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CONTROL OF IRON DEFICIENCY

DSAN-C-0045

University of Kansas Medical Center

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Narrative Summary

The objective of this AID contract is to develop intervention strategies to combat nutritional iron deficiency. The following five areas of activity were proposed in the original contract: (1) factors affecting iron bioavailability of food, (2) iron availability from diets in developing countries (LDC diets), (3) development of iron fortification systems, (4) food technology, and (5) pilot fortification programs. With few exceptions, these studies involved measurements of radioiron absorption in man. A total of four hundred and sixty iron absorption measurements have been performed during the past year, using 115 volunteer subjects.

In the area of factors affecting iron bioavailability of food, investigations of egg protein and soy products were extended. Additional evidence was obtained indicating that fresh egg white contains a heat labile inhibitor of iron absorption, the effect of which is increased by the spray-drying process which yields egg albumen. With reference to soy protein, it was shown that the soy component of the infant food supplement corn-soya-milk has an appreciably greater inhibitory effect on iron absorption than do the corn or milk components of this food. This area of

research was extended by examining the effect of two new classes of dietary constituents on iron absorption. The fiber component was studied by measuring the effect of three fiber sources: bran, cellulose, and pectin. Only bran showed an appreciable inhibitory effect. Finally, possible interaction with the lipid component of a meal was examined in semisynthetic meals containing corn oil, soy oil, coconut oil, or butter. Iron was less well absorbed in the presence of soy oil and coconut oil.

The second major area of investigation related to the development of fortification systems. Our extensive studies designed to characterize the properties of NaFeEDTA (reported in the previous annual report) were completed and further evidence of the advantage of NaFeEDTA in inhibitory meals was obtained by demonstrating that NaFeEDTA was better absorbed than ferrous fumarate when used as the iron supplement in CSM. On the other hand, we have previously found that EDTA blocks the enhancing effect of ascorbic acid on nonheme iron absorption. However, contrary to our earlier observations, the enhancing effect of meat appears to be unaffected.

The use of ascorbic acid remains an important means of improving iron absorption. Initial observations (previously reported) suggested that ascorbyl palmitate, a more stable fatty acid ester of ascorbic acid, might have an advantage in baked food products. However, a study carried out during the current year demonstrated no difference between ascorbic acid and ascorbyl palmitate.

An important potential delivery system for fortification iron was studied. The absorption of iron from rice coated with ferrous sulfate in a manner which makes fortified grains indistinguishable from unfortified rice in the uncooked state was

measured. It proved possible to design a coating that ruptured during the cooking process, releasing iron into the cooking water. The iron was then reabsorbed into the total rice pool in the meal. While these studies demonstrate the feasibility of coating rice with iron, one disturbing observation was made. It appears that iron absorbed into rice may be poorly absorbed and relatively little affected by enhancers of iron absorption present in the meal. This is regarded as an important area for further investigation.

In studies of iron availability from diets in developing countries, diets typical for Egypt and for Thailand were studied. The quantity of iron absorbed in each case was less than that required to maintain normal iron balance.

A. General Background

Iron deficiency anemia is widely recognized as the most prevalent nutritional deficiency in the world. While the impact of the anemia which accompanies iron lack has not been fully defined, severe iron deficiency is known to impair work capacity, increase the risk of maternal and infant morbidity and mortality, reduce resistance to infection, and impair the physical and mental growth of school-age children.

Two major approaches have been discussed as intervention strategies to combat iron deficiency anemia. The most direct approach is iron supplementation or the supply of medicinal iron to children or pregnant women. However, iron supplementation programs are costly to administer and usually fail due to lack of compliance. An alternative to iron supplementation is some form of dietary manipulation; either adding more iron to the diet (iron fortification) or adding a substance that increases food iron availability (enhancement fortification). A critical question at present concerns the efficacy of either or both of these procedures because their effectiveness has never been clearly established. Furthermore, because no single method of iron delivery will be suitable in all LDCs, several fortification systems should be made available which could be applied in specific situations with minimal modification.

The purpose of the present project is to develop methods of iron delivery that would be suitable for field testing and eventual implementation on a national level. The optimal approach will depend on the level of dietary iron and the degree of its availability. At one extreme, diets with low-iron content but high availability would be best suited to iron fortification. On the other hand, a diet with high content but low assimilation would be more suitable for enhancement fortification. The best strategy may be to employ both of these approaches in tandem.

B. Statement of Project Objectives

The development of an effective food intervention strategy to combat iron deficiency requires consideration of the absorption of a particular form of iron, its stability when added to a particular food vehicle, and the effect that it may have on the vehicle itself. The selected food vehicle must be consumed in sufficient quantities by the deficient population, be compatible with the form of added iron, and be centrally processed to permit adequate regulation of the fortification process.

Before selecting a particular fortification strategy, a number of studies must be conducted in human subjects with radioisotopes to determine basal iron availability of the diet, to measure absorption of fortification iron, and to estimate the gain in iron balance that would be achieved with implementation of the strategy. Field trials will eventually be required to establish the effectiveness of a given strategy but such trials are costly and should be limited to those approaches that appear most promising.

The present project as originally submitted was to conduct research in 5 closely related areas:

(1) Evaluation of factors affecting food iron availability.

(2) Measurement of iron availability from LDC diets.

(3) Development of effective systems of iron or enhancement fortification.

(4) Food technology studies to establish iron/vehicle compatibility.

(5) Pilot fortification trials to measure the efficacy of the intervention strategy.

There has been no major change in the basic objectives as defined in the original contract. Efforts in developing iron fortification systems depend largely on isotopic studies of food iron absorption. These studies are still the core of the present contract. As compared with the initial year, a much greater investment was placed during the second year of this contract in the development of delivery systems for iron fortification although factors affecting food iron availability continue to be of interest. Studies were initiated during the past year to measure iron availability from diets in two LDC's, Egypt and Thailand. Other areas of potential collaboration of this type include Guyana, the Caribbean, and Cameroon.

C. Accomplishments to Date

The body of this progress report is contained in the following appendices:

Appendix A: Iron absorption studies
Appendix B: Publications
Appendix C: LDC collaborations

A detailed description of 12 separate iron absorption studies conducted during the past year are contained in Appendix A. The studies are presented under the headings of Summary, Rationale, Design, Results, and Comment. The following is a list of these studies:

No.	Pg.	Subj.	Title
27	25	9	Iron availability from rice coated with ferrous sulfate. I.
28	32	8	Evaluation of NaFeEDTA as the iron supplement for CSM.
29	39	9	Effect of fiber on iron absorption. I.
30	45	7	Iron availability from Egyptian meals.
31	52	13	Effect of ascorbyl palmitate of iron availability from muffins containing soy flour.
32	58	11	Effect on iron absorption of cooking spray-dried or fresh egg white.
33	65	11	Effect of fats on iron absorption.
34	72	10	Availability from iron fortified rice. II.
35	78	9	Effect of fiber on iron absorption. II.
36	84	12	Effect of beef on iron absorption from $FeCl_3$ and NaFeEDTA.
37	91	10	Effect of components of CSM on iron absorption.
38	98	6	Iron availability of Thai meals.

Five of the twelve studies performed during the past year were directed at identifying dietary factors affecting food iron bioavailability. Two of these represent extensions of investigations carried out in the first and second years and were designed to answer some of the questions raised in those studies. Evidence that conalbumin, a constituent of egg albumen, inhibits nonheme iron absorption was obtained in previous studies, and the conclusion drawn in last year's report was that the results were consistent with a modest inhibitory effect of the protein. Cooking destroys the iron binding properties of conalbumin, but in these earlier studies difficulty was encountered in ensuring proper cooking of the spray-dried material while maintaining sufficient palatability to allow its inclusion in a meal. In Study 32 these investigations were extended to examine the effect of cooked and uncooked fresh egg white and egg albumen (spray-dried egg white). The results indicate that egg white contains a heat labile inhibitor of iron absorption and that the inhibitory effect is accentuated by the spray-drying process. The iron binding properties of conalbumin suggest that this is the factor involved. The heat labile nature of the effect would make egg white unlikely to be of importance in most normal diets.

Studies demonstrating the inhibitory effect of soy products on iron absorption have been described in detail in previous annual reports. Current studies were an extension of our original observation that iron availability from the infant food supplement corn-soya-milk (CSM) is very low. The role of soy in contributing to the low availability of this supplement was examined in Study 37. Inorganic calcium and phosphorous present in the product are in part responsible for low iron bioavailability. However, when calcium and phosphorous are omitted, absorption remains relatively low. In Study 37 we examined the effect of selectively omitting the major components of CSM. The omission of soy resulted in a twofold increase in iron absorption

while the deletion of corn or milk had no significant effect. It was concluded that soy was the major inhibitor present. It should be noted, however, that even when soy was omitted, percentage absorption remained relatively low. This study stresses the need for careful evaluation of iron availability in products like this and for a clear definition of practical methods for improving the amount of iron absorbed. The omission of soy from infant food supplements is not a practical consideration because it is an important source of high quality protein. Further study of the nature of its inhibitory effect on iron absorption is necessary to determine the best way of ensuring adequate iron nutrition in people consuming large quantitites of soy products.

Two new important aspects of the effect of dietary factors on food iron availability were examined during the past year. The first related to the possible effects of fiber on iron absorption. It has been assumed in the past that fiber would be inhibitory to iron absorption. In studies 29 and 35 the effect of three fiber sources, bran, cellulose, and pectin, was tested. Both studies demonstrated essentially similar effects although absorption in the absence of fiber in Study 29 was very low, thereby reducing the sensitivity of this study for detecting an inhibitory effect. Bran caused significant inhibition in Study 35. However, the purified fiber sources cellulose and pectin had no effect. This is an important observation and suggests that the inhibitory effect previously associated with fiber may be limited to specific fiber sources. Alternatively, the inhibition of iron absorption may be related to a factor(s) associated with fiber rather than fiber itself. The effect of fiber requires more detailed evaluation.

Although considerable attention has been paid to the interaction between various protein sources and iron absorption, little

effort has been devoted to the examination of the effect of the other major components of the diet. In Study 33 the semi-synthetic meal was used to evaluate the influence of four fat sources with widely varying composition. The highest absorption values were obtained with butter and corn oil, when soy oil or coconut oil were used, absorption was significantly less. This initial study suggests that the fat component of the diet may also influence iron absorption. However, at this stage, the possibility of protein or carbohydrate contamination of these oil sources cannot be excluded since both the soy oil and coconut oil were prepared by a cold pressing procedure. It is planned to further evaluate the possible influence on fats by using lipid sources prepared by solvent extraction.

The second group of investigations were directed at the development of iron fortification systems. Two studies were done to complete the series of investigations designed to define precisely the advantages and/or disadvantages of NaFeEDTA for iron fortification. This form of iron is still of key importance because of its use in fortification of sugar in field studies in Guatemala and because it can be added to food without unwanted reactions such as discoloration and rancidity. In Study 28 the possibility of substituting NaFeEDTA for ferrous fumarate in CSM was examined. It has previously been demonstrated that NaFeEDTA tends to be better absorbed than other iron salts from meals containing inhibitory factors. In this study, NaFeEDTA was significantly better absorbed than ferrous fumarate. While NaFeEDTA would lead to an appreciable improvement in iron assimilation, iron in this product would still be relatively unavailable.

It was reported last year that while ascorbic acid produces a sharp increase in iron absorption from most iron salts, it has little effect on NaFeEDTA. This would place severe limitations

on its usefulness in diets containing large quantities of ascorbic acid. In an extension to these studies, the effect of beef, another important enhancer of iron absorption, on NaFeEDTA was examined in Study 36. Unlike ascorbic acid, 100 g beef produced a 3.2-fold increase in iron absorption from an SS meal containing NaFeEDTA. This improvement in iron absorption was very similar to the 3.3-fold increase observed with $FeCl_3$ indicating that NaFeEDTA is a satisfactory form of iron for fortifying meat-containing meals. These studies emphasize the importance of examining the basal availability of iron in the diet and the nature of enhancing or inhibiting factors present before choosing NaFeEDTA for fortification in any given geographic region.

Ascorbic acid is a powerful promoter of nonheme iron absorption and could play an important part in iron fortification systems by improving iron availability of both fortification iron and iron present in the food. However, it has two major disadvantages, cost and stability. Ascorbyl palmitate, a fatty acid ester of ascorbic acid, could have practical advantages since it is not as rapidly oxidized during heating (e.g., baking) as ascorbic acid. Whereas ascorbic acid baked into bread is ineffective in improving iron absorption, ascorbyl palmitate was previously shown to have a modest influence. In Study 31 the possibility of improving iron absorption from soy-enriched wheat flour by using ascorbyl palmitate was examined. Ascorbyl palmitate and ascorbic acid were baked into separate batches of soy-enriched wheat flour muffins. Neither demonstrated any improvement in iron absorption. These studies suggest that ascorbyl palmitate has no appreciable advantage over ascorbic acid with regard to the deleterious effects of baking on the enhancement of iron absorption.

