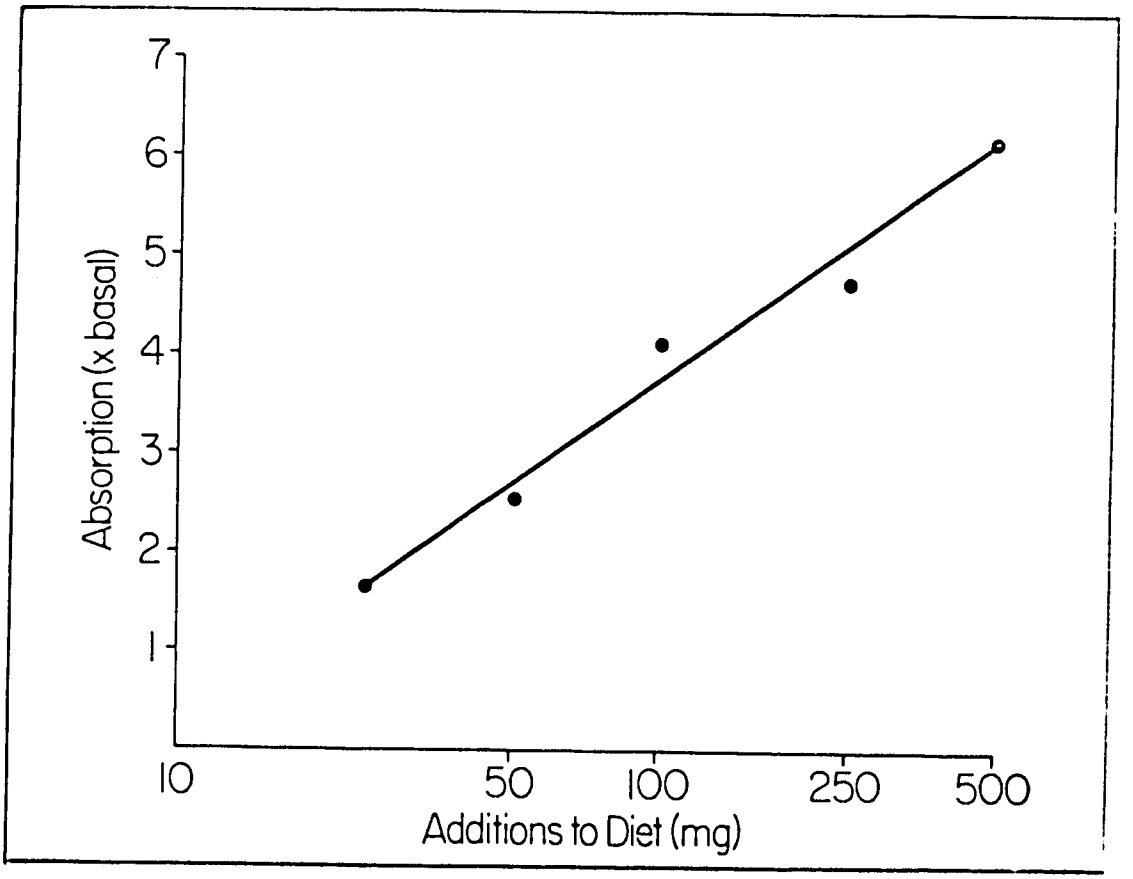

Fig. 5 Effects of increasing quantities of ascorbic acid on the absorption of non-haem iron in food

Table 8. Approximate ascorbic acid content of selected fruits and vegetables (55,56)

		Approximate amount in mg/100g
Fruits:	Guavas, fresh	326
	Lemon, fresh (juice)	37 - 50
	Orange, fresh	46
	Pineapple, fresh	37
	Mango, fresh	42
Vegetables:	Cabbage, raw	54 - 60
	Cabbage, boiled	15
	Cauliflower, raw	60 - 96
	Cauliflower, boiled	20
	Potato, raw	21
	Potato, boiled	12 - 18
	Sweet potato, raw	25 - 37
	Sweet potato, boiled	15
	Spinach, boiled	7 - 25
	Tomato, raw	20 - 26
	Turnip, boiled	17

In many rural homes vegetables and fruits are eaten only infrequently and in small amounts. Motivating families to add them to their diet can have a considerable impact. The difficulties associated with reaching this goal should not be underestimated, however. Encouraging the development of kitchen gardens and helping families to grow appropriate vegetables have been tried in the past, as in India through the Applied Nutrition Programme, with mixed results. Resolving the practical difficulties associated with translating these ideas into action might appear trivial, but such factors as the availability of water, appropriate fencing to protect what is grown, and an appreciation of the value of the produce are critical to achieving success (57).

Approximately 50-80% of the vitamin C originally present in food can be lost during cooking. In order to increase iron bioavailability, therefore, one has to begin with relatively large amounts of the vitamin. Encouraging the consumption of fruits and vegetables in raw form helps to alleviate this problem, although the risk of gastrointestinal infections from eating raw vegetables should not be ignored. Also, the vitamin C content of food that is cooked and left standing decreases considerably, with reheating reducing it still further. In many rural households a whole day's food supply is cooked only once, providing two meals as much as 12 hours apart. Under such circumstances it is difficult to ensure that enough vitamin C is retained unless a good source of ascorbic acid is added. This point is made in order to ensure that proper remedial steps are taken through nutrition education especially when provided by primary health care workers.

A number of commonly used household processing methods - germination, baking and fermentation - can change the bioavailability of iron either by increasing vitamin C content or lowering tannin and phytic acid content (58,59,60). For example, the germination of some cereals and legumes for 24-48 hours is associated with the appearance of 10-70 mg of ascorbic acid per 100 g, an 8 to 25% reduction of tannins, and a 25 to 35% fall in phytic acid. The bioavailability of iron from such germinated grains as determined in vitro increases almost two-fold, while the malting of minor millets results in a five- to ten-fold improvement.

Although the amount of iron that is bioavailable from these processed foods may be relatively small, these foods when consumed as part of a meal, will increase its ascorbic acid content and should therefore be beneficial by improving the bioavailability of the total dietary iron.

5.2.2 Preventing IDA by supplementing with medicinal iron

Supplementation with medicinal iron has the advantage of producing rapid changes in iron status and of reaching specific segments of the population in greatest need or at greatest risk. However, this approach's effectiveness is limited because of the associated gastrointestinal effects of oral iron that result in poor compliance, and the difficulty of sustaining motivation over time (2-3 months) in individuals who do not actually perceive themselves as being ill. This provides a challenge to health educators and social marketing strategists.

Because iron supplementation also requires an effective health delivery system it is unlikely that the prevalence of anaemia will be reduced in an entire community through supplementation alone given the degree of coverage that is required and the need to reach the individual, which is virtually impossible in many cases. Medicinal iron supplementation programmes have a greater chance for success when special groups are targeted, for example pregnant women, infants, and preschool-age children, or such "captive audiences" as school children or plantation workers.

Pregnant women: Pregnant women should be considered a priority target group for iron supplementation; the responsibility for identifying this group and distributing iron tablets to each pregnant woman falls within the competence of primary health care workers. In supervised situations it has been demonstrated that the use of two tablets, each containing 60 mg of elemental iron plus 250 ug of folate, per day during the second half of pregnancy will prevent the development of anaemia (the recommended dose can either be reduced or increased to ensure compliance or bioavailability). Supplementation should focus on the second half of pregnancy because it is then that iron needs are greatest. Morning sickness during early pregnancy constitutes an impediment to iron supplementation at that time and should therefore be avoided.

A major drawback to iron supplementation is poor subject compliance, which may be due to side effects but also quite often to a lack of awareness of any real need for taking iron. Mere delivery of the message and the tablets is not enough to ensure success; a major effort is needed to motivate women by educating them about the importance of iron for their health. Compliance is closely related to socioeconomic and sociocultural characteristics and attempts to improve compliance call for their modification. The training of primary health care workers and paramedical personnel in the techniques of communication and motivation is by and large a negelected area which needs to be emphasised.

Preschool children: Supplementation of preschool children also requires special consideration. This may be accomplished relatively easily through, for example, the networks of child-care centres that exist in many developing countries. Compliance is perhaps a problem of even greater concern than in the case of pregnant women; it can be partly solved by supervising the supplement intake when the child visits a health centre. Supplements can be administered by those responsible for the centres, thereby freeing the primary health worker to undertake other tasks.

Supplementation strategies are generally based on the subject taking one or several iron tablets daily. Although such an approach is necessary in individual cases of severe endemic anaemia, it is doubtful whether daily supplements are required to control mild-to-moderate anaemia in this age group. To ensure more uniform iron coverage and higher compliance rates than those provided by daily, short-term supplementation, it may be more practical to reduce the frequency of supplementation but spread it throughout the year.

The optimal frequency and dosage may vary from one community to another. Thirty mg of elemental iron in tablet or liquid form, depending on the age of the child, is usually sufficient when administered daily over a short period.

School-age children usually do not have the same high prevalence of anaemia as preschool-age children. They can be reached at schools if the primary health worker can establish the necessary links with teachers and ensure the regular intake of tablets under the latters' supervision.

Infants: Preventing anaemia in late infancy and early childhood through primary health care includes protecting and promoting breast-feeding for as long as possible given the high absorbability of breast-milk iron, and encouraging the timely introduction of weaning foods that are either promoters of iron absorption or that have been fortified with iron. Among rural populations in most developing countries successful and prolonged breast-feeding is the rule; the contrary is more typical of urban areas, which is where breast-feeding promotion efforts should be concentrated. Breast-milk appears to be adequate to cover the dietary iron requirements of normal birth weight infants up to the age of 6 months. For LBW infants, they may require iron supplementation from the age of 2 months on.

Controlling anaemia by the use of commercially prepared iron-rich weaning foods is likely to have only limited success in rural communities, since they are often expensive and beyond the reach of most people. Iron-rich weaning foods are not difficult to prepare in the home. However, mothers need educating and motivating to do so and to increase the vitamin C content of weaning diets.

Because iron intake and bioavailability as well as health structures differ so much from one area to another, it may be useful to proceed step-wise in the implementation of a supplementation programme. A preliminary small scale field trial may help to determine optimum iron dosage and deal with any problems concerning acceptability and logistics. This allows for the proper planning of the supplement delivery strategy.

The risk of iron overload is a concern in supplementation programmes, among those populations in whom genetic abnormalities of haemoglobin synthesis or iron metabolism are common as in Burma, Thailand etc. Due attention must be paid to this aspect of the problem, although the benefits accruing from the treatment and prevention of IDA generally far outweigh the dangers stemming from possible iron overload.

5.2.3 Food fortification

Food fortification is one of the most effective approaches to preventing iron deficiency. It can be targeted to reach some or all segments of the population depending on the objective, and it does not necessarily require cooperation by the individual. The initial cost is modest and recurring expenses are less than that of supplementation. The major difficulty associated with the iron fortification of food, is the identification of a suitable food vehicle. Identification of a form of iron that is both adequately absorbed and does not alter the appearance or taste of the food vehicle is equally important. The target population needs to be accustomed to and economically capable of purchasing the fortified food.

Iron fortification is technically more difficult than with other nutrients because bioavailable forms of iron are chemically reactive and often produce undesirable effects when added to the diet. Since a population is unlikely to accept a fortified vehicle if the added iron can be detected, inert iron compounds, which are poorly absorbed and therefore more or less ineffective, are commonly used.

Bioavailable sources of iron pose serious problems. For example, soluble ferrous salts often produce colour changes by forming complexes with sulfur compounds, tannins, polyphenols, and other food substances. Discoloration is a particular problem when fortifying foods that are white in colour. Also, reactive iron compounds catalyze oxidative reactions, resulting in undesirable odours and flavours.

Ferrous sulfate has been used extensively in the fortification of such foods as bread and other bakery products, which are stored for only short periods. Ferrous fumarate is used to fortify a corn-soya-milk preparation (CSM) that is distributed as a weaning or supplementary food by the United States Agency for International Development in its food assistance programmes. The use of ferrous lactate and ferrous gluconate are limited by cost considerations to the fortification of such products as milk and soy-based infant formulas. Elemental iron is commonly used in the fortification of wheat flour and bread in North America and Western Europe.

Among the most commonly fortified food products in industrialized countries are wheat flour and bread, corn meal and grits, milk products including infant formulas, and weaning foods (infant cereals). Breast-feeding promotion is an important concern in PHC programmes, unsupplemented breast feeding helps to prevent IDA. Nevertheless, after 6 months of age, an additional source of iron should be introduced to all breastfed infants. Infant formula can be effectively fortified with iron sources such as ferrous sulfate, ferrous gluconate and ferrous lactate. In addition, because of the limited amount that needs to be fortified, ascorbic acid can be included at reasonable cost to enhance bioavailability.