An important aspect of this year's investigation was the study of rice as a potential delivery system for fortification iron. Widespread use of rice as a staple in many developing countries reinforces the relevance of considering it as a potential vehicle. In studies 27 and 34, the value of a new rice-coating procedure was examined. This method makes it possible to coat rice with ferrous sulphate in a manner that makes it indistinguishable from unfortified rice in the uncooked state. Moreover, the coating is not dissolved when the rice is washed as is the common practice in Southeast Asia. The method for preparing rice in Southeast Asia employs the use of sufficient rice to absorb the total quantity of water used for cooking. The coating is designed to rupture during cooking thereby releasing the fortification iron into the water for absorption into all the rice grains present in the meal. Therefore, iron absorption would not be affected materially if the consumers removed the coated rice grains because of a residual change in appearance. In Study 27 iron coated onto the rice was far less available for absorption than that in the common pool. Moreover, its absorption was not improved by the presence of enhancers of iron absorption. This was attributed to the use of a coating that failed to release the iron during cooking.

In Study 34 a thinner coat was used which ruptured during cooking and released the fortification iron. In a meal composed solely of rice, iron from the coated grains was absorbed to exactly the same extent as iron added to the cooking water, indicating that the fortification rice had entered the common nonheme pool. However, when the absorption of iron cooked onto the rice was compared with iron added to the meal after the preparation of the rice, a marked difference in absorption was encountered. This observation suggests that it is possible that iron cooked into

rice does not enter the nonheme common pool and is therefore less available for absorption. This finding would have major implications for our understanding of iron deficiency in rice-consuming populations and be of considerable importance in the design of fortification strategies. It is planned to extend these studies during the coming year to define the effect of rice on the nonheme common pool.

The third objective of studies conducted during the past year was the assessment of iron bioavailability in the diets of developing countries. Meals from two further countries were studied. In Study 30 iron absorption was measured from three typical Egyptian meals. Values of 1.41, 1.45, and 1.49% were obtained. From the iron content of these meals, it was calculated that the daily intake of iron would be approximately 10 mg and that people with absent iron stores would be expected to absorb only 0.3 mg nonheme iron daily. In the Egyptian diet, containing little or no meat, this amount of iron would not maintain normal iron balance. The absorption of iron from three typical Thai meals was measured in Study 38. Percentage absorption from these meals was relatively good (2.41, 4.95, and 6.22%), which was presumably the result of the presence of meat in two of the meals and a comparatively high ascorbic acid content in the other. Despite the relatively high percentage absorption, the very low iron content of these meals meant that the quantity of iron assimilated would again be insufficient to meet dietary iron needs. It is planned to conduct studies in Egypt and Thailand using similar meals to study iron absorption in volunteers drawn from the native population. Our preliminary studies suggest that major attention should be directed towards improving the availability of iron in the Egyptian diet whereas the primary problem in the Thai diet appears to be an inadequate quantity of iron.

D. Dissemination and Utilization of Research Results

The findings that have been developed under this contract have been communicated through formal presentations at national and international meetings, through exchange on an informal basis between investigators visiting the iron nutrition unit in Kansas City, and by reciprocal visits by investigators in LDCs. The following is a summary of these various avenues of communication.

1. Presentations

a. Dr. Skikne presented a poster display on the "Inhibition of Nonheme Iron Absorption by Cimetidine" at the 18th Congress of the International Society of Hematology in Montreal, Canada; August 1980.

b. Dr. Cook presented an overview of the findings obtained during the first two years of the contract at the annual meeting of the International Nutritional Anemia Consultative Group (INACG). Talks entitled "Food Iron Absorption: An Update" and "Quantitative Measurements of Iron Status" were presented by Dr. Cook at the meeting in Bangkok, Thailand; October 1980.

c. Dr. Cook spoke on "Food Iron Availability" and "Uptake on Iron Nutrition in Man" to the Department of Foods and Nutrition at Kansas State University; January 1981.

d. Dr. Cook presented a paper entitled "Dietary Inhibitors of Iron Nutrition" at the Florida Symposium on Micronutrients in Human Nutrition held at the University of Florida; February 1981.

e. Dr. Cook presented a paper entitled "Effects of Protein on Iron Absorption in Man" at the American Chemical Society annual meeting in Atlanta, Georgia; March 1981.

f. Dr. Morck presented a paper entitled "Iron Availability from Infant Food Supplements" at the Federation of the American Societies for Experimental Biology (FASEB) annual meeting in Atlanta, Georgia; April 1981.

g. Dr. Cook attended "The Control of Anemia in the Commonwealth Caribbean" Technical Workshop in Kingston, Jamaica at which he presented a talk on "Fortification in the Control of Anemia"; June 1981.

h. Dr. Cook presented a paper entitled "Biochemical Determinants of Food Iron Absorption" at the XII International Congress of Nutrition in San Diego, California; August 1981.

i. Dr. Morck presented a paper entitled "Inhibition of Food Iron Absorption by Soy Products" at the XII International Congress of Nutrition in San Diego, California; August 1981.

j. Dr. Lynch presented a paper entitled "An In Vitro Method for Predicting Food Iron Availability" at the Fifth International Conference on Proteins of Iron Storage and Transport in La Jolla, California; August 1981.

2. Scientific Publications

The following articles have been prepared during the third year of this contract and are in the process of being published (as indicated). Preprints are included in Appendix B.

a. Cook JD, Morck TA, Skikne BS, Lynch SR. Dietary inhibitors of iron absorption in man. Academic Press (in press).

b. Morck TA, Lynch SR, Skikne BS, Cook JD. Iron availability from infant food supplements. Am J Clin Nutr (in press).

c. Cook JD, Morck TA, Lynch SR. The inhibitory effect of soy products on nonheme iron absorption in man. Am J Clin Nutr (in press).

d. Cook JD, Morck TA, Lynch SR. Biochemical determinants of iron absorption. Elsevier-North Holland (in press).

e. Lynch SR, Cook JD, Morck TA. In vitro measurement of food iron availability. Elsevier-North Holland (submitted).

f. Morck TA, Lynch SR, Cook JD. Reduction of the soy-induced inhibition of nonheme iron absorption. Am J Clin Nutr (submitted).

g. Lynch SR, Morck TA, Cook JD, Kibalo SM. In vitro estimation of nonheme food iron availability. Br J Haem (in preparation).

h. Morck TA, Lynch SR, Cook JD. Effect of coffee on food iron absorption. Am J Clin Nutr (in preparation).

3. Scientific Exchange

The following individuals visited our research laboratory at the University of Kansas Medical Center during the third year of the contract.

a. Malee Sritongkul, Division of Hematology, Siriraj Hospital, Mahidol University; Bangkok, Thailand; July 1980-June 1981.

b. Chaim Hershko, M.D., Department of Hematology, Shaare Zedek Hospital; Jerusalem, Israel; August 1980.

c. Fawzi El-Shobaki, Ph.D., Nutrition Laboratory, National Research Center; Cairo, Egypt; September-October 1980.

d. Gumpei Urata, M.D., Chief, Department of Nutrition and Biochemistry, Institute of Public Health; Tokyo, Japan; 10-12 March 1981.

e. Sukanya Linpisarn, Senior Medical Technologist, Research Institute for Health Sciences, Chiang Mai University; Chiang Mai, Thailand; April-July 1981.

f. V. I. Mathan, M.D., Ph.D., Head, Wellcome Research Unit, Christian Medical College Hospital; Vellore, India; May 1981.

g. Khruawon Khaikham, Medical Technologist, Siriraj Hospital, Mahidol University; Bangkok, Thailand; May-November 1981.

h. Romsai Suwanik, M.D., Professor and Chairman, Department of Radiology, Siriraj Hospital, Mahidol University; Bangkok, Thailand; June 1981.

E. Statement of Expenditures and Obligations

Category	Total Amt. Awarded**	Actual Amt. Used**	% Used
Personnel	$271,981	$178,227	66
Travel	29,480	16,833	57
Shipping	24,000	-	-
Equipment	38,644	22,100	57
Supplies	38,644	30,861	80
Volunteers	67,500	33,865	50
Contracts	12,915	4,858	38
Publication	2,500	1,986	79
	485,666	288,730	59
Indirect Costs	163,190	106,936	66
Total	$648,856	$395,666	61 %

*Total three-year award (1 October 78-31 October 81).
**Actual expenses through 31 July 81 (34 months).
+92% time (34 of 37 months).

About 30% of the total effort was invested in studying factors influencing iron availability, 50% of the effort in developing iron fortification systems and the remaining 20% in evaluating iron bioavailability of LDC diets. These proportions can be applied directly to the total figure of $395,666 in direct costs to determine the amount actually expended in these three categories of work activity. Thus, $118,700 was for factors affecting food iron availability, $197,833 for studies to develop a fortification system, and $79,133 for studying iron bioavailability in LDC diets.

F. Proposed Studies for the Coming Year

The investigations planned for the coming year represent an extension of the studies already carried out under this grant and are tabulated below in their approximate order of priority:

1. The effect of soy on heme iron absorption.

2. Study of the relationship between the quantity of soy and its effect on iron absorption from a complex meal.

3. Evaluation of the common pool in rice-containing meals.

4. Comparison of iron absorption from different legume sources, soya beans, lentils, peanuts, mung beans, navy beans, lima beans, and pinto beans.

5. Evaluation of iron availability from millet-based meals. (The millet will be obtained from Lesotho, Africa where it is staple food for a large part of the population).

6. The effect of iron status on iron absorption--a comparison of iron absorption from meals eaten with and without soy products in subjects who have been phlebotomized with parallel studies being done in iron replete volunteers.

7. The relationship between phytin content of soy and its effect on iron absorption.

8. The importance of the carbohydrate component of the semi-synthetic meal to iron absorption--substitution of corn syrup solids by potato starch, cornstarch, and wheat starch.

9. The effect of the carbohydrate component on iron absorption from a semisynthetic meal--effect of processing the carbohydrates. Comparison of corn syrup solids with cornstarch and a simple sugar mixture.

10. The effect of the lipid component of a semisynthetic meal on iron absorption--comparisons will be made between corn oil, soy oil purified by cold pressing, soy oil purified by extraction, and sunflower oil.

11. Evaluation of iron fortification of corn meal.

12. Iron absorption from more complex meals: Russian, Mexican, Greek, American, and Italian.

APPENDIX A

- IRON ABSORPTION STUDIES -

GENERAL METHODOLOGY

The following is an interim report of investigations of food iron availability performed under USAID Contract DSAN-C-0045 between 1 September 1980 and 30 August 1981. The results are presented as a series of 12 consecutively-numbered absorption studies performed in groups of 6-13 subjects each. The following aspects of methodology and data analysis are common to all of these studies.

Experimental Subjects. Each study was performed in normal male volunteer subjects between the ages of 18 and 65 years. Written informed consent was obtained from the subjects, all of whom were remunerated for their participation in the studies. None of the subjects gave a history of hematological abnormalities or diseases known to influence gastrointestinal absorption of iron. The majority of volunteers had normal iron status as gauged as serum ferritin levels; an occasional subject had mild iron deficiency due to either participation in other experimental studies or frequent blood donations.

Test Meals. Except for absorption from the reference dose of inorganic iron which is described below, the test meals contained either a mixture of food items or a combination of semipurified dietary ingredients. The methodology used to measure the absorption of nonheme dietary iron was based on the principle of extrinsic radioiron tagging. With this technic it is possible to measure absorption from the common pool of nonheme iron that is found when several food items are ingested in the same meal. Extrinsic tagging is performed by adding 0.1 mg elemental iron as labeled $FeCl_3$ to the meal. This radioiron label is either mixed with the entire meal when studying liquid diets or is added to one of the liquid items in a conventional meal.

Composition of the test meals differed in each study depending on the nature of the experiment; specific meals are described under each study. Many studies were performed with a test meal that has been used extensively in published studies from this laboratory. The meal consists of semipurified ingredients, the amount of which can be altered in each experiment so as to standardize the total content of the major dietary ingredients in each meal. This semisynthetic (SS) meal contains 37.4 g egg albumen as protein, 66.8 g dextrimaltose as carbohydrate, 34.8 g corn oil as fat, and sufficient dibasic calcium phosphate, dibasic potassium phosphate, and ferric chloride to bring the calcium, phosphorus, and iron content to 262, 361, and 4.1 mg respectively. Its nutrient composition corresponds closely to that of a typical American meal. In some studies modifications were made in its composition to improve basal iron availability.

A final test dose that was used in many studies consisted of a solution of inorganic iron and is referred to as reference (REF) absorption. This test dose contained 3 mg iron as ferrous sulfate and 2 mol freshly prepared ascorbic acid/mol iron in 50 ml iron-free water. The purpose of the REF test is to provide a measure of the intestinal avidity of iron in each subject and thereby permit standardization of absorption measurements performed in groups of subjects that differ in iron status. By expressing absorption from a given test meal as a ratio of REF absorption, the effect of differences in iron status between subjects or groups of subjects is eliminated.

Iron Absorption Measurements. In all studies four iron absorption tests were performed in each subject as two sets of parallel double isotope measurements. The day before the first meal the subjects reported to the laboratory, were told the nature of the study, and gave written informed consent. Age, height, and weight of each subject were recorded, and 30 ml whole blood was drawn for measurements of hematocrit, serum

iron, total iron-binding capacity, serum ferritin, and background blood radioactivity. On the following day, the first test meal tagged with either ^{59}Fe or ^{55}Fe was administered to fasting subjects between 8 and 10 a.m.; nothing further was allowed by mouth for 3 hours. A second meal tagged with the alternate form of radioiron was given under identical conditions the following day. Fourteen days later the subjects returned to the laboratory and 10 ml whole blood was obtained for duplicate measurements of ^{55}Fe and ^{59}Fe. A second pair of absorption tests were again performed with ^{59}Fe and ^{55}Fe on two successive days, and a final blood sample was obtained 14 days later to measure the rise in ^{55}Fe and ^{59}Fe blood radioactivity. The total dose of radioactivity administered to each subject was 4 uCi ^{59}Fe and 10 uCi ^{55}Fe.

Simultaneous measurements of ^{55}Fe and ^{59}Fe activity in 10 ml blood (day 0 x 1, day 14 x 2, day 28 x 2) were performed by liquid scintillation counting using a modification of the Eakins and Brown method. Standards representative of each test dose were processed along with the blood samples. Percentage absorption was calculated from whole blood radioactivity and total blood volume, the latter estimated on the basis of height and weight. In these iron replete volunteers it was assumed that 80% of the absorbed iron was incorporated into circulating red cells 14 days after administration of each test meal.

Statistical Analysis and Data Presentation. Iron absorption measurements expressed as a percent of the administered dose are highly skewed and the degree of skewing is particularly marked at low levels of absorption which are frequently encountered in studies of food iron availability. Previous studies of this problem have established that log transformation of percentage absorption will produce a Gaussian distribution. Statistical analysis was performed on logarithms of percentage absorption and the results transformed as antilogs to recover the original units of percent absorption.

When testing the significance of differences in mean absorption between any two test meals in a given study, analysis was performed on logarithms. Student's t was calculated to determine whether the mean difference in log absorption between any pair of meals was significantly different than zero; this is equivalent to testing in original units for a ratio different than unity.

In the main table prepared for each study, percent absorption from each meal is listed individually for each subject and the most important ratios are similarly tabulated. Also included for each study is a table which lists mean absorption ± 1 SE for all possible meal combinations. The reciprocal mean ratio (1/ratio) is also listed for each meal but not the associated ± 1 SE which can be calculated as reciprocals of $1 \pm$ SE for the original ratios. Values for Student's t and the associated p-values are also listed for each meal. In addition, correlation coefficients for absorption from each pair of meals (performed on logarithms) are listed together with the associated p-value. These correlation coefficients reflect the precision of each paired comparison. Lower correlation coefficients are usually obtained when comparing absorption from test meals administered 14 days apart rather than 24 hours apart--that is, when comparing absorption from either meals A or B with either meals C or D. Note that the meal designations (A,B,C,D) do not necessarily indicate the order in which the meals were actually administered; this order was sometimes altered in the tables to emphasize the most important comparisons in each study. Graphs are also included for each study showing percentage absorption and the important absorption ratios, both plotted on a logarithmic scale.

STUDY 27

IRON AVAILABILITY FROM RICE COATED WITH FERROUS SULFATE. I.

SUMMARY

Iron absorption from rice coated with ferrous sulfate was compared with absorption from the nonheme common pool in two meals. Iron coated onto the rice was significantly less available for absorption than iron in the common pool. This appeared to result from inadequate dissolution of the coating material during cooking and digestion in the upper small bowel.

RATIONALE

Rice is a staple food in developing countries throughout the world, many of which have a high prevalence of iron deficiency anemia. The iron status of the population in these countries could be improved if the amount of iron in the diet was increased by the fortification of a major food item. Past attempts to fortify rice with iron and other nutrients have been unsuccessful because fortification has altered the appearance of the food, thereby making it unacceptable to consumers. Dr. Alex Emodi of Hoffmann-La Roche Laboratories has developed a method for coating rice grains with ferrous sulfate that does not affect their appearance in the uncooked state. The aim of this study was to measure the bioavailability of iron coated on rice in this manner.

DESIGN

Rice (400 g short grain "California Pearl") was coated with ferrous sulfate followed by alternate layers of dried talc and shellac until the rice reached the desired degree of whiteness. The procedure used 24 g talc and 14.1 g shellac solids/400 g rice and was designed to produce a premix containing 4 mg iron as ferrous sulfate and 5 uCi ^{55}Fe/1 g rice. This premix was then diluted 1:99 with unfortified rice grains. Preliminary experiments demonstrated that washing the rice, as is customary in developing countries, led to only a 0.1% loss of radioiron.

Nine volunteers participated in this two-meal study. The first meal consisted only of 216 g cooked rice (100 g dry

weight). Rice was steam cooked in a double boiler and all of the water in the upper bowl was absorbed during the cooking process. Ferrous sulfate tagged with ^{59}Fe was added to the water to label the common nonheme iron pool and to provide an additional 4 mg iron and 2 uCi ^{59}Fe/serving.

The second meal was prepared in the manner described above except that no iron was added to the cooking water. This meal also contained a 113 g hamburger patty (uncooked weight) and 80 g applesauce. Two microcuries ^{59}Fe and 4 mg iron as $FeCl_3 \cdot 6H_2O$ was added to the applesauce.

RESULTS

By tagging the common nonheme pool with one isotope and using a second isotope to label the iron coated onto the rice, it was possible to make a direct comparison between the absorption of the two forms of iron in each meal. In meal B, containing only rice, absorption was 0.95% from the iron added to the water and absorbed by the rice during cooking. In comparison, absorption of the iron coated onto the rice was only 0.41% (A). The meal ratio A/B=0.43 was indicative of a highly significant difference between the two forms of iron in the same meal (t=9.17, p 0.001). In the second meal, containing foods known to enhance iron absorption, the difference in absorption between the iron coat and the extrinsically labeled nonheme iron pool was even more apparent (0.72% and 2.49%, meals C and D respectively). The ratio C/D=0.29 was again indicative of a highly significant difference (t=9.8, p 0.001). Direct comparison of the two meals demonstrates that the iron in the common pool was markedly susceptible to the effect of enhancing agents while this effect was much less apparent in the iron coated onto the rice (D/B=2.60, C/A=1.75).

COMMENT

The low availability of iron from ferrous sulfate-coated rice and the discrepancy in percentage absorption between this iron and that present in the common pool suggest that the coating procedure prevented the iron from entering the nonheme iron pool in the meal. Although the uncooked iron-coated grains were difficult to distinguish from other grains, after cooking they were easily identifiable by their darker color and were not as tender as the uncoated grains. It appears that the shellac coating, which sealed the iron from moisture before cooking, did not rupture and release iron into the cooking water as anticipated. Several volunteers complained of the presence of hard, uncooked grains in the meal.

The coating procedure used in this study appears to be unsuitable for fortification because it prevents release of the iron during the cooking process as well as in the lumen of the upper small bowel. Results of a test using a modified coating procedure are reported in Study 34.

Study 27. Absorption from Iron-Fortified Rice. I.

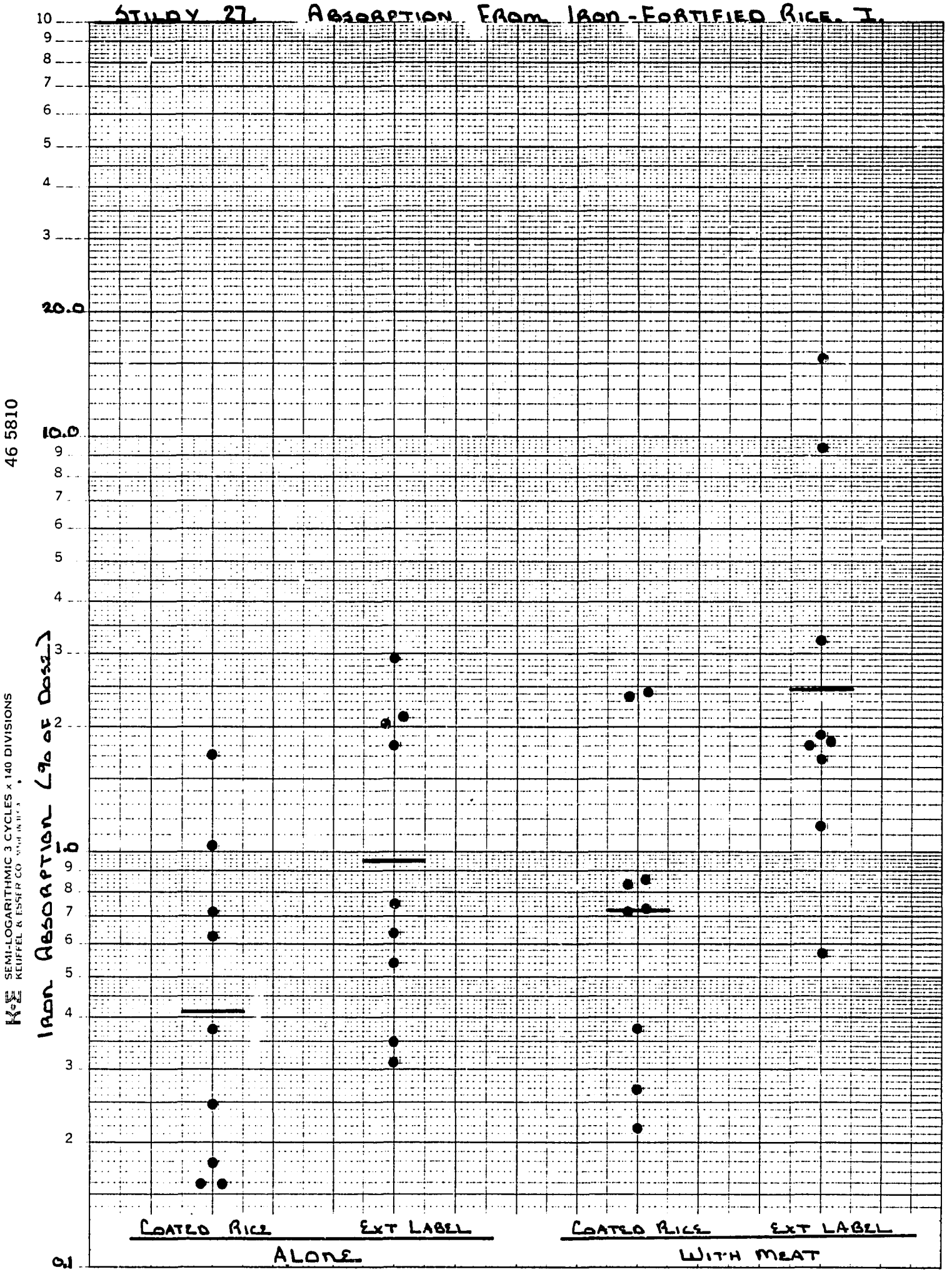
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption (% of dose): Alone, Coated Rice (A)	Alone, Extrinsic Label (B)	With Meat, Coated Rice (C)	With Meat, Extrinsic Label (D)	Absorption Ratio A/B	C/D	C/A	D/B
1	29	45	137	0.16	0.31	0.22	0.58	0.51	0.37	1.37	1.87
2	20	43	99	0.16	0.35	0.27	1.18	0.45	0.22	1.68	3.37
3	30	46	39	0.18	0.65	0.88	3.30	0.27	0.26	4.88	5.07
4	28	48	51	0.25	0.55	0.38	1.88	0.45	0.20	1.52	3.41
5	19	45	77	0.38	0.76	0.85	1.70	0.50	0.50	2.23	2.23
6	22	49	113	0.63	2.06	0.73	1.83	0.30	0.39	1.15	0.88
7	19	44	33	0.72	2.12	2.45	15.58	0.33	0.15	3.40	7.34
8	24	48	144	1.05	1.81	0.75	1.91	0.58	0.39	0.71	1.05
9	29	44	84	1.71	2.96	2.41	9.45	0.57	0.25	1.40	3.19
Mean*	24	46	77	0.41	0.95	0.72	2.49	0.43	0.29	1.75	2.60
			-1 SE	0.33	0.72	0.54	1.77	0.39	0.26	1.44	2.06
			+1 SE	0.55	1.27	0.96	3.49	0.47	0.33	2.12	3.27

*Geometric means except Age and Packed Cell Volume.