Iron and ascorbic acid fortified milk formula has been extensively investigated in Chile and found to reduce the prevalence of anaemia at 15 months of age to less than 2% compared with 28% among infants who received unfortified formula. Powdered milk is thus a good vehicle in that country, since it is distributed free by the Government to over 80% of infants and lactating mothers. Infants 0-6 months of age are given 3 kg of milk powder on a monthly basis and 2 kg between 6-12 months of age (61).

Consideration has been given to the fortification of other food vehicles in developing countries such as salt, sugar, rice, and fish sauce and fish paste. The combined use of ferric orthophosphate and sodium acid sulfate in the fortification of table salt has recently been reported to produce acceptable long-term iron bioavailability with only slight discoloration. In recent field trials in India, salt was fortified in this manner in order to provide an additional iron intake for adults of 10-15 mg/day (62). The product was tested for 12-18 months in various settings and was usually well accepted. A new formulation with ferrous sulphate, orthophosphoric acid and sodium acid sulphate, which is less expensive has been found to be equally effective. A decrease in the prevalence of anaemia was observed in areas where it had been

high initially. The cost of salt fortification, which raises the price of the salt by 20%, is estimated to be about US$ 0.07/person/year. The problem with salt as a vehicle for iron fortification is the difficulty of foreseeing all of its interactive possibilities with food because it is used in so many culinary preparations.

Sodium Iron EDTA (Ethylene diamine tetracetate) is widely used in the food processing industry and has been found to be an effective enhancer of iron bioavailability. As its iron salt (Fe Na EDTA) it has been successfully used in fortifying sugar in Guatemala (13 mg iron /100 g sugar) (63). Field trials have shown that its consumption (40 g day/person) improves the iron status of the population. Fortified sugar costs approximately 2% more than the unfortified sugar. Recent work with unrefined Egyptian wheat flour indicates that as the disodium salt, EDTA enhances the bioavailability of added ferrous sulphate by reducing both the inhibitory effects of wheat phytate and high bread baking temperatures. These properties of EDTA make it an attractive substance for future fortification programmes.

It should be recognized, however, that while generally speaking iron fortification is an easy process to undertake in industrialized countries, the necessary industrial infrastructure to implement fortification programmes does not exist in some developing countries, particularly in Africa.

5.2.4 Controlling viral, bacterial and parasitic infections

Infectious diseases

Effective and timely medical care, and family education concerning proper feeding practices during and after periods of illness are two public health interventions that are potentially capable of reducing the nutritional consequences of infectious diseases. Although in the absence of adequate preventive measures, the number of infective episodes is unlikely to be reduced, proper curative services can at least contribute to a reduction in the duration and severity of infections and thus reduce overall morbidity. This alone would contribute to improved iron status even failing increased dietary iron consumption. For the reasons indicated above, preschool-age children would take particular benefit from such interventions.

Educating the family is especially relevant where the feeding of young children is concerned since they are often placed on a semi-starvation regime during periods of illness. Changing this inappropriate practice calls for a serious effort to impart correct nutrition and health messages through health personnel. The maintenance of breast feeding does much to prevent infectious diseases, quite apart from its direct effect on iron status.

Immunization continues to gain acceptance and primary health care can strengthen this activity considerably. However, while immunization will reduce morbidity due to certain diseases, current procedures do not permit the control of morbidity due to the most frequently occurring gastrointestinal and respiratory infections. These call for preventive public health measures - the provision of safe water and improvements in environmental sanitation as well as personal hygiene.

The training of PHC workers in the above is of particular importance, especially in view of their own cultural pre-conceptions that may require modification. Training programmes should be linked to the competance, skills and workload of the workers who are to induce behavioural changes that reduce anaemia.

Parasitic infections

The role that hookworm and Bilharzia play in the etiology of anaemia through chronic blood loss has been established beyond dispute; that played by other common intestinal parasites is less certain. There is some evidence indicating that a number of parasites can interfere with the absorption of some nutrients, particularly if worm load is heavy; this has been shown for Giardia which causes a lowering of iron absorption (64). From a health and nutrition point of view, it is undesirable to harbour parasites, and recommendations that deworming form part of the primary health care inventory are frequently made. It is open to question, however, whether this is the best way to deal with the problem.

Deworming in the absence of simultaneous efforts to eradicate the reservoir of infection quickly results in reinfestation and a renewed need to deworm.

The high cost of antihelminthic drugs and logistical problems associated with large-scale deworming operations militate against the cost-effectiveness of such repeated operations. Deworming per se can be temporarily effective in decreasing the parasitic load but this is perhaps only of minor benefit for haemoglobin levels. The provision of additional iron, either through supplementation as medicinal iron or by food fortification, results in much higher increases in haemoglobin levels, even when deworming is not done.

The decision to include deworming as a routine primary health care activity in communities where hookworm anaemia is endemic should therefore be taken with caution, particularly when there is no parallel effort to reduce, or eliminate altogether, sources of reinfestation through for instance the provision of cheap footwear. Providing iron supplements may be the method of choice. Treating individual cases of severe anaemia resulting from heavy hookworm load should obviously include deworming.

VI. ECONOMIC CONSIDERATIONS

Despite wide variations in costs from country to country, for example to cover salaries of health workers, Levin has recently prepared a cost/benefit analysis of IDA prevention programmes that is based on the estimated cost of inputs required for various types of interventions (65). In the case of iron fortification there is the cost of the fortificants, stabilizers, mixing, possibly special packaging, and distribution. In India the cost of fortification increases the price of salt by about 20%, while iron added to sugar in Guatemala raises it by only about 2%. It should be noted, however, that in the case of salt, the cost of the latter is low and the amount consumed every day small so that a 20% increase is still acceptable. Levin estimates that the cost of iron per person per year in a large-scale fortification programme is roughly US$ 0.20-0.30. The addition of ascorbic acid would probably be double.

The author's calculation of costs related to supplemental medicinal iron distribution are based on delivery systems built around community- or village-based health care systems. They include salaries, medicinal iron (possibly with folate), transport and distribution. On this basis intervention costs range between US$ 2-3 per person per year for the delivery of ferrous sulfate tablets depending on the rural/urban population density and the other services being rendered by health workers.

Apart from immediate increases in physical work output, the prevention and treatment of anaemia produce a number of longer-term benefits including lower morbidity and mortality, higher productivity outside the work place, improved quality of leisure time, increased learning capacity, and an improved sense of well-being. Clearly, the purely material value of many of these benefits is impossible to measure.

Expressed in 1980 figures, the economic and social benefits resulting from increased work output in an adult population were estimated at US$ 5-7 in the case of fortification and at US$ 12-18 for iron supplementation in Indonesia, Kenya and Mexico. The cost-benefit ratio for IDA prevention programmes ranges between 6 and 58. Levine concludes that, based on a variety of mainly conservative assumptions, the cost-benefit ratio of both iron fortification and supplementation programmes exceeds unity by a considerable margin and that they therefore represent highly productive investments for developing countries.

VII. DEVELOPING AND IMPLEMENTING PROGRAMMES TO CONTROL IDA

While many countries have long-standing activities aimed at preventing and controlling anaemia, few have a coordinated anaemia strategy. The commonest measure is the distribution of iron tablets through hospitals and clinics. This has rarely been evaluated despite the fact that problems of supply and compliance are very common. The development of primary health care in many countries, when considered in the light of newer knowledge of IDA, provides a rare opportunity to develop a strategy against IDA that is direct, inexpensive and more effective.

7.1 Technical strategies

Possible corrective measures can be grouped under four technical approaches based on:

1. Food fortification,
2. Medicinal supplementation,
3. Diet modification, and
4. Control of infection.

Countries have a choice of selecting their overall strategy in relation to these approaches and those components selected will depend on the epidemiology of IDA, and the form that PHC is taking.

In general it is unwise to rely on a single technical approach. It is unlikely to have sufficient effect. Although it must be said that experience in the evaluation of anaemia control is limited and no approach or combination of approaches can be said with certainty to have this or that effect.

7.2 Situation analysis

A minimal data base is needed in order to be able to develop an appropriate prevention and control strategy against IDA. It is necessary to have some idea of anaemia prevalence by region and district, the most vulnerable groups, and most prominent types of anaemia. For example, adult males are rarely anaemic in some countries, while they are quite vulnerable in others. In some places folate and iron deficiency are prevalent, and in others the anaemia is due mainly to iron deficiency.

Public health administrators need to familiarise themselves with all the information available on the following:

(a) the epidemiology of anaemia, for example age and sex distribution, geographical distribution and main causes (diet, infections);

(b) the "administrative infrastructure". This includes the distribution of health staff and facilities, community organization, transport and storage, and the management structure of PHC including supplies, budgeting and so on;

(c) the geographical, nutritional and socioeconomic background. For example accessibility of people to services, main dietary sources (actual and potential) of iron and ascorbic acid, major inhibitors to iron absorption, customs that may influence the prevalence of anaemia, etc.;

(d) current and past experience. Experience of what has been shown to be successful or unsuccessful so far in a given country and in similar situations elsewhere can be very helpful in planning an anaemia strategy.

The information for preparing such a situation analysis will come from a variety of sources, in particular investigations and surveys that have been undertaken, but also government reports, information from nongovernmental organizations, unpublished research and personal experiences. The purpose of the situation analysis is twofold: to provide an information base for initial decision-making and to indicate information gaps that may be undermining sound decision-making.

7.3 Characteristics, objectives and goals of an anaemia strategy

Even before a situation analysis has been completed an outline of a strategy's characteristics, objectives and goals can be drawn up. They will be improved and made more specific based on the results of the situation analysis, together with the contribution of the people who carried it out. There are nevertheless a number of general characteristics that apply in most situations.

An anaemia prevention and control strategy is likely to have the following characteristics:

(a) it will be integrated into the national primary health care delivery system as far as possible so as to keep costs low through a sharing of facilities, training and supervision, and to maximize health benefits through its support to convergent services;

(b) it will depend on general scientific, epidemiological and therapeutic principles but will use technologies and approaches that are specifically adapted to the country and the problem;

(c) operational responsibilities for coordinating the anaemia stragegy will be vested in the ministry or analogous body responsible for health but the strategy itself will involve other sectors, such as agriculture and education particularly extension services, and branches of government, for example those responsible for the drafting and adoption of legislation;

(d) monitoring and evaluation including, as far as possible, screening for IDA will be incorporated into the strategy as a management tool that encourages flexibility and improvement of programme operation over time.

The anaemia strategy's objectives are unlikely to include the elimination of IDA, as this is an unachievable aim in most countries for the foreseeable future. It will, however, set levels of achievement (goals) for the most vulnerable groups and concentrate in the first instance on severe anaemias (in many countries the prime target will be women in the second half of pregnancy and preschool-age children).

These goals will be achieved through a combination of the four technical approaches outlined in section 7.1.

7.4 Fortification

The fortification of a widely consumed and centrally processed staple food is the backbone of anaemia control strategies in many countries. It mostly involves centralized decision-making, legislation and action taken in flour mills and other food processing plants. Fortification leaves little or no room for community involvement and thus cannot be considered within the direct ambit of PHC. The reader is referred to other sources (29,66) for a discussion of the use of this approach in various circumstances.

7.5 Supplementation

Supplementation through tablets or solutions containing iron or iron-folate is commonly provided in many countries, formally through the health system and informally through a range of over-the-counter preparations. The success of a supplementation programme depends on such factors as organization and coverage of the PHC system in relation to the target group, appropriateness of the supplements provided, a regular supply of supplements, low prevalence of side effects, and education and motivation of recipients in order to ensure compliance.

As PHC expands, the problem of accessibility to those in need diminishes. Tablets can be given at antenatal attendances, at post-natal and family planning checks, and especially in child health sessions, demonstrating the need for an anaemia control strategy to be integrated into such health activities as maternal and child health programmes. A supplementation strategy will be aimed at preventing anaemia in those at special risk such as pregnant women, and treating it in others. It is important to develop very clear criteria on dosage frequency, follow up, referral criteria, how to deal with side effects or non-compliance, etc., and to build these into the training of health personnel and supervisory procedures.

Type of iron supplements: The most appropriate iron salts is dealt with in section V. A balance needs to be struck that combines optimum chemical appropriateness, cost, dosage, simplicity, shelf-life and acceptability, while at the same time minimizing side-effects.

Supply: An anaemia control strategy should estimate quantities of supplements required over time and develop a logistics system to ensure regular and adequate delivery. In most countries this involves joint programming between those responsible for the anaemia strategy, the ministry of health's medical stores, and the essential drugs programme. It is vital to establish budget lines for necessary purchases. It also involves developing an appropriate packaging system - perhaps using packets similar to those developed for oral contraceptives; these can help the patient to remember to take the tablet regularly. Supplements need to be clearly labelled to remind health workers and patients of the correct dosage. In many countries it is very difficult to find appropriate containers, particularly for liquid preparations. These problems must be solved if an anaemia strategy is to be successful.