Study 27. Absorption from Iron-Fortified Rice. I

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	0.43	0.57	0.17	1.33	0.38	0.29
-1 SE	0.39	0.47	0.13	1.14	0.31	0.26
+1 SE	0.47	0.69	0.22	1.54	0.48	0.33
1/ Ratio	2.32	1.75	6.02	0.75	2.60	3.44
Student's *t*	9.17	2.91	6.63	1.88	4.15	9.80
p value	$<10^{-5}$	0.010	$<10^{-4}$	0.049	0.002	$<10^{-4}$
Correlation* (r)	0.95	0.78	0.64	0.86	0.74	0.93
p value	$<10^{-4}$	0.007	0.031	0.002	0.011	0.0001

* log scale


STUDY 27. ABSORPTION FROM IRON-FORTIFIED RICE. I.
IRON ABSORPTION (% OF DOSE)
10
9
8
7
6
5
4
3
20.0
10.0
2
1.0
0.1
COATED RICE
EXT LABEL
ALONE
COATED RICE
EXT LABEL
WITH MEAT
46 5810
K+E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO.

STUDY 28
EVALUATION OF NaFeEDTA AS THE IRON SUPPLEMENT FOR CSM

SUMMARY

Previous studies have demonstrated poor iron absorption from the infant food supplement corn-soya-milk (CSM) fortified with ferrous fumarate. In the present study we evaluated the effect of substituting NaFeEDTA for ferrous fumarate. Percentage iron absorption was significantly better when NaFeEDTA was used (1.03% vs 0.53%).

RATIONALE

Earlier studies indicated that EDTA influences the availability of iron from the nonheme common pool and that this effect is a complex one. When NaFeEDTA is used as the fortificant, iron from the complex enters the nonheme common pool. If this is the only source of EDTA in the meal the molar ratio of EDTA:Fe is of necessity less than 1. Under these circumstances, EDTA appears to reduce iron availability in meals containing important absorption enhancers such as ascorbic acid, but to improve its availability from meals that have a predominately inhibitory effect.

Our studies have shown that corn-soya-milk (CSM) as well as other infant food supplements exhibit low iron availability. Supplemental iron in these products is added as ferrous fumarate to provide 9.2 mg iron/50 g dry product. The current study was designed to compare absorption of iron from extrinsically labeled (with $^{59}FeCl_3$) ferrous fumarate and preformed Na-^{55}FeEDTA at a level of 9.10 mg iron/meal. This is the level currently used in the formulation of the product. Additionally, the absorption of iron from NaFeEDTA was compared with that from $FeCl_3$ at a reduced iron content of 3.4 mg iron/meal.

DESIGN

Eight volunteers participated in the study. Each received four meals consisting of a cooked porridge made from: 50 g dry CSM, 209 ml water, 20 g sucrose, and 1 g salt. Dry CSM, water, and salt were mixed in a blender, boiled with constant stirring for five minutes, and the sugar was then mixed in. Individual 280 g servings were weighed out and carrier iron plus radioiron dissolved in 1.0 ml of 0.01 N HCl was added just before serving. The quantity and chemical form of the iron added to the meals is shown below.

Meal	Form of Iron	Carrier Iron (mg)	Intrinsic Iron (mg)	Iron/Meal (mg)
A	Ferrous Fumarate	7.8	1.4	9.2
B	NaFeEDTA	9.0	1.4	10.4
C	$FeCl_3 \cdot H_2O$	2.0	1.4	3.4
D	NaFeEDTA	2.0	1.4	3.4

Meal A was the currently available CSM formulation (corresponding to "moderate Ca & P" in Study 2) containing 7.8 mg iron derived from ferrous fumarate in the mineral mix. In meals B and D, NaFeEDTA was added to provide 9 and 2 mg additional iron respectively. Iron (2 mg) in meal C was added as $FeCl_3$.

RESULTS

Percentage absorption from meal A (0.53%) was similar to that observed previously from this infant food supplement containing ferrous fumarate. The substitution of NaFeEDTA for ferrous fumarate in CSM (meal B) yielded a significantly higher percentage absorption, 1.03% (B/A = 1.93, $p = 0.003$). At the lower level of iron in the second two meals, a similar trend was observed. However, although the percentage absorption from NaFeEDTA was moderately higher, the difference was not statistically significant (0.65% to 0.91%).

COMMENT

The absorption value of 0.53% for meal A corresponds very well with the results obtained for similar meals in AID studies 2, 6, and 7 in which reported values were 0.40, 1.40, and 0.57% respectively. The enhancing effect of EDTA in an inhibitory

meal previously reported in Study 3 was again observed. Although the trend was similar in both meals, a significant increase in iron absorption occurred only in the meals containing the higher quantity of iron. The potential benefit of this increase in iron availability is more apparent when the absolute amount of iron absorbed from each meal is calculated as follows:

	Meal			
	A	B	C	D
Fe/Meal (mg)	9.20	10.40	3.40	3.40
Absorption (%)	0.53	1.03	0.65	0.91
Fe Absorbed (mg)	0.55	0.11	0.11	0.33

Thus, the substitution of NaFeEDTA for ferrous fumarate would be expected to increase the amount of iron absorbed from 50 g CSM as it is currently formulated by 0.06 mg in iron replete males. Since one would anticipate that percentage iron absorption would be two to three times higher in iron deficient children, the use of NaFeEDTA might improve iron absorption from the meal by almost 0.2 mg. While it is apparent that the substitution of NaFeEDTA for ferrous fumarate in CSM could produce a significant increase in available iron, it would be important to weigh this option against other methods of decreasing the inhibitory effect of CSM. For example, when we omitted calcium and phosphate salts from the preparation (Study 2), percentage iron absorption increased 2.25 times.

Study 28. Absorption of Fortification Iron from CSM.

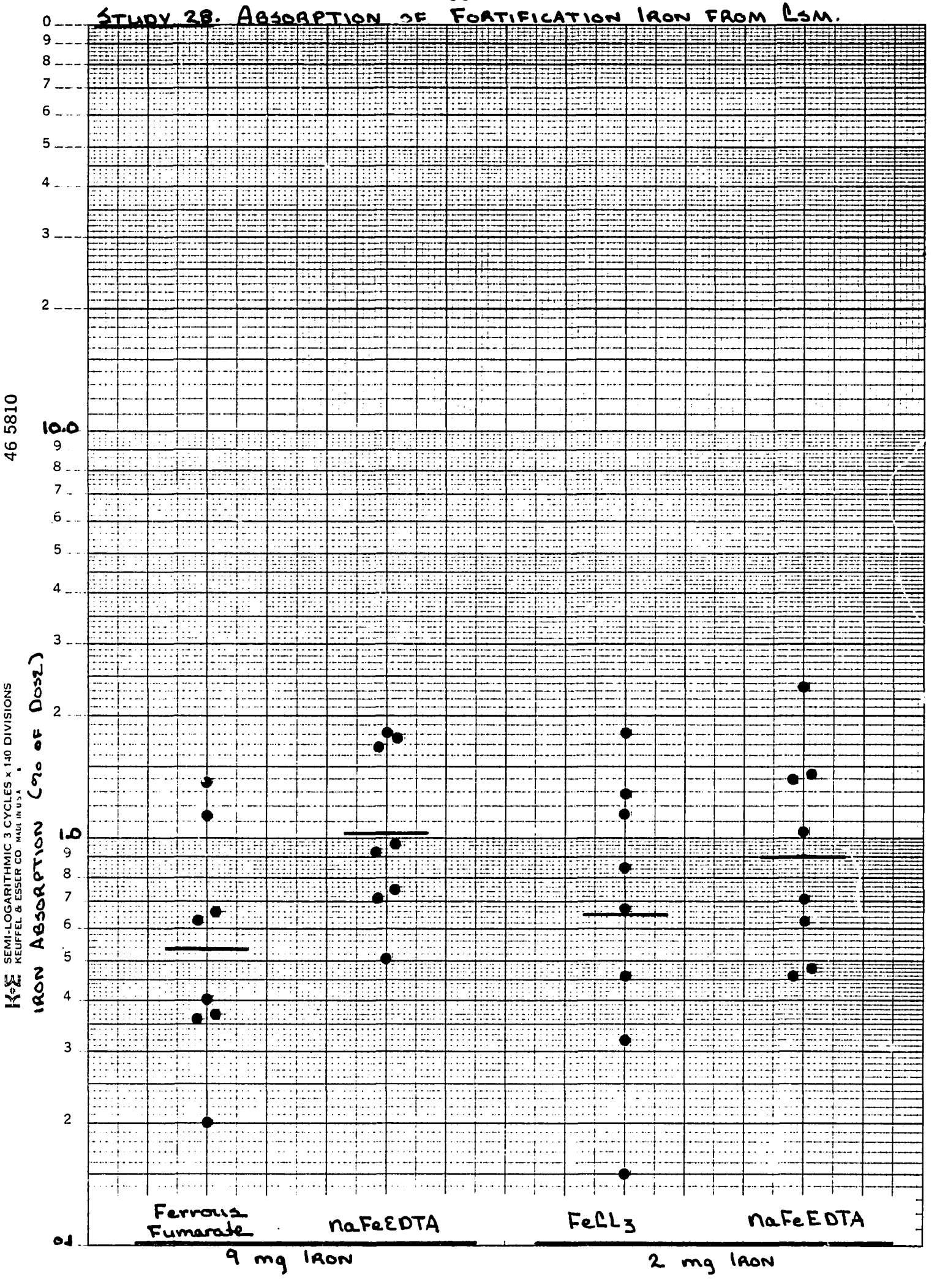
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption (% of dose) 9 mg Iron Ferrous Fumarate (A)	9 mg Iron NaFeEDTA (B)	2 mg Iron Ferric Chloride (C)	2 mg Iron NaFeEDTA (D)	Absorption Ratio B/A	C/A	D/B	D/C
1	22	44	141	0.20	0.51	0.32	0.48	2.55	1.60	0.90	1.50
2	22	42	163	0.36	0.71	0.67	0.46	1.97	1.86	0.64	0.68
3	23	47	63	0.37	1.78	1.15	1.40	4.81	3.10	0.78	1.21
4	24	46	106	0.40	0.97	0.85	1.05	2.42	2.12	1.08	1.23
5	27	45	107	0.63	0.92	0.46	0.62	1.46	0.73	0.67	1.34
6	30	45	82	0.66	0.75	0.15	0.71	1.13	0.22	0.94	4.73
7	19	43	35	1.13	1.80	1.81	1.43	1.59	1.60	0.79	0.79
8	32	43	30	1.37	1.67	1.28	2.37	1.21	0.93	1.41	1.85
Mean*	25	44	78	0.53	1.03	0.65	0.91	1.93	1.22	0.88	1.40
			-1 SE	0.42	0.87	0.48	0.74	1.63	0.91	0.81	1.13
			+1 SE	0.67	1.22	0.87	1.12	2.28	1.63	0.97	1.72

*Geometric means except Age and Packed Cell Volume.

Study 28. Absorption of Fortification Iron from CSM.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	0.52	0.82	0.59	1.58	1.13	0.72
-1 SE	1.63	0.61	0.50	1.31	1.03	0.58
+1 SE	2.28	1.09	0.70	1.90	1.24	0.88
1/ Ratio	1.93	1.22	1.70	0.63	0.88	1.40
Student's *t*	3.91	0.69	3.12	2.48	1.35	1.60
p value	0.110	0.26	0.008	0.021	0.110	0.080
Correlation* (r)	0.68	0.39	0.69	0.80	0.90	0.69
p value	0.033	0.168	0.028	0.008	0.001	0.029

* log scale


STUDY 28. ABSORPTION OF FORTIFICATION IRON FROM LSM.
IRON ABSORPTION (% OF DOSE)
10.0
1.0
0.1
Ferrous Fumarate
NaFeEDTA
FeCL3
NaFeEDTA
9 mg IRON
2 mg IRON
46 5810
K+E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

STUDY 29

EFFECT OF FIBER ON IRON ABSORPTION. I.

SUMMARY

This study was designed to examine the effect of two purified fiber sources, cellulose and pectin, and wheat bran on the absorption of iron from wheat flour muffins. Percentage absorption values for all the meals were very low ($<1\%$). None of the fiber sources demonstrated a statistically significant effect.

RATIONALE

Previous studies have suggested that fiber has an inhibitory effect on iron absorption, but the nature and magnitude of this effect have yet to be determined. This study was designed to assess the effect of two purified fibers, cellulose and pectin, and of wheat bran on iron absorption from wheat flour muffins.

DESIGN

Four test meals were administered to each of nine volunteers. The meals consisted of two muffins and two pats of margarine. The muffins were halved, and a 1.0 ml dose of 0.01 N HCl containing 3 mg iron as $FeCl_3$ and either 5uCi ^{55}Fe or 2uCi ^{59}Fe were pipetted drop-wise onto the four halves. The margarine was then spread on the muffins which helped seal in the radioiron and reduced crumbling. Water was allowed ad libitum with the muffins. Three sources of food fiber, wheat bran (from red hard wheat), citrus pectin, and α cellulose, were added to the batter used to prepare the three separate batches of muffins. Absorption of iron from these muffins was compared to that from plain wheat flour muffins.

RESULTS

Only 0.60% of the iron in the plain muffins was absorbed (meal A). The addition of 12 g bran reduced absorption to 0.32% (meal B), but this 47% reduction was not statistically significant. No effect was seen with 8 g pectin (meal C, 0.53%) and the highest absorption, 0.84%, occurred when 8 g α cellulose was added (meal D).

COMMENT

Absorption values for all meals in this study were very low. The value of 0.60% for meal A (plain muffins) is much lower than the 2.39% absorption for the same meal plus a milkshake reported by Simpson, Cook, and Morris (Am J Clin Nutr, in press). In the latter study, only one-fourth as much iron was absorbed when 12 g bran was added to the muffins. The failure to demonstrate a similar effect in the present study may be a reflection of reduced sensitivity of the test resulting from the low absorption obtained even in plain muffins.

Study 29. Effect of fiber on Iron Absorption. I.

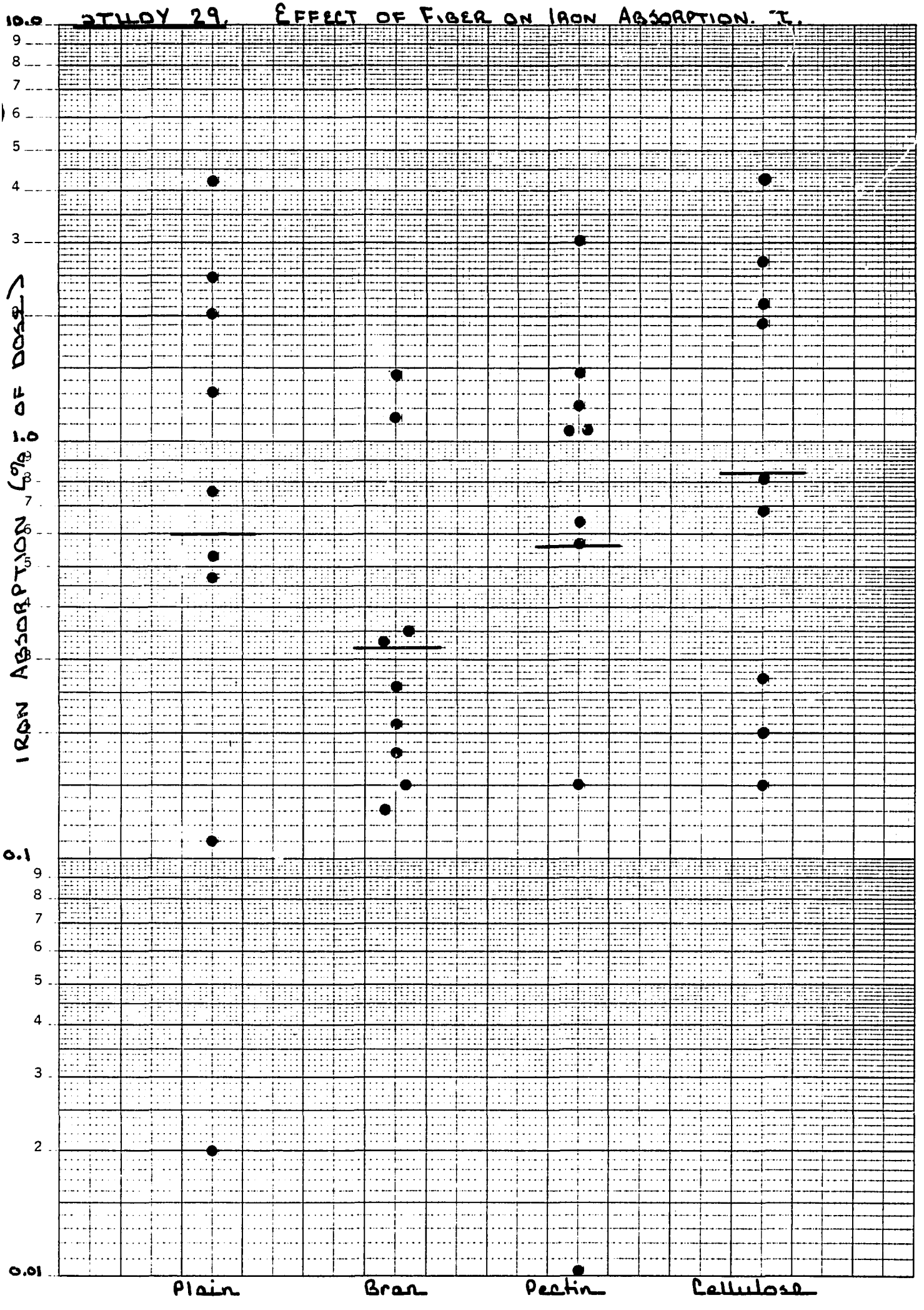
Subj.	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption Plain (A)	Bran (B)	Pectin (C)	Cellulose (D)	Absorption Ratio B/A	C/A	D/A	B/C	B/D	C/D
				(% of dose)									
1	24	47	124	0.02	0.35	0.57	0.15	17.50	28.50	7.50	0.61	2.33	3.80
2	26	44	48	0.11	0.15	0.01	0.27	1.36	0.09	2.45	15.00	0.55	0.03
3	26	41	116	0.47	0.13	0.15	0.20	0.27	0.31	0.42	0.86	0.65	0.75
4	22	43	83	0.53	0.18	0.65	0.68	0.33	1.22	1.28	0.27	0.26	0.95
5	28	43	66	0.76	0.21	1.07	1.92	0.27	1.40	2.52	0.19	0.10	0.55
6	24	45	27	1.31	1.15	1.48	0.81	0.87	1.12	0.61	0.77	1.41	1.82
7	37	42	978	2.03	0.26	1.21	4.30	0.12	0.59	2.11	0.21	0.06	0.28
8	28	43	27	2.50	1.43	3.01	2.70	0.57	1.20	1.08	0.47	0.52	1.11
9	22	45	75	4.21	0.33	1.07	2.16	0.07	0.25	0.51	0.30	0.15	0.49
Mean*	26	44	84	0.60	0.32	0.53	0.84	0.53	0.87	1.38	0.60	0.38	0.63
			-1 SE	0.34	0.24	0.30	0.56	0.31	0.51	1.02	0.39	0.26	0.41
			+1 SE	1.06	0.42	0.93	1.26	0.89	1.49	1.88	0.94	0.57	0.98

*Geometric means except Age and Packed Cell volume.

Study 29. Effect of Fiber on Iron Absorption. I.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	1.90	1.15	0.72	0.60	0.38	0.63
-1 SE	1.12	0.67	0.53	0.39	0.26	0.41
+1 SE	3.22	1.95	0.98	0.94	0.57	0.98
1/ Ratio	0.53	0.87	1.38	1.66	2.62	1.58
Student's *t*	1.21	0.25	1.05	1.15	2.42	1.06
p value	0.130	0.403	0.162	0.142	0.021	0.161
Correlation* (r)	0.36	0.55	0.84	0.65	0.38	0.64
p value	0.171	0.062	0.002	0.030	0.157	0.030

* log scale



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K&E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

STUDY 30
IRON AVAILABILITY FROM EGYPTIAN MEALS

SUMMARY

Percentage iron absorption from three typical Egyptian meals was measured. The mean values obtained were 1.41%, 1.45% and 1.49% while absorption from ferrous ascorbate was 21.11%. Based on these values and a calculated daily iron intake of approximately 10 mg, people with early iron deficiency would be expected to absorb about 0.3 mg nonheme iron daily. This is far below the amount required to maintain normal iron balance.

RATIONALE

Iron deficiency anemia is common among women and children in Egypt. There is little meat in the diet, the staples being rice, lentils, and beans. The availability of iron from three typical meals was determined using the extrinsic tag technic. In each subject iron absorption was related to the absorption of the reference dose of iron ascorbate to allow us to make extrapolations for calculated iron absorption in iron deficient people in Egypt.

DESIGN

Eight men participated in this study. The results obtained from one subject were omitted from the final calculation because his serum ferritin value of 654 ug/l suggested that he had an iron-loading disease.

The three meals were designed and prepared by Dr. Fawzi El-Shobaki in the manner customary for Egyptians. Meal A ("Koshari") consisted of rice, macaroni, and lentils garnished with tomato sauce, lemon juice, and fried onions. Meal B was a lentil soup containing dehulled lentils and grated carrots. Meal C ("Foul Medames") was a sandwich made of a paste of cooked beans and lentils mixed with lemon juice and olive oil, and spooned into one-half of a "pita" bread loaf (also called pocket or Syrian bread). The fourth meal (D) was the standard reference meal (REF) consisting of 3 mg iron as ferrous sulfate plus 18.9 mg ascorbic acid (2:1 molar ratio AA:Fe) in 50 ml water.

RESULTS

Percentage iron absorption from the four test meals was remarkably similar, ranging from 1.41% to 1.49%. The REF absorption of 21.11% (meal D) is typical for male subjects with normal iron stores.

COMMENT

The extremely close values for the three Egyptian meals suggest that lentils (a major component of each meal) may have an overriding effect on iron absorption. Based on the mean ratio meal:REF of 0.07 for the three meals, absorption adjusted to a 40% REF value (the level that is indicative of absent iron stores in most people) would be only 2.8%. The approximate iron content of the meals ranged from 1 to 3 mg. Assuming that most people eat three meals a day and that most meals contain the higher level of iron (3 mg), the daily dietary intake of nonheme iron would be approximately 10 mg. The average absorption of 2.8% calculated for people with early iron deficiency would mean that only 0.3 mg nonheme iron would be absorbed daily. This amount is not sufficient to meet the requirements of men, much less those of women and children.

Table 30-2

NUTRIENT COMPOSITION OF EGYPTIAN MEALS

Ingredient	Amt	Energy	Protein	Fat	Total CHO	Fiber	Nonheme Iron	Vit C
	g	kcal	g	g	g	g	mg	mg
Meal A								
Rice, enrich., cooked	66	72	1.3	.1	16	0.1	0.6	-
Lentils, cooked	34	36	2.7	0	6	0.4	0.7	-
Macaroni, enrich., cooked	40	44	1.4	0.2	9	0	0.4	-
Tomato sauce	3							
Lemon juice, fresh	0.5	2	0.1	0	0	0	0.1	1.2
Onions, fried	3							
Total	146.5	154	5.5	0.3	31	0.5	1.8	1.2
Meal B								
Lentils, de-hulled, cooked	120	127	9.4	0	23	1.4	2.5	
Carrot, boiled	10	3	0.1	0	1	0.1	0.1	
Onion, fried	3	1	0	0	0	0	0	
Lemon juice, fresh	0.5							0.1
Total	133.5	131	9.5	0	24	1.5	2.6	
Meal C								
Beans, (Vicia faba)	100	92	8.2	0.5	15	1.7	1.8	26
Lentils, cooked	50	53	3.9	0	10	0.6	1.1	0
Olive oil	5	44	0	5.0	0	0	0	0
Lemon juice, fresh	0.5	0	0.1	0	0	0	0	0
Bread, Syrian 1/2 loaf	28	76	2.5	0.9	14	0.1	0.7	0
Total	183.5	265	14.7	6.4	39	2.4	3.6	26.2

Study 30. Iron Availability from Egyptian Meals.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption Meal A*	Meal B*	Meal C*	REF D	Absorption Ratio A/D	B/D	C/D
				(% of dose)						
1	18	47	93	0.78	0.63	0.86	19.85	0.03	0.03	0.04
2	18	43	46	0.90	0.71	1.42	32.65	0.02	0.02	0.04
3	31	49	75	1.07	0.86	0.76	16.68	0.06	0.05	0.04
4	18	46	64	1.55	1.52	0.90	17.87	0.08	0.08	0.05
5	19	46	70	2.10	2.46	3.33	22.31	0.09	0.11	0.14
6	18	45	80	2.33	2.77	2.16	13.42	0.17	0.20	0.16
7	21	46	58	2.96	2.85	2.31	32.31	0.09	0.08	0.07
Mean+	20	46	68	1.49	1.41	1.45	21.11	0.07	0.07	0.07
			-1 SE	1.23	1.10	1.17	18.61	0.06	0.05	0.06
			+1 SE	1.81	1.81	1.80	23.94	0.09	0.09	0.09

*Meal contents are described in Summary.