Compliance: Compliance is a major problem worldwide and no standard solution has been found. It is related to the long period over which supplementation needs to be continued and to side-effects. The latter are, as described earlier, dose-related. A minimum therapeutic dose needs to be taken (with meals only if there are side-effects) and subjects should be alerted that changes in the stools are normal. Reinforcing advice to keep on taking the supplements may be helpful. It may also be useful to study the problem of compliance in selected populations using such social marketing techniques as focused group discussions.

7.6 Diet modification

A diet modification strategy is based on four principles: (a) increased consumption of haem- and non-haem iron; (b) concurrent consumption of non-haem iron absorption enhancers; (c) concurrent avoidance of inhibitors of non-haem iron absorption; and (d) unsupplemented breast-feeding during the first 4-6 months of life and a proper complementary diet added thereafter.

It will be seen that principles (a) and (d) are consonant with the more general nutrition advice that is likely to be given through PHC, and thus a strategy to prevent and control anaemia can link with other nutrition activities. It could be incorporated into a national food and nutrition policy or strategy, for example, where such exists.

Regarding (b), the consumption of enhancers, many fruits are excellent sources of ascorbic acid - for example citrus, pawpaw and mango. Reference to a food composition table will provide many more enhancers that are appropriate to each country and season. An anaemia strategy can include the development, for example, of a fruit-tree distribution programme in collaboration with the agricultural extension services or forestry department.

In some countries iron absorption inhibitors, such as tea when drunk with meals, contribute to the development of anaemia. An education programme to change such habits may be necessary.

7.7 Control of infection

The objective of an infection control strategy will be to lower the prevalence of infective morbidity and heavy parasitic infections to a level below public health significance in populations who are at risk of anaemia. It will involve improving faecal and solid waste disposal, better hygiene, and improved access to clean water.

7.8 Public education

With the possible exception of fortification, the success of all four technical approaches depends on the active participation of the population, and thus requires a public education support strategy. It is important in this respect to identify the behaviours required, and particularly changes in behaviour, to achieve each aspect of these approaches.

The major behavioural changes needed centre on compliance, i.e. continuing to take supplements regularly, and dietary modification. Because of the cost and difficulties of inducing such change, efforts should be focussed on identified target groups such as pregnant women. Specific educational messages will need to be designed. These can then be incorporated into the training of health and other workers who deal directly with the public and adopted as part of a media strategy. Some of the older lessons learnt from community

development and the newer methodologies adapted from advertising can be used to derive messages that seek to identify the true motivating factors of the target group. Many past failures in anaemia control programmes are, in large part, attributable to a failure to develop an appropriate educational strategy based on these new insights and techniques.

There is a growing body of educational experience relating to nutrition and health in general which needs to be tapped in developing an anaemia prevention and control strategy. Such approaches can be enhanced by involving communities in the definition of an anaemia control strategy and motivating them to decide on the activities they will perform and the promotion they will undertake by themselves with support from the service providers.

Monitoring and evaluation

A continuing monitoring and evaluation strategy is best incorporated into a country's existing health information system, becoming part of the established routine.

There are still many problems in developing a technology for community anaemia screening. Studies are underway to develop a reasonably accurate and rapid method for identifying persons with anaemia that is at the same time inexpensive and involves apparatus that does not break easily and that is not dependant on electricity. Section IV provides the scientific basis for understanding this question.

A monitoring and evaluation strategy should not only measure the prevalence and severity of anaemia but should also indicate how the anaemia strategy is progressing in technical and managerial terms. The supply of haematinics, for example, should be monitored and evaluated. This is often best done through the monitoring and evaluation system developed for a country's essential drugs programme. Problem areas such as compliance need to be monitored so that failures can be anticipated before they become serious and remedial measures taken in good time. Monitoring and evaluation data should be used to improve and adjust the programme operation.

The role and activities of the various components of the primary health care systems in areas where moderate or severe IDA exists can be summarized as follows:

LEVEL	DUTIES
1. Community level, community health worker	Screen and treat all cases of anaemia. If screening is not possible, supplement with iron (+ folate) those age/sex groups where risk of IDA is high. All pregnant women should receive iron + folate during the second half of pregnancy, even without screening. Carry out health and nutrition education. Improve levels of personal and community hygiene.
2. First level health facilities health centre	Screen and treat all patients with anaemia. Refer those with severe anaemia to district hospital for treatment. Supplement all pregnant women with iron + folate during second half of pregnancy. Treat patients with hookworm, bilharzia and giardia. Carry out health and nutrition education.
3. First referral level, rural or district hospital	Diagnose and treat all cases of anaemia Diagnose and treat all cases of intestinal infections
4. District health officer	Determine the epidemiology of IDA Organize treatment and prevention of IDA at all levels of the primary health care system (integrate with other disciplines)

	Organize the training of health personnel, including community health workers for the treatment and prevention of IDA, related nutrition education, and the control of relevant parasitic diseases Mobilize district resources such as education, agriculture, community or political groups.
5. Provincial or National Health administrator	Monitor and evaluate the programmes in its technical and logistic aspects. Ensure an efficient flow of supplies. Mobilize resources to deal with problems.

Conclusion

Measures that will help to reduce the prevalence of iron deficiency anaemia include increasing iron intake and improving the bioavailability of dietary iron through suitable health and nutrition education, reducing morbidity by controlling infection and parasitic infestations, distributing iron supplements and motivating compliance in their use. The limitations inherent in their application should not be underestimated, however. Dietary modifications, for example, are easy to suggest but difficult to follow. The effective delivery of messages intended to stimulate changes in attitudes that are necessary for altering habits require a level and type of skills not readily found in primary health care workers. Primary health care can improve abilities to deal with episodes of infection and to provide timely curative care. Inadequacies in these areas must be corrected if primary health care is to make a significant contribution to control of anaemia.

Obviously, most if not all of these measures are equally relevant to overcoming most other health and nutrition problems. The control of anaemia through primary health care should therefore not be seen as an isolated activity but rather as part of total health care that is, in turn, integrated into the overall socioeconomic development process.

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APPENDIX 4

X INACG Meeting

Quito, Ecuador

INACG

International
Nutritional Anemia
Consultative
Group

Chairman:
Dr. E.M. DeMaeyer, WHO

Secretary
Dr. Samuel G. Kahn, AID

Secretariat:
The Nutrition Foundation
1126 16th St. NW, 3rd Floor
Washington, D.C. 20036
Cable: NUTRITION WASHINGTONDC
Telex: "NUFOUND" 6814107
Phone: (202) 659-9024

MINUTES OF THE X INACG MEETING QUITO, ECUADOR QUITO INTERCONTINENTAL HOTEL 12-15 MAY, 1987

Dr. Edouard DeMaeyer chaired, except where otherwise stated.

Dr. Edouard DeMaeyer, Chairman of the International Nutritional Anemia Consultative Group (INACG), started the program by thanking Dr. Wilma Freire on behalf of INACG for arranging the meeting in Quito. He commented that previous INACG meetings in South America had been held in Campinas, Brazil and Santiago, Chile but that this was the first meeting at which the results of a national nutrition survey were to be presented. He hoped that this recently completed survey would serve as a basis for preventing iron deficiency and cited the example of Chile to point out that the control of iron deficiency is not impossible.

Dr. Jorge Enriquez, Director of the National Development Council (CONADE), then welcomed the group and formally opened the meeting. He expressed the hope that the meeting would help to find the means for reducing the prevalence of nutritional anemia throughout the world and in Ecuador.

Dr. Eduardo Rodriguez, Director of International Relations at the Ministry of Health, next welcomed the group and expressed his wishes for the success of the meeting. He pointed out the importance of an international exchange of ideas and the relevance of the INACG discussions to the entire developing world.

Dr. Wilma Freire, Director of Nutrition of CONADE, expressed her pleasure at having the INACG meeting here. She indicated that she was proud to have the opportunity to present the results of the Nutrition Survey of Ecuador at the meeting and hoped that the findings would be of mutual benefit.

Dr. Carlos Daza, Representative of the Pan-American Health Organization (PAHO), next wished for the success of the meeting in helping to meet the needs of lower income groups, many of whose problems deal with food consumption.

Mr. William Goldman, Health Officer of the United States Agency for International Development (USAID), outlined the present programs in Ecuador. A marked increase in immunization coverage and the use of oral rehydration have helped to reduce infant mortality. These efforts were partly instrumental in Ecuador receiving one of two awards to be given to a country this year by the Council on International Health.

Dr. Christian Wahli, Director of LATINRECO, expressed pleasure that LATINRECO was able to collaborate in the Ecuador Nutrition Survey by helping to plan and implement the biochemical analyses. He hoped that this would be a model for morecollaborations, not confrontations, between private companies and governmental agencies. His comments closed the introductory portion of the program.

Dr. Sharyn Sutton, Vice President of Doremus, Porter and Novelli, Washington, D.C., U.S.A., next reviewed a three day workshop on "Social Marketing" of Iron Deficiency that was sponsored by INACG in Washington, D.C., in August 1986.

Dr. Sutton first described some of the principles of social marketing as applied to iron deficiency anemia (IDA). One problem involves "positioning" or presenting the priority of a program to prevent IDA in relation to other competing programs, such as immunization and oral rehydration, to policy makers, health workers, and the general public. She emphasized the principle of identifying key policy makers and promoting their support of an IDA prevention program in a manner that reflects favorably on them.

A key need is to express the problem of IDA in easily comprehended and compelling terms. Some of the selling points are as follows.

The Problem: IDA is the most common nutritional problem in the world today with over 700 million affected people, especially children and women in developing countries.

The Causes: include growth and high iron requirements in children and adolescents and blood loss and iron loss to the fetus in women. It was pointed out that health programs directed to these segments of the population now have a high priority with granting agencies.

Consequences in infants and children include impaired motor and mental development, a decrease in resistance to infection, and a decline in physical activity. Iron deficiency in pregnancy results in increased fetal morbidity and mortality. In both men and women, there is a decreased work and earning capacity and fatigue.

Benefits: include an improvement in the above-mentioned consequences that can be achieved at very low cost of less that $0.20/capita/year. The cost/benefit ratio of fortification has been calculated to be substantially more favorable than in programs for vitamin A and iodine fortification.

Types of Interventions to be planned will include fortification, supplementation, and education.

Marketing Objectives include an increase in annual funding by $ 4 million to reduce IDA by 50% by 1995.

Target Audiences are USAID and other bilateral agencies, WHO, UNICEF, development banks, industry and government legislators.

Marketing Advantages are the high prevalence of IDA, the relative low cost of intervention, and the severe consequences for the two most vulnerable groups. Treatment is important for workers and employers can be a target for information. The necessary technology for fortification is available and the example of success stories can be used as a selling point.

Obstacles include the facts that IDA is probably not a principal cause of mortality and that visible benefits of prevention are not dramatic. Supplements may have side effects and require prolonged use. Additionally, frequent self medication decreases the prestige of iron supplements.

Marketing strategies and suggested tactics. After outlining the marketing objective, target audiences, and the marketing opportunities and problems, Dr. Sutton concluded by identifying marketing strategies or approaches to achieving the objective. Five marketing strategies are as follows, in order of their perceived importance and chronology.

1) Consolidate the image of IDA with a logo, theme and graphic identify, and define key benefits to the target audience.
2) Increase the perceived importance of IDA among donor agencies.
3) Increase industry participation in the development of effective programs to decrease IDA in the developing world.
4) Increase the demand for IDA resources/services by developing countries.
5) Support research to improve the salability of IDA (e.g. the relationship to infection and disease, effects on mental development and productivity, and new technologies).

Dr. Allan Forbes of the U.S. Food and Drug Administration opened the afternoon session of May 12 with a discussion of the results of the INACG Bioavailability Research Project. The project was sponsored by INACG and supported by contributions from industry and U.S. government agencies. Laboratories involved in the project were Nestlè, U.S. Department of Agriculture, ICCNA at Kansas University Medical Center, and the U.S. Food and Drug Administration. Three forms of iron were studied: ferrous

sulphate as a standard of reference, electrolytic iron (a size that passes a 20 mesh) of a type that is commonly used in food fortification in the U.S., and iron orthophosphate, which appears to have poor bioavailability under most circumstances. The purpose of the study was to determine the comparability of in vitro, animal and human iron bioassay methods.

The results of the isotopic iron absorption studies in man showed electrolytic iron to be absorbed 75% as well as ferrous sulphate; orthophosphate was absorbed 25% as well as ferrous sulphate. Three types of rat studies and three types of in vitro studies were evaluated to determine which best match the human results. This is an important issue because the human studies are expensive and difficult to perform, and less expensive methods are needed to evaluate new forms of iron used in fortification.

Among the rat methods, the closest to human results was a modified, non isotopic Association of Official Analytical Chemists (AOAC) method that measures the rate at which a rat recovers from iron deficiency anemia. This method showed averages of 72 and 29%, respectively for electrolytic iron and iron orthophosphate, compared to a reference value of 100% for ferrous sulfate. The best in vitro method was a dialysis procedure which resulted in values averaging about 65 and 35%, respectively, for electrolytic iron and iron orthophosphate. This method, however, produces somewhat variable results and requires further standardization.

Dr. Peter Dallman, University of California, San Francisco, next discussed the assessment of iron deficiency in infants and children. He emphasized that growth was the major factor contributing to iron deficiency in children as opposed to menstrual and fetal iron losses in women. Consequently, the periods of highest prevalence of iron deficiency in children are late infancy after 4 months of age (when neonatal iron stores have been depleted and growth is rapid) and adolescence (when children gain 9 to 10 Kg. per year during their growth spurt).

Strategies for detecting anemia include: (1) Early identification before altered behavior and other abnormalities in body function have become prominent. It is important to catch anemia when it is still mild, even though it is easier to diagnose when more advanced. (2) Screening for anemia should be incorporated into regular health care visits with ages 6 to 12 months and the adolescent growth spurt as periods of highest priority. (3) Screening for anemia should be done when there is no concurrent or recent infection. Studies have shown that even mild infection can cause anemia and also confound interpretation of the other tests for iron deficiency.

The spectrophotometric analyses of hemoglobin by the cyanmethemoglobin method continues to be the preferred method and is most readily done in a centralized laboratory. Improved instruments for use in the field are being developed. Ideally such instruments should be sturdy, not require an electrical supply, not require a measured volume of blood, have low maintenance needs, and have low initial and running costs. One new instrument that has most of these features was described. Its major disadvantage is the high cost of the dry reagent-containing disposable cuvettes. There is a good possibility that this cost will come down.

Dr. Tomas Walter, of the Instituto de Nutricion y Tecnologia de los Alimentos (INTA), Santiago, Chile, reported on recent studies of iron in infant and child development. Infants were studied between 12 and 15 months of age. They were classified as (iron deficient) anemic, sideropenic, and "normal" by a completebattery of laboratory studies and response to iron treatment. After an initial evaluation by the Bayley Scale (a test of mental and psychomotor development), they were given iron or placebo and retested after 10 days. Then all infants were treated with iron for 75 days after which they were tested a third time by the Bayley Scale.

Initially, the anemic infants had significantly lower Bayley scores than normal infants. Below a hemoglobin of 10.4 g/dl the deficit was substantial, at concentrations of 10.5-10.9 g/dl the deficit was slight and above 11.0 g/dl there were no significant differences compared to normals, even if other tests of iron deficiency were abnormal. Infants with anemia of prolonged duration had a greater deficit.

The deficit in the anemic children was not reversed by 75 days of iron treatment. This discouraging finding is in accord with results from other groups and the findings next reported by Dr. Pollitt.

Dr. Ernesto Pollitt, University of California, Davis, next reported on the related topic of iron deficiency and school performance, a collaboration with Dr. Valyasevi of Thailand.

2171 children attending the 16 largest schools, in the most accessible areas of Cholburi province, Thailand, participated in this double-blind clinical trial with measurements of educational achievement initially and after 10-12 weeks of treatment. Fifty mg. of ferrous sulphate or a placebo was given for roughly 15-20 days. In this preliminary report 2101 children between 9 and 12 years of age were classified as iron deficient with anemia, iron depleted, or iron replete.

The results of the Thai language test before treatment showed that iron deficient anemic children (subsequently placebo or iron treated) had significantly lower mean scores than the iron replete children. There was no overlap between the iron-deficient anemic and iron replete groups.

The results of the mathematics test were strikingly similar to those of the Thai language test; the highest and lowest performances were observed among the iron replete and the iron deficient anemic children, respectively. In respect to the IQ data, the iron deficient anemic children again performed significantly poorer than the iron replete children. In neither case were the differences between the iron depleted and iron replete children statistically significant.

The analyses showed that there was a striking difference in school test performance between iron deficient anemic and iron replete children; this difference, however, persisted after 2 to 3 weeks of iron treatment.

Dr. Wilma Freire, Chief of the Nutrition Unit at CONADE, next discussed the use of hemoglobin as a predictor of response to iron therapy. Dr. Freire studied the sensitivity (a positive result with disease) and specificity (a negative result in absence of disease) at various cut-off levels for hemoglobin concentration, using the response to iron treatment as an end-point. It was anticipated that the predictive value of the hemoglobin concentration would increase in a population with a very high prevalence of iron deficiency. The trial involved 412 women around the 26th week of pregnancy in Quito, Ecuador, at an altitude of 2,800 meters. Iron was provided as ferrous sulphate at a dose of 39 mg. elemental iron twice a day to 210 women and 202 women received a placebo. Correction for regression to the mean was accomplished by comparing hemoglobin response in the two groups at every initial hemoglobin level. Hemoglobin values were corrected for altitude. Based on response to treatment, the prevalence of iron deficiency in the sample was 62%, even though the initial prevalence of anemia was only 9%. Thus, the classic definition of anemia would vastly underestimate the number that responded to iron by having a rise in hemoglobin concentration.

The considerations involved in setting a better cut-off value to decide on iron treatment were discussed. If most potentially responsive people were to be treated without budgetary constraints, a cut-off value of 13.3 g/dl (1.0 g/dl higher than the anemia cut-off) seemed satisfactory for the group studied because it would correctly identify 76% of responders. However, 54% would unnecessarily be treated. Budgetary constraints might require a lower cut-off level which would identify fewer responders. The results emphasize the importance of prevalence

in decisions to modify the cut-off value at which a treatment decision is made.

Dr. Rodolfo Florentino presented the report of Dr. Florentino Solon, who was unable to attend. He discussed iron and food supplementation delivery for pregnant women within the framework of primary health care in the Philippines.

The Department of Health and the Nutrition Center of the Philippines had embarked on a project to determine the feasibility and effectiveness of iron and food supplementation delivery within the existing framework of Primary Health Care. The objective was to demonstrate whether such an approach would be effective in reducing anemia and improving pregnancy outcome among pregnant women.

The project was conducted in La Union Province in northeastern Philippines. Each of the 20 Rural Health Units in the province were allocated prospectively into one of 4 treatment groups: (1) Food group, where 400 Kcal/d. food supplements weredistributed monthly by village health workers (VHW) to all pregnant women from 7 months of pregnancy to term; (2) Iron group, where iron tablets (120 mg. iron daily) were distributed weekly to pregnant women from the fifth month on; (3) Combined food and iron group, where both food and iron supplements were given, and (4) Control group. In addition, a nutrition information and education program was administered to all 4 groups, including control.

Problems included low compliance to supplementation by target pregnant women and adherence to program protocols by VHWs during the implementation of the project. On the average only about 31 to 50% of the planned food packets and 20% of the planned iron tablets were taken by the pregnant women. In spite of these problems, however, the preliminary results based on two validation studies and data from the monitoring system, seem to point to positive effects of supplementation to pregnancy outcome. Among women from the combined food and iron group, where levels of implementation and compliance were relatively higher, the supplementation resulted in a significantly higher incidence of favorable pregnancy outcome, i.e., normal birth-weight, full-term age of gestation and improved nutritional status of the infant at the end of the first month of life. Of particular interest were the preliminary findings that indicated a significant reduction in the percent of low birth weight infants when pregnant women were supplemented with iron.

Results from the research component (pre- and post-surveys) are currently being processed and analyzed.

Dr. Eva Hertrampf of INTA, Chile next discussed the effect of a therapeutic trial of iron on the assessment of iron status in a field trial. The purpose of the study was to evaluate the problem of the hemoglobin overlap between responders (> 1.0 g/dl) and non-responders to iron treatment with a different experimental design from that of Dr. Freire's study. The four dietary groups and their hemoglobins before and after 3 months of iron treatment (3-5 mg/Kg/d) were:

	12 mo.	15 mo.
1) Iron and ascorbate fortified milk after early weaning	12.6	12.7
2) Non-fortified milk after early weaning	11.2	12.4
3) Hemoglobin-cereal with prolonged breast feeding	12.2	12.8
4) Regular solids with prolonged breast feeding	11.7	12.6

Cumulative distribution curves at 12 months showed that the entire hemoglobin distribution curve was shifted downward, most markedly in group 2 but also substantially in group 4 (the two unfortified groups), and to a lesser degree in group 3. Response to the therapeutic trial was far more common than would have been predicted from prevalence of anemia at 12 months. Even group 1 with no anemia had a 12% response rate, and group 2 with 37% of anemia had a 50% hemoglobin response rate. In both groups 3 and 4, a hemoglobin response was about twice as common as anemia. The analysis of additional tests of iron status (MCV, EP, Fe/TIBC and ferritin) were of little help in predicting a hemoglobin response.

Dr. Tomas Walter started the 13 May morning session with the country report from Chile. He began by paying tribute to Dr. Abraham Stekel who was responsible for much of the success of the INTA program and who died unexpectedly in the spring of 1985.

Dr. Walter described an infant rice cereal fortified with hemoglobin in an amount to provide 1 mg. absorbed iron in a 40 g. serving. Due partly to its low fat content, the cereal has a shelf life of 2 years. As shown in Dr. Hertrampf's talk, the cereal was relatively effective in preventing anemia and low serum ferritin at 12 months of age (though not as effective as the iron and ascorbate fortified milk).

The hemoglobin cookie program was described next. A program of supplying cookies and milk or a milk substitute to school children during their mid-morning break was in existence prior to 1982. At that time the Ministry of Education accepted the use of cookies as a means of delivering a bioavailable form of iron (hemoglobin) to a large population of children. The cookies are

well accepted and in a comparison study of two adjacent provinces were found to significantly raise hemoglobin and serum ferritin in all children, particularly in post menarchial girls. About 1 million children are now covered by the program at an estimated cost of $0.50/child/year. A 5 month trial of these cookies in Ecuador resulted in an impressive decrease in prevalence of anemia from 34 to 4%, with a rise in mean hemoglobin of 0.8 g/dl.

Both programs are effective, relatively low in cost and potentially applicable in many countries.

Dr. Osman Galal, Director of the Nutrition Research Institute in Cairo, gave the country report for Egypt. Dr. Galal started by describing the very high prevalence of anemia among preschool children (31%) and pregnant and nursing women (25%). Anemia is most common in rural areas where schistosomiasis probably plays a role. Hookworm, however, is much reduced.

There are 4 ongoing programs in Egypt that will contribute to combat anemia: (1) Wheat flour fortification is proposed since 50% of calories come from flour, 90% of which is used for bread. Setting up the methodologies for monitoring iron status is in progress. A study of the effect of iron fortification on the acceptability of the bread is underway. (2) A beverage for school children ("Kool Aid" fortified with 4.5 mg iron and 60 mg ascorbate per serving) has been tested for 70 days in 232 school children aged 6 to 12 years. The youngest children had the highest prevalence of anemia initially. After 70 days the mean hemoglobin rose from 11.8 to 13.5 g/dl. The study showed the effectiveness of a fortified beverage for school children. (3) As part of the Child Survival Project, a cereal and iron product is being used and will be produced using local ingredients. (4) Eradication of schistosomiasis is being implemented as a program in the near future. The four programs are expected to achieve a decline in prevalence of iron deficiency anemia in the country as a whole. In addition, the Nutrition and Health education program includes a specific component dealing with how to avoid iron deficiency by changing food consumption patterns.

Dr. Rodolfo Florentino reported on programs related to iron deficiency in Philippines:

1) Recent data on prevalence of iron deficiency anemia. Results of the most recent nutrition surveys carried out by the Food and Nutrition Research Institute (FNRI) in 1986 in 6 out of 13 regions of the country showed that anemia was most prevalent in the Visayas regions (38% of all age groups) and lowest in the Mindanao regions (25%). Compared with the second national nutrition survey in 1982, the prevalence increased in the National Capital Region and the Visayas regions, while a slight decrease was found in Mindanao.

2) Control Program of the Department of Health (DOH). Under the structured Health Care Delivery System of DOH and utilizing the core strategy of Primary Health Care, the Department is prepared to undertake a nationwide nutritional anemia control program. The program calls for the distribution of iron pills to pregnant women (100-120 mg iron daily from midpregnancy to term) and of iron syrup to moderately and severely malnourished preschool children (60 mg. iron daily for 20 days). The distribution will be carried out in 1991 Rural Health Units and Health Centers and 9600 Barangay (village) Health Stations throughout the country by the midwives and Barangay Health Workers.

Implementation of the program will be monitored through the monthly monitoring system of DOH. The impact of the program will be evaluated through the periodic nutrition surveys of FNRI.