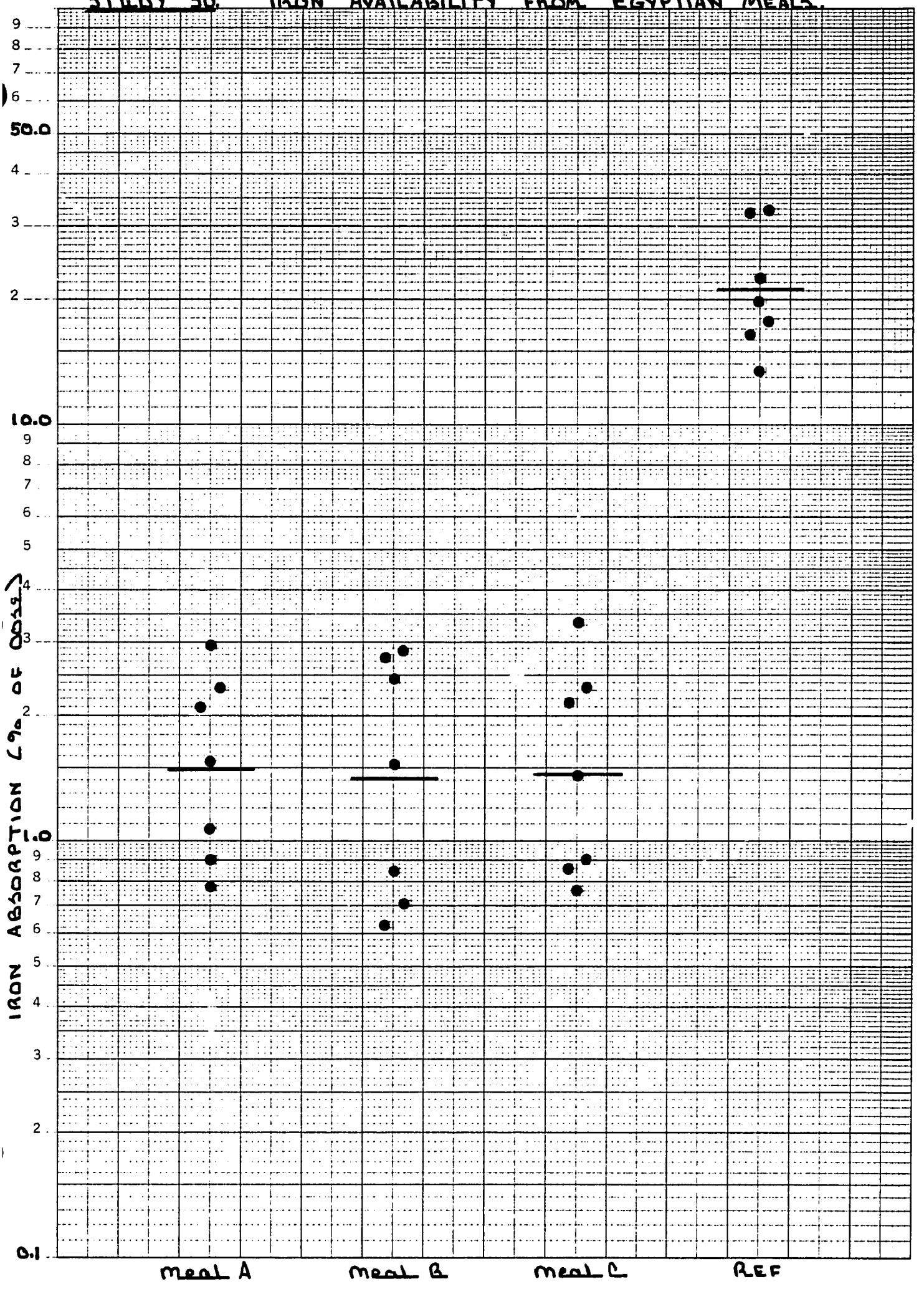
+Geometric means except Age and Packed Cell Volume.

Study 30. Iron Availability from Egyptian Meals.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	1.06	1.03	0.07	0.97	0.07	0.07
-1 SE	0.99	0.89	0.06	0.83	0.05	0.06
+1 SE	1.13	1.19	0.09	1.14	0.09	0.09
1/ Ratio	0.95	0.97	14.10	1.03	14.92	14.50
Student's *t*	0.85	0.19	11.46	0.18	9.31	12.21
p value	0.213	0.428	$<10^{-4}$	0.431	$<10^{-4}$	$<10^{-5}$
Correlation* (r)	0.99	0.75	0.000	0.78	0.09	0.28
p value	$<10^{-4}$	0.026	0.500	0.020	0.423	0.274

* log scale

STUDY 30. IRON AVAILABILITY FROM EGYPTIAN MEALS.



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STUDY 31

EFFECT OF ASCORBYL PALMITATE OF IRON AVAILABILITY FROM MUFFINS CONTAINING SOY FLOUR

SUMMARY

Ascorbyl palmitate is a fatty acid ester of ascorbic acid which is not as rapidly oxidized during baking as ascorbic acid itself. However, while 100 mg ascorbic acid sprinkled onto two muffins made from soy-enriched wheat flour increased absorption from 1.04% to 2.28%, the addition of either ascorbic acid or ascorbyl palmitate before baking had no effect.

RATIONALE

Ascorbic acid (AA) has been shown to reduce the inhibition of iron absorption due to soy products. L-ascorbyl-6-palmitate (AP), a fatty acid ester of AA, shares this property but is less rapidly oxidized during the baking process. In Study 8 we demonstrated that when AP was added to bread before baking the increase in iron absorption was similar to that seen when AP was added after baking. The present study was designed to determine the effects of AP on iron absorption from muffins containing soy flour which is used in bread products to increase protein content and to produce a darker brown crust.

DESIGN

Four test meals, consisting of two muffins and two pats of margarine, were given to each of 13 volunteers. Each muffin contained 50.6 g enriched white wheat flour and 16.9 g full fat soy flour. Meal A was the control meal with no additions. Meal B contained 236 mg AP per two muffin meal (molar equivalent of 100 mg AA) added to the batter using a monodiglyceride carrier. Meal C contained 100 mg AA per meal added to the batter before baking and in meal D, 100 mg AA was sprinkled on the four halves of the muffins.

RESULTS

There was no significant difference in iron absorption between meals A (1.04%), B (1.11%), and C (1.17%) made with batter containing no addition, AP, and AA respectively. The addition of AA after baking increased iron absorption to 2.28% (p 0.001).

COMMENT

The enhancing effect of ascorbic acid on nonheme iron absorption from a meal containing soy was again demonstrated (meal D). However, it is apparent that this influence is lost during the baking process whether AA or AP is used. The results suggest that the heat and oxidizing conditions present during the baking process destroy the iron enhancing properties of both ascorbic acid and ascorbyl palmitate and that the use of ascorbyl palmitate would have no advantage for iron absorption.

Study 31. Effect of Ascorbyl Palmitate (AP) and Ascorbic Acid (AA) on Iron Absorption from Soy Muffins.

Subj.	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption (% of dose): Muffin Alone (A)	Before Baking: AP (B)	Before Baking: AA (C)	Baked: AA (D)	Absorption Ratio: B/A	C/A	D/A	B/C	D/B	D/C
1	19	45	38	0.32	0.78	1.26	0.91	2.43	3.93	2.84	0.61	1.16	0.72
2	20	44	65	0.43	0.66	0.67	0.75	1.53	1.55	1.74	0.98	1.13	1.11
3	20	46	71	0.48	0.42	0.55	0.98	0.87	1.14	2.04	0.76	2.33	1.78
4	19	45	39	0.71	1.55	1.11	1.83	2.18	1.56	2.57	1.39	1.18	1.64
5	19	46	76	0.72	0.46	1.02	2.01	0.63	1.41	2.79	0.45	4.36	1.97
6	22	48	79	0.93	0.96	0.53	1.71	1.03	0.56	1.83	1.81	1.78	3.22
7	19	49	80	0.97	1.23	0.90	2.62	1.26	0.92	2.70	1.36	2.13	2.91
8	21	45	30	1.10	1.77	1.42	2.21	1.60	1.29	2.00	1.24	1.24	1.55
9	19	44	71	1.13	1.32	1.03	2.55	1.16	0.91	2.25	1.28	1.93	2.47
10	20	46	56	1.26	0.75	1.47	2.65	0.59	1.16	2.10	0.51	3.53	1.80
11	19	46	80	2.00	1.75	1.88	5.12	0.87	0.94	2.56	0.93	2.92	2.72
12	19	43	13	3.68	4.51	5.71	7.11	1.22	1.55	1.93	0.78	1.57	1.24
13	18	47	57	5.36	1.63	1.43	7.78	0.30	0.26	1.45	1.13	4.77	5.44
Mean*	20	46	52	1.04	1.11	1.17	2.28	1.06	1.12	2.18	0.95	2.05	1.94
			-1 SE	0.83	0.93	0.99	1.86	0.91	0.94	2.06	0.84	1.78	1.68
			+1 SE	1.31	1.33	1.39	2.80	1.24	1.33	2.31	1.06	2.36	2.24

*Geometric means except Age and Packed Cell volume.

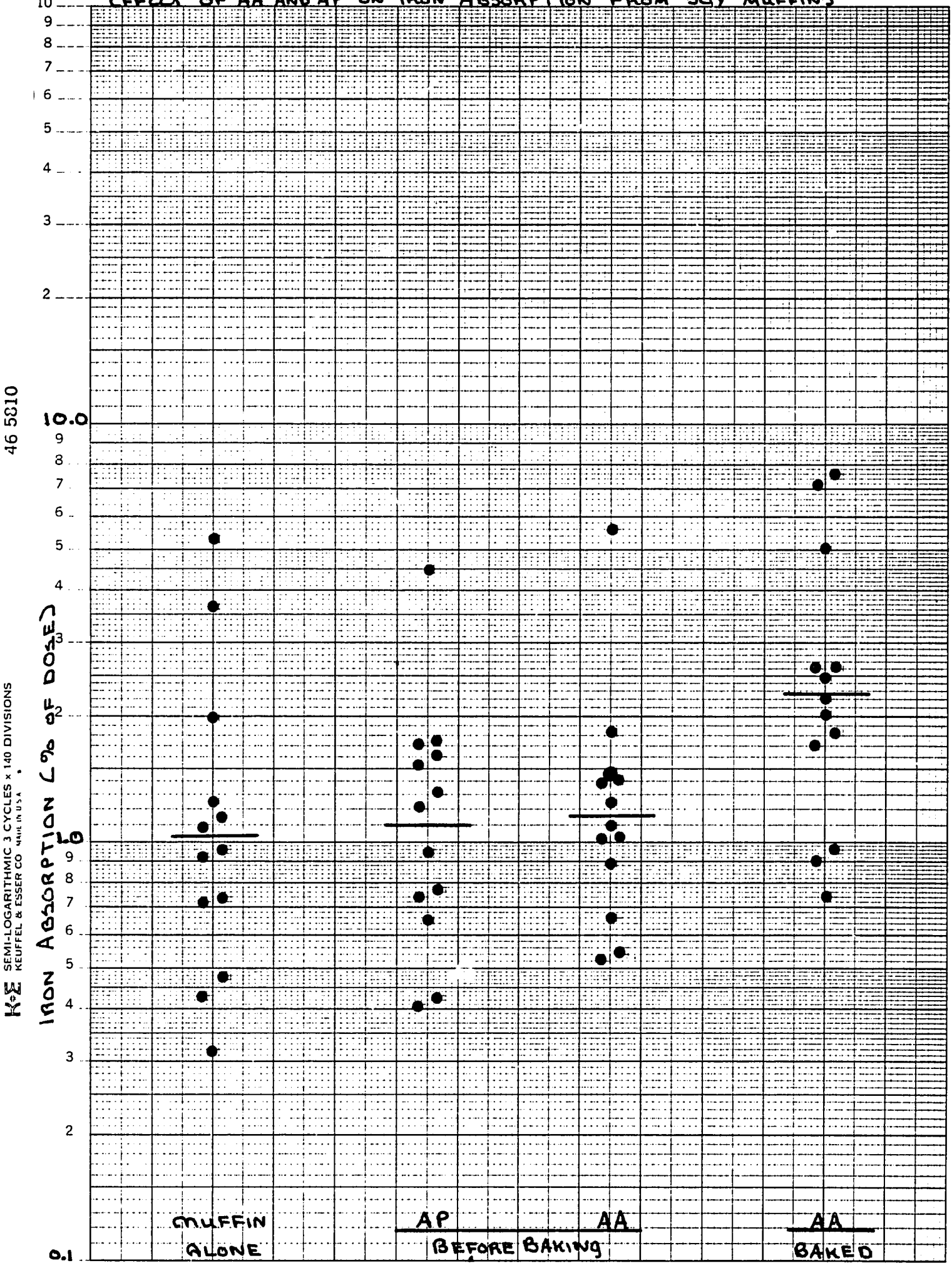
Study 31. Effect of Ascorbyl Palmitate (AP) and Ascorbic Acid (AA) on Iron Absorption from Soy Muffins.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	0.94	0.89	0.46	0.95	0.49	0.51
-1 SE	0.81	0.75	0.43	0.84	0.42	0.45
+1 SE	1.10	1.06	0.49	1.06	0.56	0.59
1/ Ratio	1.06	1.12	2.18	1.06	2.05	1.94
Student's *t*	0.38	0.66	13.70	0.46	5.15	4.62
p value	0.35	0.26	$<10^{-5}$	0.33	0.0001	0.0003
Correlation* (r)	0.72	0.65	0.97	0.78	0.74	0.72
p value	0.003	0.008	$<10^{-5}$	0.0008	0.002	0.003

* log scale

STUDY 31.

EFFECT OF AA AND AP ON IRON ABSORPTION FROM SOY MUFFINS



STUDY 32

EFFECT ON IRON ABSORPTION OF COOKING SPRAY-DRIED OR FRESH EGG WHITE

SUMMARY

Iron absorption was measured from semisynthetic meals in which the protein component was either spray-dried egg white (egg albumen) or fresh egg white. Percentage iron absorption was greatest in the meal containing cooked egg white (10.93%), with values of 4.71% and 2.76% being obtained with uncooked egg white and egg albumen respectively. This study indicates that egg white contains a heat labile inhibitor of iron absorption. Spray drying accentuates the inhibitory effect.

RATIONALE

Egg albumen (EA), the protein source for the semisynthetic (SS) meal inhibits nonheme iron absorption. Conalbumin, a transferrin-like protein, makes up 12% of the total protein in EA. Iron added to EA becomes bound to conalbumin and it seemed possible that this binding might make the iron unavailable for absorption. Cooking destroys the iron binding properties of conalbumin. Since EA is simply spray-dried egg white, this study was designed to determine the effect on iron absorption of cooking EA and fresh egg white.

DESIGN

Eleven volunteers took part in this study. Each of the four test meals had the composition of the previously described SS meal. No additional calcium or phosphate salts were added. The protein was provided as EA in meals A and B and as fresh egg white in meals C and D. The components were blended and taken as a drink in meals A and C. The egg protein in meals B and D was cooked to destroy the iron-binding properties of conalbumin. In meal B this was accomplished by cooking a water solution of dried EA in a double boiler until a uniform temperature of 70°C had been reached. Afterward, the coagulated product was mixed with the other SS meal components in a blender and served as a drink. For meal D, fresh egg whites were mixed with the corn oil component of the SS meal and cooked as an omelet. The corn syrup solids were diluted with water and the mixture served as a drink along with the omelet. The radioiron tag, together with 3.7 mg carrier iron, was added to the liquid portion of each meal to provide a total iron content of 4.1 mg.

Because of the unpalatability of meal B (cooked EA) most of the subjects were unable to consume more than half of the total meal.

Therefore, results from meal B are not directly comparable to those from the other three meals.

RESULTS

Mean iron absorption from the standard SS meal was 2.76% (meal A). When fresh egg white was used in meal C, absorption was 70% higher (4.71%, p 0.05). Cooking the egg white increased iron absorption significantly, to 10.93%. This represents a 2.3-fold increase over iron absorption from the SS meal containing uncooked egg white (p 0.001). Absorption from EA also doubled, from 2.76 to 5.57%, when it was cooked (meal B). However, an increase of this magnitude has also been attributed to halving the protein content of the SS meal. Therefore, since only half the meal was eaten by most subjects, the effect of cooking cannot be distinguished from the effect of reducing the protein level.

COMMENT

The value of 2.76% for iron absorbed from the SS meal is similar to the values of 2.49% and 3.00% reported in AID studies 3 and 10. In Study 3, iron absorption was improved 2.5-fold by cooking EA, whereas no effect was seen at the lower level of iron (4.1 mg) used in meals A and B of Study 10. At the higher iron level of 8.2 mg (Study 10, meals C and D,) absorption increased only 30% when the EA was cooked. The difference between these two earlier studies was attributed to incomplete cooking of the EA in Study 10. The results of the present study demonstrate that cooking improves iron absorption from a meal containing fresh egg white. It is of interest that percentage absorption was higher in the meal containing fresh egg white (4.71%) than it was with EA (2.76%) which is normally used in the SS meal. The explanation for this difference is not readily apparent.

Monsen and Cook (1979) demonstrated that EA has a marked inhibitory effect on iron absorption. Omitting EA from the SS meal increased absorption from 3.8% to 9.6%. This 2.5-fold rise was similar in magnitude to the effect of cooking EA seen in Study 3 and only slightly greater than that observed with fresh egg white in this study. The information derived from all these studies when taken together indicates that egg white contains a heat labile factor(s) that inhibits iron absorption. The iron binding properties of conalbumin point to its being the constituent responsible for this effect.

REFERENCE

Monsen ER, and Cook JD. Food iron absorption in human subjects. V. Effects of the major dietary constituents of a semisynthetic meal. Am J Clin Nutr 32:804,1979.

Study 32. Effect of Cooking Spray Dried and Fresh Egg White on Iron Absorption.

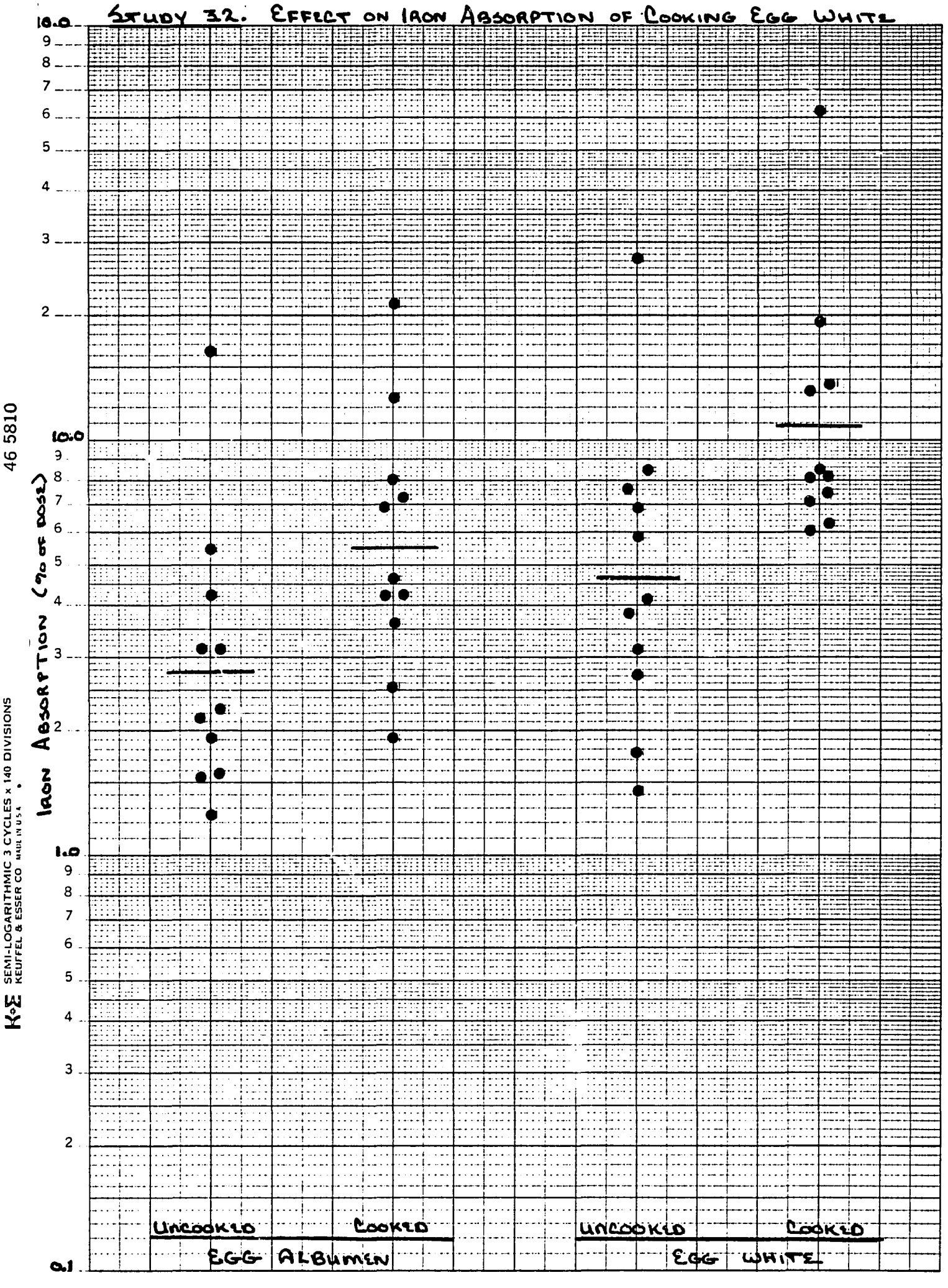
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption (% of dose): Egg Albumen Uncooked (A)	Egg Albumen Cooked (B)	Egg White Uncooked (C)	Egg White Cooked (D)	Absorption Ratio B/A	D/C	C/A	D/B
1	20	48	115	1.25	4.27	2.71	8.50	3.41	3.13	2.16	1.99
2	19	46	134	1.52	8.00	5.96	13.25	5.26	2.22	3.92	1.65
3	19	45	72	1.56	12.68	8.43	13.93	8.12	1.65	5.40	1.09
4	19	43	263	1.91	2.53	3.15	7.18	1.32	2.27	1.64	2.83
5	19	45	40	2.13	4.60	4.13	8.20	2.15	1.98	1.93	1.78
6	20	48	58	2.51	6.85	1.78	6.05	2.72	3.39	0.70	0.88
7	21	47	97	3.15	3.66	6.85	8.11	1.16	1.18	2.17	2.21
8	19	45	95	3.16	7.37	7.75	19.42	2.33	2.50	2.45	2.63
9	19	42	54	4.26	4.28	1.43	6.33	1.00	4.42	0.33	1.47
10	19	47	46	5.40	1.92	3.85	7.60	0.35	1.97	0.71	3.95
11	20	43	10	10.62	21.06	27.61	62.97	1.98	2.28	2.59	2.99
Mean*	19	45	69	2.76	5.57	4.71	10.93	2.01	2.32	1.70	1.96
			-1 SE	2.28	4.52	3.67	8.89	1.56	2.08	1.33	1.71
			+1 SE	3.35	6.86	6.04	13.43	2.60	2.58	2.18	2.24

*Geometric means except Age and Packed Cell Volume.

Study 32. Effect of Cooking Spray Dried and Fresh Egg White on Iron Absorption.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	0.50	0.59	0.25	1.18	0.51	0.43
-1 SE	0.38	0.46	0.21	0.97	0.45	0.39
+1 SE	0.64	0.75	0.31	1.44	0.58	0.48
1/ Ratio	2.01	1.70	3.95	0.85	1.96	2.32
Student's *t*	2.73	2.16	6.87	0.86	5.00	7.80
p value	0.011	0.028	$<10^{-4}$	0.205	0.0003	$<10^{-5}$
Correlation* (r)	0.18	0.40	0.50	0.65	0.79	0.90
p value	0.298	0.112	0.061	0.015	0.002	$<10^{-4}$

* log scale


STUDY 32. EFFECT ON IRON ABSORPTION OF COOKING EGG WHITE
IRON ABSORPTION (% OF DOSE)
10.0
9
8
7
6
5
4
3
2
1.0
0.1
UNCOOKED
COOKED
EGG ALBUMEN
UNCOOKED
COOKED
EGG WHITE
46 5810
K+E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

STUDY 33

EFFECT OF FATS ON IRON ABSORPTION

SUMMARY

Iron absorption from four semisynthetic meals containing egg albumen, dextrimaltose, and one of four different fat sources was compared. The highest percentage absorption values were obtained with corn oil and butter (5.75% and 5.85% respectively). Iron absorption was significantly less when soy oil and coconut oil were used (2.48% and 2.76% respectively).