3) Research Program. More recent data on non-hemoglobin iron availability from meals consumed in various regions of the Philippines have been obtained. Using the in-vitro radio-chemical method, a total of 39 meals (representing breakfast, lunch and supper from 13 regions) were studied. Non-hemoglobin iron availability ranged from 2.4% to 13.4% with a geometric mean of 5.5% $\pm$ 0.22. The bioavailability was found to diminish with increasing tannic acid content of the meal. Surprisingly, no relationship between iron availability and phytic acid, meat/fish/poultry and ascorbic acid of the meals was apparent.

Another project about to be launched is designed to evaluate the effectiveness of iron and vitamin A enriched rice in improving iron and vitamin A status of preschool children under controlled clinical setting, and if successful, under field conditions.

Dr. S. G. Srikantia, of the Central Food Technological Research Institute, Mysore, gave the country report for India. He described the strategies of iron tablet distribution and education that have been applied for over a decade and noted the difficulties in estimating their benefit. Baseline data for prevalence of iron deficiency anemia in India are scant, partly due to a strong cultural aversion to blood sampling. Surveys have indicated that among those populations that are supposed to receive iron tablets, fewer than 27% of pregnant women, 17% of lactating women and 2% of children are covered by the distribution programs. Of those who received the tablets, it is estimated that less than half used them. The most promising program to prevent iron deficiency by fortification involves the addition of iron to salt. The program has not yet been implemented because the fortification of salt with iodine to prevent goiter has received a higher priority. The fortification

of salt with both iron and iodine has posed technical problems that have not been resolved until recently. Dr. Srikantia was of the opinion that educational programs to improve iron nutrition have not been very effective. As evidence he cited surveys showing no change in the consumption of greens in the the last decade.

Dr. B. S. Narasinga Rao, Deputy Director of the National Institute of Nutrition, Hyderabad, next elaborated on the double fortification of salt with iron and iodine and on a pilot project for iron fortified salt in Tamil Nadu, India. A stabilizer has been developed that ensures the stability of iodine in the presence of iron. The final salt formula includes 1% stabilizer, 1000 ppm iron as ferrous sulfate, and 40 ppm iodine as KI. The bioavailability of both iron and iodine appear to be good, and stability is satisfactory, even after a year of storage. The estimated cost increase will be 30%. It is planned to use the doubly fortified salt in a pilot project in several months.

Dr. Rao next outlined the production and distribution of iron fortified salt in two districts of Tamil Nadu. The salt distribution will be promoted through an educational program and the effectiveness will be monitored with hemoglobin and serum ferritin. A simple field kit has been developed to verify that salt reaching the consumer contains the appropriate amount of iron.

The next country report was that of Dr. Adeline W. Patterson, Director of the Caribbean Food and Nutrition Institute. Dr. Patterson described a population study of anemia prevalence conducted in 1985. There was an unusually high prevalence of anemia in all age groups. The lowest value was 14% among young men and the highest values were 80% in infants 12 to 24 months and 63% in pregnant women. In June 1986, a workshop was convened to discuss the results of the survey and develop a strategy for the control of anemia in Grenada. The recommended solutions included the following: (1) Supply management of iron supplements including record keeping and transportation. (2) Logistics and technology of supplementation programs with emphasis on ante and postnatal clinics and child health clinics, as well as collaboration with the Ministry of Education to organize and monitor school feeding programs. (3) Regular hemoglobin screening in infants and women during pregnancy and postpartum. (4) Looking into the adequacy of dietary bioavailable iron and various alternatives for improving bioavailability. The status of the project will be evaluated in 1988.

The last country report was that for China by Dr. Zhi Nan Zhang, Chief of the Division of Hematology, Peking Union Medical

College. Iron deficiency anemia is common in China, especially in rural areas, and constitutes an important health care problem. The prevalence in preschool children varies from 17% to 80%, according to location, and about 22% of pregnant women are anemic at term. The government is concerned about the problem but it is difficult to mount a nationwide campaign with a uniform protocol. Most studies are currently carried out in separate medical institutes in collaboration with local health centers. The goals of the current program at the Peking Union Medical College are to standardize methods for the design of studies, laboratory assessment of nutritional status and evaluation of interventions. There is great interest in increasing international collaboration and communication.

During the afternoon of May 13, the preliminary results of the NATIONAL NUTRITION SURVEY OF ECUADOR - 1987 were summarized by Dr. Wilma B. Freire, Director of Nutrition at CONADE (the National Council for Development of Ecuador), and by Dr. Henri M. Dirren, who heads the division of Nutritional Epidemiology at the Nestle Research Department in Switzerland.

Dr. Freire opened the discussion by outlining the purposes and scope of the survey. The purposes included providing a baseline for the design and implementation of interventions to combat malnutrition and to measure changes in nutritional status when programs are implemented. The objective was to characterize malnutrition by region and socioeconomic group, with an emphasis on protein-energy, protein, iron, vitamins, trace elements and thyroid status.

Analyses were planned to determine the relationships between nutritional status and socioeconomic group, diarrhea prevalence, family composition, maternal education, breast feeding and infant feeding practices, and family food consumption.

The survey focussed on children from birth to 60 months of age, since this group was considered to be at highest risk. A national probability sample was selected, based on 1982 census figures, by region, in clusters, and stratified according to urban, rural, coastal and highland locations and socioeconomic group. The total size of the sample was 8100 children selected from 360 census sectors. Biochemical analyses were restricted to a subsample of 1620 children. Technical assistance was provided by PAHO, LATINRECO, AID, CDC, and CEAS. Planning for the survey began in February 1982. The analyses are currently in their preliminary stages and it was emphasized that all of the results presented were on unweighted data, that is, they are not yet corrected for differences in selection probabilities.

The anthropometric data were striking in showing that more than 60% of the children over the age of 12 months were stunted (decreased height for age) by WHO criteria. Weight for age was decreased in about 50% in the same age range. Decreased weight for height (reflecting acute malnutrition) showed a peak of about 25% between 12 and 24 months, with much lower values at earlier and at later ages. Infants under the age of 6 months showed the lowest prevalence of malnutrition by all of the three measures. The prevalence of malnutrition by anthropometric criteria was greatest in the rural highlands, followed in order of frequency by rural lowlands, urban highlands, and urban lowlands.

Dr. Dirren next described the collaboration between CONADE and Nestle that made it possible to include the biochemical component of the nutrition survey. He reviewed the methods with particular emphasis on measures related to iron nutrition. These included hemoglobin, hematocrit, erythrocyte protoporphyrin, serum ferritin, and serum iron and iron binding capacity. Two acute phase reactants, C reactive protein and alpha 1 acid glycoprotein, were also measured since the presence of infection can confound the laboratory diagnosis of iron deficiency.

Dr. Freire then summarized some of the preliminary results pertaining to iron deficiency. Of major importance in the analysis was the correction of hemoglobin values for altitude. Hemoglobin < 11.0 g/dl (corrected for altitude), protoporphyrin > 2.8 ug.g Hb, transferrin saturation < 12%, and serum ferritin < 10 ug/L were considered abnormal. Anemia was most common in the coastal regions: 19% vs. 11% in the highlands (above 2000 m). In both areas, about 25% of children had low serum ferritin values.

Dr. Dirren next discussed the hemoglobin correction for altitude and the role of infection and inflammation in anemia. The altitude correction was virtually identical to that described by Hurtado and coworkers in 1945 (Arch Int Med 75:284). In the survey population, an altitude correction was derived from those children who had two or fewer abnormal measures of iron nutrition. Contrary to what is usually recommended in the literature (a 4% increase per 1000 m elevation), the correction for the first 1000 m was only 1.9%. However the correction rose in a curvilinear fashion with increasing altitude: 2.8% between 1000 and 2000 m and 3.9% between 2000 and 3000 m.

About 13% of the children had an infection or inflammation on the basis of an abnormality in one or both acute phase reactants. There was a downward shift of 0.5 g/dl in the hemoglobin distribution curve of infected compared to non-infected individuals. Furthermore, 33% of those with infection were anemic compared to only 15% of those without

infection. The availability of the acute phase reactant will make it easier to distinguish infection from iron deficiency as causes of anemia.

The conclusions to date are that there is a moderate prevalence of iron deficiency anemia among preschool children in Ecuador and that this prevalence is sufficiently great to warrant interventions and a look at other high risk groups such as women during childbearing years. When the analysis is complete, there should be a sufficient basis for planning programs to prevent iron deficiency.

Dr. Samuel Kahn chaired the morning 14 May session

Dr. Edouard DeMaeyer first discussed the Guidelines and Recommendations for Developing Strategies. He pointed out that when anemia is first identified as a problem, its causes should be identified. Different local factors include use of IUDs, parasitic infestation, insufficient food, low iron density in the diet (milk in infants), low bioavailability of iron in diet (diets low in meat and/or ascorbic acids) or inhibition of iron absorption (tannins in tea). Iron fortification must be monitored to make sure that the food is actually fortified as specified by regulations when it reaches the consumer. Iron supplementation is less reliable but may specifically be needed in special populations such as pregnant women.

Dr. Samuel Kahn next discussed the Background for Strategy Development under three headings: whole country factors, population factors, and administrative factors.

Whole country factors include

1) Does IDA exist? What is the target group, its size, and location?
2) What are existing programs and how effective are they?
3) What other operational systems can be used (e.g., agricultural, educational, labor ministries)?
4) Which are the options for delivering bioavailable iron and what are their costs?
5) What support activities are needed?
6) What is the nature of pharmaceutical industry?
7) What is the nature of food industry (is there centralized production)?

Population factors include the perception of benefits by the consumer, delivery systems for the target population, food consumption patterns, health practices, and the existence of public advocacy.

Administrative factors involve ministries, public and private sectors, identification of leadership, and financial resources. Monitoring and evaluation of the program should be an integral component. In some cases, regulation and legislation and import vs. local production issues must be considered. In addition, development of infrastructures and program sustainability is important. Overall strategies are ideally set up so that they can be maintained over time.

Dr. Carlos Daza of PAHO next discussed the Role of PAHO and WHO in implementation of Latin American Programs. The levels of organization are as follows:

The worldwide level of organization is the WHO Nutrition Unit in Geneva; the regional level is the Food and Nutrition Unit in Washington; subregional units include CFNI in the Caribbean and INCAP in Guatemala; intercountry units include that in Lima for the Andean Region.

Donor agencies include FAO, WFP, UNICEF, UNESCO, USAID, CIDA, GTZ, IDB, the World Bank, and foundations. PAHO has programs with distinct food components and nutrition components. The first includes education and fortification whereas the second includes monitoring of growth and feeding of children and monitoring maternal nutrition. Additional factors involve diagnosis and epidemiology.

Some of the implementation strategies include:

1) Mobilization of local and international resources
2) Dissemination of information.
3) Research promotion.
4) Training of personnel.
5) Development of guidelines and norms.
6) Direct technical assistance.

A regional network of Food and Nutrition institutes has been developed. The sites include Chile, Mexico, Brazil, Colombia, Guatemala, Jamaica, and the United States.

Iodine deficiency is the target of a special program in the Andean countries and Paraguay, vitamin A deficiency in Guatemala, iron deficiency in Chile and Guatemala, and fluoride deficiency in Costa Rica. Fortification programs include iodine and fluoride in salt, iron in sugar, and iron in cereal.

Mr. Richard Manoff reviewed some additional aspects of Social Marketing. He emphasized that having a solution to a problem does not mean that it is solved. For example, in family planning, access does not mean use of services, and growth charts

for preventing malnutrition are of little use if mothers do not understand them. One needs to create demand among the consumers. Supply alone does not create demand. These principles were first recognized by the commercial sector which went on to develop techniques to create demand. Perceptions of consumers are first investigated by interactive methods. Maximum awareness of health programs can be promoted by education, motivation of health workers, and use of public sector. Identification of obstacles in terms of cultural habits is important. Media must be used to promote the product.

An example of social marketing was the promotion of iodized salt in 1973 in Ecuador. This was fostered by 6 radio messages, one of which Dr. Manoff presented in detail. Messages were broadcast several times a day for a year. These messages had to change the popular perception of goiter from a normal condition to its recognition as a disease. Without this change in perception, the program would have failed. Health workers often do not fully appreciate that the consumer does not see a problem such as goiter as a serious issue. Distribution of iodized salt samples in conjunction with radio messages helped to assure acceptance when iodized salt was first introduced in two highland provinces of Ecuador.

On May 14, after the mid-morning break, Dr. Kahn divided the participants into three working groups to develop strategies for the control of iron deficiency anemia as a component of maternal and child health programs in Ecuador. The working groups were constituted as follows:

	TECHNICAL	MARKETING	OPERATIONAL
Chair	Walter	Manoff	Florentino
Rapporteur	Hertrampf	Gurney	Srikantia
	Aguirre	Forbes	Andrade
	Dirren	Galal	Clark
	Esteves	Patterson	Daza
	Gatel	Pollitt	Freire
	Rao		Jacome
	Schirla		Olivares
	Zhang		

The groups prepared preliminary consensus statements during the remainder of May 14, and these were presented to the whole group for discussion on the morning of May 15. During the late morning and early afternoon, the final consensus reports were prepared, and these are appended as an appendix to the minutes.

Dr. DeMaeyer presided over the concluding session of the meeting late on the afternoon of May 15. Dr. Freire first thanked the group for its focus on the problem of iron deficiency anemia in Ecuador, and hoped that there would be a continued interaction with INACG. Dr. Daza next stressed the importance of the Ecuador survey and anticipated that the results would lead to programs to prevent iron deficiency. Dr. Kahn thanked Dr. Freire and the other hosts for a successful meeting.

In his closing comments, Dr. DeMaeyer stressed that progress in preventing iron deficiency anemia in the context of maternal and child health was being made in many countries. He cited the Ecuador survey as a model and as a sound basis for implementing programs. He hoped that such programs would ensue, and that monitoring would show a decline in iron deficiency as has been seen in Chile . He thanked Dr. Freire for all the excellent preparations and arrangements for the meeting. Appreciation was also expressed for the help of the local secretariat staff, Laurie Lindsay and Anne Hill of INACG, the interpreters, and the rapporteur. Lastly he thanked the Government of Ecuador, LATINRECO, and INACG for the financial support that made the meeting possible. The meeting was then officially closed.

Respectfully submitted,

Peter R. Dallman,
Rapporteur

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INACG

International
Nutritional Anemia
Consultative
Group

Chairman:
Dr. E.M. DeMaeyer, WHO

Secretary
Dr. Samuel G. Kahn, AID

Secretariat:
The Nutrition Foundation
1126 16th St. NW, 3rd Floor
Washington, D.C. 20036
Cable: NUTRITION WASHINGTONDC
Telex: "NUFOUND" 6814107
Phone: (202) 659-9024

Working Group Reports

X INACG Meeting

Quito, Ecuador

14-15 May, 1987

INACG Editorial Committee
C. O. Chichester, Ph.D.
Samuel G. Kahn, Ph.D.
Laurie Lindsay, R.D.

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EXECUTIVE SUMMARY

Iron deficiency anemia is an important public health problem in Ecuador and deserves attention as such. The recent Ecuadorian Nutrition Survey has shown that one out of every five preschool aged children suffers from iron deficiency anemia, with the prevalence greatest in the coastal areas of Ecuador. Early studies have reported that 30% of non-pregnant fertile women in Quito are anemic and the prevalence of anemia in pregnant women may be 60%. The deleterious effects of iron deficiency anemia are: (1) potential impairment of infant motor and mental development as well as the academic achievement of school children, (2) exposure of a pregnant woman to higher risk at delivery and greater likelihood to have a low birth weight newborn, (3) possible decreased resistance to infection, and (4) known adverse effect on work capacity and performance. These widespread adverse effects are likely to take an immediate and long-term toll on the well-being and progress of the community. Fortunately, today there is technical information that makes possible the correction, control and prevention of iron deficiency anemia. Specific high prevalence groups can be reached with targeted programs of food enrichment and/or the provision of supplemental iron medication during short periods of time. In essence, iron deficiency anemia in Ecuador is correctable.

This report of the tenth INACG meeting recommends several intervention measures that should be considered for alleviating iron deficiency anemia in children of preschool age and in pregnant women. Recommendations for both short-term and long-term supplementation and fortification strategies are given and include brief comments on training, monitoring, education and infrastructural organization. A separate section is given to recommendations regarding the social marketing of programs to combat iron deficiency anemia.

REPORT FROM THE TECHNICAL COMMITTEE

Chairman: Dr. Tomas Walter

Rapporteur: Dr. Eva Hertrampf

Members: Dr. Z. Zhang
Dr. B.S. Rao
Dr. H. Dirren
Dr. H. Aguirre
Dr. J. Schirla
Dr. M. Gatel
Dr. E. Esteves

Iron deficiency is the most widespread nutritional deficiency in the world. Approximately one billion people suffer with iron-deficiency anemia (IDA). It affects principally infants, children, and women in fertile age, particularly during pregnancy. About half of all women and children in the developing countries have anemia. The basis for this nutritional deficiency lies mainly in the low quantity of absorbable iron in the diet coupled with the increased requirements for iron in the rapidly growing child, the menstruating female and the needs of the fetus during pregnancy.

The deleterious effects of anemia and iron deficiency are:

1. An anemic pregnant woman is exposed to higher risk at delivery and is more likely to give birth to a smaller newborn. Recent reports indicate that the low birth weight can be reversed with appropriate iron supplementation during pregnancy.

2. Experimental evidence suggests that there is a decreased resistance to infection in iron deficiency and that this is related to a diminished function of certain components in the immune system.

3. It has long been observed that iron deficient children show apathy, irritability and difficulty in concentrating. Recent research findings indicate that iron deficiency anemia can impair infant psychomotor and mental development as well as the academic achievement of school children. Short-term iron therapy that corrects for anemia does not readily reverse these impairments.

4. It is known that iron deficiency anemia has an adverse effect on work capacity and performance. Experimental, field, and economic reports substantiate that anemia limits individual productivity thereby decreasing potential income and self-esteem.

These widespread adverse effects are likely to take an immediate and long-term toll on the well-being and progress of the community. Additionally, the doubts remaining about the reversibility of certain deficits once anemia is established should make the prevention of iron deficiency a major thrust of public health programs.

The types of interventions available may be employed individually or in combination. They are:

1. supplementation - the distribution and administration of iron-containing tablets, capsules, elixirs, or powders;

2. fortification - the addition of iron to foods, condiments, or beverages; and

3. education - the modification of the eating practices of vulnerable groups to increase the consumption of absorbable iron.

A careful assessment of local needs and resources must be conducted if the details of an effective intervention strategy are to be developed. The strategy must have the flexibility to allow adaptation to local conditions and the inclusion of new, innovative iron deficiency systems. The intervention system must include monitoring of the response, careful recording of the effects, and evaluation of the benefits. In addition, the system should be conducted with other programs, e.g., existing health services, and with the agencies and authorities managing these systems. This may require modification of current techniques to make these more effective and efficient. Technical knowledge is currently available to assist in the design of specific fortification and supplementation interventions. Also, education and social marketing approaches have been developed that can be applied in local situations.

Factors influencing the selection of an appropriate intervention are prevalence and severity of the problem and the intended targeted population group. Daily iron supplementation is the preferred approach when iron needs are high, such as in pregnancy. The supplementation of all women with iron is standard practice during the last two trimesters of pregnancy. There are major obstacles to effective supplementation (occurence of side effects and reduced compliance) but potential new iron preparations currently being developed and effective marketing and educational programs can overcome these obstacles.

Iron fortification is a long-term approach to the prevention of iron deficiency. Several foods have been used successfully as vehicles for iron fortification. Though this approach represents a most satisfactory long-term solution, often technical and local logistical difficulties preclude its effectiveness. Nevertheless, there is a considerable body of technical information and experience in fortification that is available to be utilized. The success of a fortification program depends on:

1. use of a bioavailable iron source,

2. use of an appropriate food vehicle,

3. use of an effective distribution system,

4. the acceptability and use by the target population, and

5. an appropriate application of resources.

Programs of fortification may be targeted toward a specific group or the population in general. Examples of the former are infant foods and formula; examples of the latter are cereal products and wheat flour. A dramatic response in infants may be seen within a few months; adults respond more slowly. However, it may take several years before an expected full benefit is perceived in an entire population.

Data from a recent well-executed and designed survey in Ecuador show the prevalence of anemia in children 6-20 months of age to be near 17%. It is likely that the figure for infants 9 to 25 months of age is even higher. Anemia at the beginning of pregnancy has been found to be 10% reaching 45% to 60% at delivery. Non-pregnant fertile women in Quito have a prevalence of anemia close to 30%. These figures closely coincide with dietary information and merit the recognition that iron deficiency is an important public health problem in Ecuador and deserves attention as such.

Today's technical information makes possible the control and prevention of iron deficiency anemia. The general population can be reached by a program of enrichment of a staple food. Specific high prevalence groups can be reached with targeted programs of food enrichment and/or the provision of supplemental iron medication during short periods of time. In essence, iron deficiency anemia in Ecuador is correctable.

REPORT FROM THE OPERATIONAL WORKING GROUP

Chairman: Dr. R. Florentino

Rapporteur: Dr. S.G. Srikantia

Members: Dr. Carlos Daza
Dr. Wilma Freire
Dr. M. Olivares
Ms. Beatriz Andrade
Dr. Diana Jacome
Mr. Douglas Clark

The preliminary data from the Ecuadorian Nutrition Survey have shown that among children below the age of five years, the prevalence of Iron Deficiency Anemia (IDA) is about 17% for the country as a whole, with variations between coastal and highland areas, as well as between rural and urban areas. It has previously been found that 60% of pregnant women in an urban area have evidence of iron deficiency. There are, so far, no data on the prevalence of IDA in other age and population groups. On the basis of data from children and pregnant women, it is presumed that IDA is a public health problem in the country requiring control and preventive measures.

The group discussed possible intervention measures and suggested the following:

1. Children of preschool age: Distribution of an iron-fortified food prepared for preschool children was the strategy of choice to control and prevent anemia in this age group. Until recently Ecuador had a food distribution program intended to cover all children below the age of five years as well as pregnant and lactating women. The food distributed was either Leche-Avena (milk and oats) or Leche-Arroz (milk and rice). They were intended to prevent and control protein-energy malnutrition. Irregular and insufficient supplies prevented this program from being fully effective. Since the infrastructure for the manufacture of these products is already available, this program could be revived and strengthened. Additionally, the products could be fortified with appropriate amounts of iron and ascorbic acid. This would have the added advantage that with a marginal increase in cost, two nutrient deficiencies would be simultaneously controlled.

Alternatively, the successful Chilean strategy of distributing iron (hemoglobin) fortified cookies to school children can be adapted to preschool children in Ecuador. This may be less expensive than reviving the Leche program. Moreover, it may have the added advantage that its production and distribution can be targeted to those areas in the country with a high prevalence of anemia instead of to the entire country.

Irrespective of the food chosen for fortification, a critical factor in this strategy is adequacy of the food delivery system. This strategy should be considered only after a thorough assessment of the existing system (with or without modifications) shows that reasonably good distribution of the fortified food can be achieved. Product acceptability by the consumer also must be shown.

2. Pregnant women There is need for both long-term and short-term strategies for pregnant women.

Long-term: The long-term strategy envisages building women's iron reserves through early childhood and adolescence so that these reserves can fulfill the increased iron demands during pregnancy. This is best achieved through fortification of a suitable food. Possible vehicles for iron fortification are wheat flour, baked goods, pasta, sugar, and salt.

Both wheat flour and salt are consumed universally in Ecuador. Though most of the wheat is imported, it is centrally processed in a few mills and lends itself to fortification. Salt production is under total government control and it is potentially a suitable vehicle for fortification. More detailed information should be collected on the pattern of wheat flour consumption and salt intake in order to establish the appropriate level of iron fortification.

Bread consumption is rising steadily but before its suitability for fortification can be determined more detailed information is needed on the percentage of families that consume it, the socio-economic profile of consumers, intra-household consumption patterns, quantities eaten by consumers, and frequency of consumption.

Short-term: Fortified food, by itself, will not be able to meet the increased iron demands during pregnancy when body storage iron levels are often depleted. Thus there is a need for a short-term strategy to cover pregnant women who currently have poor iron stores and a high risk of developing IDA. Distribution of iron supplements is recommended for this purpose. This suggestion is made despite discouraging experiences in some countries. Three main reasons have contributed to the disappointing results: administrative weaknesses, inadequate distribution systems and poor subject

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compliance with tablet consumption. It should be possible to strengthen administration and distribution systems. Use of appropriate social marketing techniques may help improve compliance.

This strategy should be tried as a pilot project in a limited area before implementation at the national level. It was understood that two provinces--one coastal and one in the highlands--would soon be regionalized and consequently would have stronger health and other services. These provinces can be used to test the feasibility of a tablet supplementation strategy. In addition, it can be attempted in two or three hospitals which provide prenatal care for many pregnant women. Any project undertaken should include a motivation component as part of social marketing.

Currently, Ecuador has no community level program for the distribution of iron tablets to pregnant women, but pregnant women who come to hospitals for antenatal check-ups receive iron tablets. The regularity and frequency of iron tablet consumption is unknown. Coverage may be 40-50% of pregnant women who live close to hospitals.

As in the case of preschool children, this strategy of tablet distribution at the community level should be considered only after a thorough assessment of the existing or proposed health delivery systems in the two provinces.

The group discussed the possibility of the adoption of the Chilean strategy of distributing full-fat powdered milk fortified with high levels of iron. This program was effective in alleviating anemia in Chile however it was concluded that it is unlikely to work, because it would require very large quantities of imported milk.

3. Infrastructural set up and coordination: There are several agencies in Ecuador currently involved in nutrition activities. Apart from the nutrition unit of CONADE, these include the Nutrition Unit of the Ministry of Health (MOH), the Institute of Nutrition Research and Social Medicine (also of the MOH), the National Institute of Infant and Child (INFA) under the First Lady, the Ecuadorian Institute for Social Security under the Ministry of Social Welfare, and the Biochemistry and Nutrition Research Laboratory of the School of Medicine.

To integrate the programs and activities of all these units it is recommended that a national coordinating body should be organized, intersectional in composition, with active participation of both the academic and private sectors. The MOH could take the lead in this body, with the same ministry providing the secretariat. An alternative would be for the present Intersectional Committee of the National Program for Food Security to serve as the nucleus for such a body.

Each participating agency will be responsible for implementing its specific role in combatting anemia, employing primary health care systems at the community level when appropriate.

4. Overall strategy: The program for the control of IDA will have to be an integral part of a broader national nutrition program, under the coordination of the national coordinating body described earlier. As a first step, the body may be established through the initiative of CONADE. The plan and strategies, including definition of roles of the various participating agencies and guidelines for implementation, should then be formulated. The programs, though constituted horizontally at various levels, can be implemented vertically by the participating agencies. The strategy will need the involvement of existing community structures to maximize community participation.

5. Training of personnel: To develop the capacity for implementation of some of these strategies, and for subsequent monitoring and evaluation of programs, it is necessary to have ongoing training programs for functionaries at various levels. Existing facilities in schools of medicine, nutrition institutes, schools of nursing and dietetics, and postgraduate departments in universities can be used for this purpose. It is also necessary to instruct primary and secondary school teachers in nutrition education.

6. Monitoring and evaluation: The national coordinating body will take the overall responsibility for monitoring and evaluating programs. Implementation of the monitoring and evaluation systems may reside either with the nutrition unit of CONADE, which has already completed the National Nutrition Survey, or with the Institute of Nutrition Research and Social Medicine. The systems will include the periodic gathering and analysis of primary and secondary indicators of program benefits and effectiveness.

7. Nutrition education: The promotion of consumption of iron-rich foods can serve to supplement the strategies mentioned earlier. This can be pursued through both informal and formal channels under current information and education programs of various ministries and agencies. It is suggested that the promotion of consumption of iron-rich foods be integrated into exisiting programs, such as OTIDES. Under this program, health educators promote good health practices in schools.

Also, there is need for a formal nutrition education component in agricultural schools. This should have joint input by the ministries of health, education, and agriculture. The curriculum should provide adequate nutrition instruction so as to upgrade agriculture students and faculty in the nutritional aspects of food as well as food consumption practices.

SOCIAL MARKETING TO PREVENT ANEMIA

Chairman: Mr. Richard K. Manoff

Rapporteur: Dr. Michael Gurney

Members: Dr. Allan Forbes
Dr. Osman Galal
Dr. Adeline W. Patterson
Dr. Ernesto Pollitt

ASSUMPTIONS

INACG over the years has developed guidelines for the reduction of iron deficiency anemia (IDA) that can be used with adaptation in Ecuador.

These are as follows:

1. Focus on prevention of anemia.
2. Emphasize iron fortification* of a selected food substance.
3. Accept that iron supplementation* has value, especially in pregnancy and in other situations where no food vehicle for fortification is available. (Supplementation is ancillary to fortification in many situations because it has severe acceptance difficulties.)
4. Promote that breast feeding adequately protects the vast majority of infants from anemia in the initial 4 to 6 months and that the period of protection is shorter for infants weighing less than 2 1/2 Kg at birth.
5. Encourage nutrition/diet education to promote the use of foods rich in bioavailable iron and/or enhancers of iron absorption.
6. Develop preventive and therapeutic strategies.

FORTIFICATION

Fortification and the consumer

With most products fortification does not affect organoleptic or other factors that affect consumers. Thus

*In accord with recent INACG and WHO publications, the term fortification will be applied to the addition of a nutrient (iron) to a food, whereas supplementation will refer to use of that nutrient in liquid or tablet form.

there is no need to attempt to influence consumers and there is no need for social marketing. When a choice is available between fortified or unfortified products social marketing has an important role for influencing consumers. In general, it is better to avoid such choices so as to avoid difficulties with acceptance. Put in marketing terms there should be a "no-choice" strategy for both fortification of basic staples and fortification of foods for targeted groups.

Attempts to influence consumers are only necessary if resistance to the newly fortified product can be anticipated, for example, from religious, natural food, or political groups. In such cases social marketing may be necessary. However, this approach should not be taken if the problem is unlikely.

Influencing policy-makers

The program needs to be seen as contributing to objectives of major concern to the policy-makers. Examples could be linked to any political priorities concerning the rights of children, including, for example, the currently popular "child survival and development revolution," or improving mental and motor development of preschool and school children and learnihg at school, or safe motherhood.

Prevention of IDA itself may be an attractive objective for planners when compared with other competing objectives. The marketing positioning should therefore be limited to other possible consequences of IDA such as those mentioned above.

A major presentation is thus needed to policy-makers that includes the "no-choice" option and emphasizes that fortification does not involve any major disruption in administration, manufacturing processes, etc. This assumes that a vehicle for fortification is available that meets the technologic requirements, i.e., the vehicle must be centrally processed and is consumed by the targeted population on a frequent basis.

1. Government ministries: Advantages to each ministry's program need to be emphasized. For example, the ministry of education may be influenced by the better job satisfaction achieved by teachers teaching "brighter" children. Deal with each ministry's "own backyard" -- in short, appeal to each ministry's interest and responsibility.

   Emphasize the cost/benefits of iron programs. For example, at a relatively low cost, alleviating anemia in school children can result in improved school performance.

3. Other key influencers of policy decisions: The positions of high level gate-keepers vary between countries. They must be identified and influenced as a key part of the marketing strategy.

4. The industrial producers: It is usually best to start with the large producers, moving later to the small ones, and allowing them to advertise their products as "new" or "improved."

5. The scientific community: The scientific and technical nature of the data is important for influencing the scientific community. Presentation and use of these findings are of great importance. However, the quality and the quantity of such data may be as important as the manner and the style in which they are presented to influence policy makers.

SUPPLEMENTATION

Need for feed-forward research

The general use of the word nutritional "supplementation" is in the context of non-therapeutic use. However, an iron product for use in pregnancy can well be for both prevention and treatment. The approaches are concurrent. This involves active participation by the target group -- in marked contrast to fortification of basic staples.

Thus qualitative research is needed among the target population before deciding on a program (to estimate chances of success) and before embarking on the program (to help program design). This quantitative "feed-forward" research contrasts with the feedback that forms part of program monitoring and evaluation.

The focus group interview technique is a useful tool. It surfaces issues and questions particularly regarding attitudes and concepts and indicates new ones that require more complex studies. However well-informed, well-intentioned, sympathetic, or empathetic the program designer and however apparently simple the message, such an approach is desirable to minimize preventable errors in program decisions and design.

There is experience in these techniques in Ecuador and this should be tapped. The techniques are easy and accurate only after training and experience. They must be designed specifically to address the issue in question: in this case product acceptability and message design for supplementation therapy.

Message design

The insights gained by focus group interviews enable message design to be more directed, more relevant, and more likely to lead to the behaviorial change needed, i.e., active, continuing compliance in supplementation and therapy. Effective message design cannot be done without recourse to target group(s) participation through qualitative feed-forward research: in this case probably pregnant woman or women of child-bearing age.

Findings of such research need to be fed back to program planners for use in program improvement.

SOCIAL MARKETING STRATEGY DEVELOPMENT FOR SUPPLEMENTATION

On the basis of qualitative research with consumers:

1. Set specific objectives for the communications program to inform pregnant women of the importance of iron supplements and to motivate their acceptance and compliance.

2. Identify the geographical area for the venture and describe primary, secondary and tertiary target audiences and their component segments in terms of why they behave as they do and how to motivate the desired change.

   Examples in the health care delivery system include

   a. physicians,
   b. pharmacists,
   c. health workers, and
   d. social workers.

3. Analyze "resistance points" to supplementation, as uncovered in qualitative research, for means to deal with them and to resolve them.

   a. Analyze for implications on product characteristics and acceptability

   b. Develop into concepts and content elements for use in formulating

      i. message strategies,

      ii. media strategies, and

      iii. research strategies for in-process evaluation.

c. Identify positive facilitators who would enhance credibility and foster adoption of the desired behavior (e.g. authority figures, popular personalities, etc.).

d. Identify all possible delivery systems and support structures in the community for collaborating in the social marketing effort for message harmony

e. Determine media exposure habits of target groups and identify all appropriate media and media materials for message delivery

f. Determine research needs for in-process monitoring for the marketing component

NUTRITION/DIETARY EDUCATION

Social marketing for prevention of IDA cannot be carried out in isolation. It needs to be incorporated and/or linked into the nutrition and health education program(s). It is vital that social marketing objectives complement each other, and that their integrated methodologies are cost effective. The anemia prevention strategy can only succeed if it is integrated with related strategies without losing its specificity.

Furthermore nutrition/diet education of the public for adequate dietary intake of iron and ascorbic acid assures that there is an ongoing effort to direct dietary behavior in the right direction. Over time, the achievement of this goal would significantly reduce the need for therapeutic approaches to this problem, thus reducing the burden on the health care delivery system.

SOCIAL MARKETING STRATEGY DEVELOPMENT FOR THERAPY

Follow the same steps as described above in Social Marketing Strategy Development for supplementation with emphasis, of course, on concepts and content related to nutrition/diet education.

In addition to these considerations of fortification, supplementation, and nutrition/diet education, the need for therapy strategies remains of vital importance. A social marketing component is needed to support this therapy strategy aimed at both policy-makers and potential consumers and should be designed in accordance with social marketing concepts already discussed.

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PRIME SOCIAL MARKETING TARGETS FOR THE DIFFERENT APPROACHES IN ANEMIA PREVENTION AND CONTROL

Strategy	Target Group					Example
	Scientific Community	Policy Makers	Industry	Health Care Delivery Systems	Consuming Public	
Fortification of a basic staple	X	X	X			Wheat flour
Fortification of products for target groups	X	X	X	X	X	Weaning foods
Supplementation (Therapy)	X	X	X	X	X	Iron tablets/ capsules
Nutrition/ Diet Education	X	X		X	X	Iron & Vitamin C in diet
Example:	Hematologists, Nutritionists, Pediatricians, Obstetricians	Government, Ministries, "Gatekeepers"	Food and pharmaceuticals, flour millers, cereal manufacturers	PHC workers, nurses, pharmacists, physicians, educators, social workers	At-risk groups, primarily young children and women	

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Revised 23 September, 1987

LIST OF PARTICIPANTS
1987 ANNUAL INACG MEETING
QUITO, ECUADOR
12 MAY - 15 MAY

Aguirre, Dr. Harold
Contraloria General del Estado
Departamento de Salud
Calles Montalvo s/n y 6 de Diciembre
Quito, Ecuador
Telephone: 239-220

Alvarez, Dr. Marco
MEGRAME
Ministerio de Salud
Juan Larrea 446 y Riofrio
Quito Ecuador
Telephone: 521-411

Andrade, Economista Beatriz
CONADE
Edificio Consejo Provincial
P. O. Box 21-92
Arenas y Manuel Larrea
Quito, Ecuador
Telephone: 563-666 (office)
435-354 (home)

Becker, Dr. Roberto Augusto
Special Programs Secretary
INAN
SQS 316/BLI/AP 202
70.387 Brasilia, DF, Brazil
Telephone: 061-273.42.01

Blanco, Dr. Boris
Representante UNICEF
Apartado Postal 0 134 CEQ 16
Quito, Ecuador
Telex: 1208 UNICEF ED

Bonilla, Dra. Rosario
Servicio de Hematologia y
Banco de Sangre
Hospital Andrade Marin IESS
Calles Portoviejo y Ayacucho
Quito, Ecuador
Telephone: 526-144

Calle, Dr. Andres
Laboratorio de Investigaciones de Bioquimica
Facultad de Medicina - Universidad Central
Iquique y Sodiro s/n
Quito, Ecuador
Telephone: 528-810

Chichester, Dr. C. O.
Director International Programs
INACG Secretariat
1126 Sixteenth Street, N.W.
3rd Floor
Washington, DC 20036
U.S.A.
Telephone: 202-659-9024

Clark, Dr. Douglas
CARE
Colon 237 y Yanez Pinzon
Quito, Ecuador
Telephone: 234-550

Dallman, Dr. Peter
Professor
School of Medicine
Department of Pediatrics
Box 0106
University of California - SF
San Francisco, CA 94143
U.S.A.
Telephone: 415-476-3079

Darquea, Dr. Luis
Gualaquiza No. 941
Quito, Ecuador
Telephone: 532-068

Daza, Dr. Carlos
Coordinator
Food and Nutrition Programs
PAHO/WHO
525 Twenty-third Street, N.W.
Washington, DC 20037
U S.A.
Telephone: 202-861-4348

DeMaeyer, Dr. Edouard M.
Nutrition Consultant
World Health Organization
Avenue Appia
1211 Geneva 27
Switzerland
Telephone: 22-913326

Dirren, Dr. Henri M.
Head, Human Nutrition Section
Nestlè Research Department
P. O. Box 353
1800 Vevey
Switzerland
Telephone: 021-33-86-24

Enriquez, Dr. Jorge
Director
CONADE
P. O. Box 537-C
Quito, Ecuador
Telephone: 563-666

Esteves, Dr. Edmundo
Facultad de Medicina
Universidad Central
Calles Iquique y Sodiro
Quito, Ecuador
Telephone: 528-810

Estrella, Dr. Ramiro
Professor de Bioquimica
Universidad Central
Iquique y Sodiro s/n
Quito, Ecuador
Telephone: 528-810

Florentino, Dr. Rodolfo
Director
Food and Nutrition Research Institute
Pedro Gil Street corner Taft Avenue
Ermita, Manila
Philippines
Telephone: 59-49-98

Freire, Dra. Wilma B.
Chief of the Nutrition Unit
National Development Council
CONADE
P. O. Box 537-C
Quito, Ecuador
Telephone: 563-666 ext. 182 Or 328

Forbes, Dr. Allan
Director
Office of Nutrition and Food Science
Center for Food Safety and
Applied Nutrition
Food and Drug Administration (FDA)
200 C Street, S.W.
Washington, DC 20204
U.S.A.
Telephone: 202-245-1561

Galal, Dr. Osman
Director
Nutrition Institute
16 Kasr Aini Street
Cairo, Egypt
Telephone: 847476

Gatel, Dr. Marie
CARE
P. O. Box 2097
Quito, Ecuador
Telephone: 231-574

Granda, Dr. Edmundo
CEAS
Edificio Cadena
Luis Sodiro y Cadena
Quito, Ecuador
Telephone: 554-897 (office)
243-039 (home)

Grijalva, Dr. Jaime
Departamento de Hematologia
Hospital Carlos Andrade marin
Portoviejo y Ayacucho
Quito, Ecuador
Telephone: 526-144

Goldman, Dr. William
AID/Ecuador
Edificio Computec
Avenida Colombia 1573
Quito, Ecuador
Telephone: 238-790; 521-100

Gurney, Dr. Michael
Senior Program/Officer (JNSP)
Nutrition Unit
World Health Organization
Avenue Appia
1211 Geneva 27
Switzerland
Telephone: 91-33-17

Hertrampf, Dra. Eva
Hematology Unit
INTA
Universidad de Chile
Casilla 15138
Santiago 11, Chile
Telephone: 2215962

Jacome, Dr. Diana
Edificio de la Cruz Roja
Ave. Colombia 110 y Elizalde
Quito, Ecuador
Telephone: 233-894; 215-998

Kahn, Dr. Samuel G.
Senior Nutrition Advisor
Office of Nutrition
Agency for International Development
Room 324
Department of State
Washington, DC 20523
USA
Telephone: 713-235-9087

Lindsay, Laurie, R.D.
INACG Secretariat
The Nutrition Foundation
1126 Sixteenth Street, N.W.
3rd Floor
Washington, DC 20036
U.S.A.
Telephone: 202-659-9024

Lopez, Dr. Patricio
Laboratorio de Investigaciones
Facultad de Medicina
Iquique y Sodiro s/n
Quito, Ecuador
Telephone: 528-690 ext. 165

Manoff, Dr. Richard
President
Manoff International
950 3rd Avenue, 23rd Floor
New York, NY 10022
U.S.A.
Telephone: 212-758-7710

Molina, Dr. Eduardo
Ministerio de Salud Publica
Juan Larrea 446 y Riofrio
Quito, Ecuador
Telephone: 521-411

Nelson, Dr. David
Consultor de Premi
Ministerio de Salud
Buenos Aires 340 y Manuel Larrea
Quito, Ecuador
Telephone: 550-708

Olivares, Dr. Manuel
Hematology Unit
INTA
Universidad de Chile
Casilla 15138
Santiago 11, Chile
Telephone: 2215962

Ortega, Dr. Fernando
Director ININMS
Ministerio de Salud
Buenos Aires 340 y Manuel Larrea
Quito, Ecuador
Telephone: 544-597 (office)

Paez, Dr. Ruben
Punaes No. 906 y Cestaris
Quito, Ecuador
Telephone: 210-512 (home)

Patterson, Dr. Adeline W.
Director - CFNI
Caribbean Food and Nutrition
Institute
P. O. Box 140
Kingston 7, Jamaica
Telephone: 809-92-71108

Pettigiani, Dr. Carlos
Representante de la OPS/Ecuador
Apartado Postal 8982
Sucursal 7
Avenida Naciones Unidas 1204 - 4º Piso
Quito, Ecuador
Telephone: 456-800; 459-609
Telex: 2370

Pollitt, Dr. Ernesto
Professor of Human Development
Department of Apride Behavioral
Science
University of California, at Davis
Davis, CA 95615
U.S.A.
Telephone: 916-752-6156

Rao, Dr. B. S. Narasinga
Director
National Institute of Nutrition
Indian Council of Medical Research
Hyderabad 500 007
India
Telephone: 71083

Robles, Dr. Roberto
Director Medico
Abbott Laboratorios del Ecuador
Via Duran Tambo, Km. 4-1/2
Guayaquil, Ecuador
Telephone: 801-531

Rodriguez, Sr. Dr. Eduardo
Director, Relaciones Internacionales
Ministerio de Salud Publica
Quito, Ecuador

Sacoto, Dr. Fernando
SAVE THE CHILDREN
Ricardo Jarmillo 439
Quito, Ecuador
Telephone: 247-197

Sghirla, Dr. Juan
Presidente de la Sociedad
Ecuatoriana de Hematologia
Edificio Cruz Roja Ecuatoriana
P. O. Box 2119
Avenida Colombia y Elizalde 110
Quito, Ecuador
Telephone: 215-998

Srikantia, Dr. S. G.
Honorary Professor
P. G. Department of Foods and Nutrition
130 Matagalli P. O.
Mysore 570 016 India
Telephone: 25104

Sutton, Dr. Sharyn
Vice President
Doremus-Porter-Novelli
1001 30th Street, N.W.
Suite 200
Washington, DC 20007
U.S.A.
Telephone: 202-342-7000

Troya, Dr. Guillermo
Consejo Nacional de Ciencia y Tecnologia
CONACYT
Avenida Patria y 10 de Agosto - 9º Piso
Edificio Banco de Prestamo
Quito, Ecuador
Telephone: 550-735; 550-553

Vallejo, Dr. Francisco
Save the Children Alliance
P. O. Box 9317
Sucursal 7
Avenida 6 de Diciembre 2256
Quito, Ecuador
Telephone: 262-955
Telex: 21016 DEJUCO ED

Walter, Dr. Tomas
Head, Hematology Unit
INTA
Universidad de Chile
Casilla 15138
Santiago 11, Chile
Telephone: 2215962

Wahli, Dr. Christian
Gerente
LATINREFCO
Cp 6053 CCI
Quito, Ecuador
Telephone: 355-886

Weilbauer, Dr. Frank
Apartado Postal 6260
Quito, Ecuador
Telephone: 242-177; 235-101
Telex: 2662 CRUZROJA

Yepez, Dr. Rodrigo
Decano Facultad de Medicina
Universidad Central
P. O. Box 8105
Sucursal 8
Calles Iquique y Sodiro
Quito, Ecuador
Telephone: 526-024

Zhang, Dr. Zhi Nan
Professor of Medicine
Chief, Division of Hematology
Peking Union Medical College Hospital
1 Shuaifuyuan
Beijing, China
Telephone: 553731 ext. 303 or 745

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Final Agenda
X INACG Meeting
Quito, Ecuador
12-15 May, 1987
Location: Inter-Continental Hotel, Quito

12 May, Tuesday - Morning
09:30

I. Welcome
Introductions - Edouard DeMaeyer, Chairman, INACG
Director of CONADE - Jorge Enriquez
Director of International Relations, Ministry of Health - Eduardo Rodriguez
Director of Nutrition, CONADE - Wilma Freire
Chairman, INACG - Edouard DeMaeyer
Representative of U.S. AID Mission - William Goldman
Representative of LATINRECO - Christian Wahli
Representative of PAHO - Carlos Daza

BREAK 10:30 - 10:50

Approval of agenda and announcements

II. Review of INACG Workshop Report on "Social Marketing" of Iron Deficiency Anemia and Discussion of Implementation
- Sharyn Sutton

III. Review and Discussion of INACG Iron Bioavailability Research Project
- Allan Forbes

LUNCH 12:00 - 13:30 On your own

12 May, Tuesday - Afternoon
13:30

IV. Reports on Ensuring Safe Motherhood and Infant Well-being through Prevention of Iron Deficiency
(20 min each)
A. Assessment of iron deficiency in infants and children
- Peter Dallman
B. Iron in infant and child development
- Tomas Walter

BREAK 15:00 - 15:30

C. Iron deficiency and school performance
- Ernesto Pollitt

D. Iron deficiency in pregnant women
   - Wilma Freire
E. The effect of iron on pregnancy outcome
   - Rudolpho Florentino
F. The effect of a therapeutic trial of iron on the assessment of iron status in a field trial
   - Eva Hertrampf

(Questions/discussion including announcement by Edouard DeMaeyer regarding Safe Motherhood Document)

RECEPTION 19:00 - 21:00 - Inter-Continental Ballroom, hosted by LATINRECO

13 May, Wednesday - Morning
09:00

V. Presentations of Country Strategies or Programs (15 min each)
   A. Chile - Tomas Walter
   B. Egypt - Osman Galal
   C. India - S.G. Srikantia
   D. India - B.S. Narasinga Rao
   E. Philippines - Rodolfo Florentino

BREAK 10:40 - 11:00

   F. Caribbean - Adeline Wynante Patterson
   G. China - Zhi Nan Chang

LUNCH 12:00 - 13:30 On your own

13 May, Wednesday - Afternoon
13:30

VI. Ecuador National Nutrition Survey

   A. Report of the Ecuador National Nutrition Survey
      - Wilma Freire - Design
      - Henri Dirren - Biochemical/Data Analysis Process and Management

BREAK 15:30 - 15:50

   B. Discussion of the implications of the survey results
      - Wilma Freire - Preliminary results
      - Henri Dirren - Infection/Inflammation

14 May, Thursday - Morning
09:00

VII. Discussion of the Guidelines and Recommendations in Developing Strategies
   - Edouard DeMaeyer

VIII. Background for Strategy Development
- Samuel Kahn

IX. Role of PAHO and WHO in the Implementation of Latin American Strategies for Nutritional Anemia Programs
- Carlos Daza

X. Concepts of Social Marketing
- Richard Manoff

BREAK 10:30 - 10:50

XI. Workshop on Development of a Strategy for Andean Countries to Control Iron Deficiency Anemia within Programs to Increase Awareness of Maternal Health and Child Well-being
Chair: Michael Gurney
A. Working groups
1. Technical
Chair: Tomas Walter
2. Marketing
Chair: Richard Manoff
3. Operational
Chair: Rodolfo Florentino

LUNCH 12:00 - 13:30 On your own

14 May, Thursday - Afternoon

XII. Andean Country Strategy Development (continued)

B. Discussion and consensus statements by working groups
1. Technical - Tomas Walter
2. Marketing - Richard Manoff
3. Operational - Rodolfo Florentino

TOUR LATINRECO and Quito 14:30 - 18:00

15 May, Friday - Morning
09:00

XIII. Workshop for Development of a Global Strategy to Control Iron Deficiency Anemia and Increase Awareness of Maternal Health and Child Well-being
Chair: Edouard DeMaeyer
A. Working groups
1. Technical - Rodolfo Florentino
2. Marketing - Richard Manoff
3. Operational - S.G. Srikantia

BREAK 10:30 - 10:50

LUNCH 12:00 - 13:30

15 May, Friday - Afternoon
13:30

B. Discussion and consensus statements by working groups
   1. Technical - Rodolfo Florentino
   2. Marketing - Richard Manoff
   3. Operational - S.G. Srikantia

BREAK 15:00 - 15:20

XIV. Concluding Remarks

TOUR of city and Convent de San Diego 19:00

DINNER at Convent de San Diego, hosted by Coca-Cola of Ecuador
20:00

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