RATIONALE

Previous investigations have demonstrated that changes in the quantity of corn oil present in a semisynthetic meal containing egg albumen and dextrimaltose have little effect on iron absorption. However, the influence of different fat sources has not been carefully studied in humans. The present study was designed to examine the effect of fats with differing fatty acid compositions (Table 33-1). Corn and soy oils consist almost exclusively of long-chain fatty acids, the predominant one being linoleic. Coconut oil and butter, on the other hand, contain many short-chain fatty acids producing polyunsaturated/saturated ratios of 1:54 and 1:34 respectively. This is in contrast to values of 1:02 and 1:03 for corn and soy oils.

METHOD

Semisynthetic meals containing 29.4 g egg albumen, 66.8 g dextrimaltose (corn syrup solids), and 34.8 g of a fat/oil source were served to eleven volunteers on four occasions. Corn oil was used as the fat source in meal A, soy oil in B, coconut oil in C, and butter in D. No calcium and phosphate salts were added; $FeCl_3$ was incorporated to bring the total iron content to 4.1 mg/meal.

RESULTS

Iron absorption from the meal containing corn oil (A) was 5.75%. Substitution with soy oil or coconut oil significantly decreased absorption by 57% (B) and 52% (C), (meal values 2.48% and 2.76% respectively). Absorption from the meal containing butter was almost the same as that containing corn oil (5.85%).

COMMENT

The results of the present study indicate that soy oil and coconut oil have a significantly inhibitory effect on nonheme iron absorption when compared with corn oil and butter. While these results demonstrate that the fat component of a meal cannot be overlooked when assessing iron availability, they do not provide an explanation for the variation seen with different fat sources. Corn oil and soy oil have a similar composition and yet differed in their effect on iron absorption. The same observation applies to coconut oil and butter. It is possible that the effects observed are a property of non-fat contaminants of the oil and not the fat sources themselves. The soy and coconut oils were produced by a cold pressing technic rather than solvent extraction and evaporation. Protein or carbohydrate contamination could possibly account for part of the effect on iron absorption attributed to these fat sources.

Table 33-1

Fatty Acid Composition of Fats and Oils Tested.

Carbon #: Double Bonds	Name	Corn oil	Soy oil	Coconut oil	Butter
4:0	Butyric				3.5
6:0	Caproic			0.8	1.4
8:0	Caprylic			5.4	1.7
10:0	Capric			8.4	2.6
12:0	Lauric	0.1		42.4	4.5
14:0	Myristic	0.2	0.4	15.5	14.6
16:0	Palmitic	11.4	9.7	10.7	30.2
16:1	Palmitoleic	0.5	1.0	0.5	5.7
18:0	Stearic	2.0	4.0	2.7	10.5
18:1	Oleic	27.5	24.2	6.8	18.7
18:2	Linoleic	57.1	51.7	1.6	2.1
18:3	Linolenic	1.5	7.9		
20+ higher		0.2	2.4		1.6
P/S ratio (polyunsaturated/ saturated fatty acids)		1:0.2	1:0.3	1.54	1.34

(Reference: Table A-6, p. 1060: Goodhard RS and Shils ME, Modern Nutrition in Health and Disease, 5th Ed., Lea and Febiger, Philadelphia, 1974.)

Study 33. Effect of Fats on Iron Absorption.

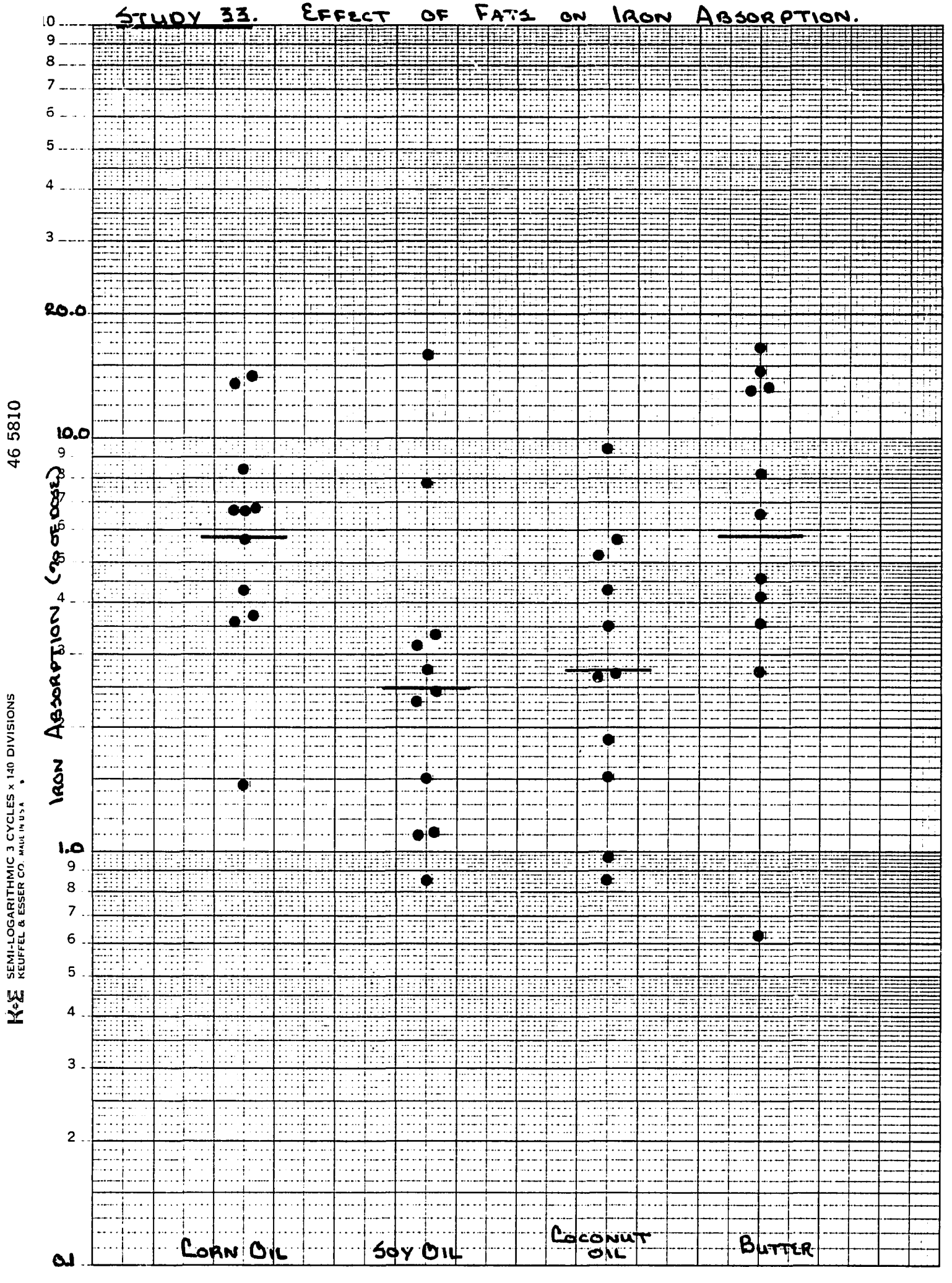
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption: Corn Oil (A) (% of dose)	Soy Oil (B)	Coconut Oil (C)	Butter (D)	Absorption Ratio: B/A	C/A	D/A
1	19	48	36	1.46	1.10	5.71	14.80	0.74	3.91	10.13
2	32	44	139	3.60	2.30	1.52	3.57	0.63	0.42	0.99
3	23	46	107	3.73	3.15	2.68	8.22	0.84	0.71	2.20
4	22	47	114	4.32	1.51	0.97	2.76	0.34	0.22	0.63
5	22	46	51	5.65	2.45	4.30	4.16	0.43	0.76	0.73
6	19	45	94	6.65	1.13	0.86	0.63	0.16	0.12	0.09
7	21	48	100	6.71	3.37	2.70	4.57	0.50	0.40	0.68
8	22	42	35	6.78	2.76	1.88	13.13	0.40	0.27	1.93
9	22	48	118	8.42	0.85	3.51	13.07	0.10	0.41	1.55
10	22	46	88	13.42	10.60	5.21	6.60	0.78	0.38	0.49
11	21	46	41	14.01	7.83	9.45	16.73	0.55	0.67	1.19
Mean*	22	46	75	5.75	2.48	2.76	5.84	0.43	0.48	1.02
			-1 SE	4.74	1.95	2.20	4.36	0.35	0.37	0.72
			+1 SE	6.97	3.14	3.47	7.82	0.53	0.63	1.44

*Geometric means except Age and Packed Cell Volume.

Study 33. Effect of Fats on Iron Absorption.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	2.32	2.08	0.98	0.90	0.42	0.47
-1 SE	1.90	1.60	0.70	0.71	0.31	0.39
+1 SE	2.83	2.70	1.39	1.13	0.59	0.57
1/ Ratio	0.43	0.48	1.015	1.12	2.36	2.11
Student's *t*	4.21	2.78	0.05	0.47	2.64	3.87
p value	0.0009	0.010	0.48	0.33	0.012	0.002
Correlation* (r)	0.59	0.22	0.02	0.49	0.26	0.75
p value	0.028	0.255	0.472	0.064	0.216	0.004

* log scale


STUDY 33. EFFECT OF FATS ON IRON ABSORPTION.
IRON ABSORPTION (% OF DOSE)
20.0
10.0
1.0
0.1
CORN OIL
SOY OIL
COCONUT OIL
BUTTER
46 5810
K+E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

STUDY 34
AVAILABILITY FROM IRON FORTIFIED RICE. II.

SUMMARY

A modified coating process was used to fortify rice with ferrous sulfate. Absorption of iron from the coated rice was compared with that from the nonheme common pool in two meals, one consisting of rice only and the other containing rice, beef, and a source of ascorbic acid. Approximately equal quantities of iron were absorbed from the coated rice and the nonheme common pool in the rice-only meal. In the presence of beef and ascorbic acid, percentage absorption from the nonheme common pool increased almost threefold while absorption from the iron-coated rice showed a small, but statistically insignificant rise. The results suggest that iron absorbed by rice grains during cooking may fail to enter the nonheme common pool and therefore not be affected by factors that enhance nonheme iron absorption.

RATIONALE

The feasibility of fortifying rice with a ferrous sulfate coating without altering the appearance of the rice was first tested in Study 27. However, the fortified product was poorly absorbed. The coating appeared to remain intact during cooking and the iron was not released in time for absorption to occur in the upper small bowel.

In this study, a thinner coat was applied. Preliminary studies demonstrated that this coat protected the iron during washing in cold water but ruptured during cooking which allowed the rice grains to release the contained iron. Two radioiron tags were again used in each of two meals to compare absorption from fortified rice with that in the nonheme common pool in the meal.

DESIGN

Rice was coated in a manner similar to that described in Study 27 except that only six to eight coats of shellac were used. The premix again contained 4 mg iron as ferrous sulfate and 5 uCi ^{55}Fe/gram rice. This premix was then diluted 1:99 with unfortified rice grains. Ten volunteers participated in this two-meal study. The first meal again consisted of 216 g cooked rice (100 g dry weight). Ferrous sulfate labeled with ^{59}Fe was added to the water used in cooking the rice to provide an additional 4 mg iron and 2 uCi ^{59}Fe/serving. All the water was absorbed by the rice during the steam cooking process. The second meal was prepared in the manner described above, except that no iron was added to the cooking water. This meal also contained a 113 g hamburger patty (uncooked weight) and 80 g applesauce. Two microcuries ^{59}Fe and 4 mg iron as $FeCl_3 \cdot 6H_2O$ was added to the applesauce.

RESULTS

Percentage absorption values of 0.71% for iron coated onto the rice and 0.75% for iron added during cooking were very similar, suggesting that the two forms of iron had been absorbed from a common pool. The second meal contained two powerful enhancers of iron absorption, beef and ascorbic acid. However, while the absorption of iron from the common pool (D) increased nearly threefold (2.22%), absorption from the iron coated onto the rice (C) was not significantly changed (0.92%, $p=0.06$). Absorption of the two labels in the second meal was significantly different ($C/D = 0.42$, $p < 0.001$).

COMMENTS

The fortified rice grains in this study were soft after cooking and the coating appeared to have ruptured. The almost identical absorption values obtained from the two isotopes in the first meal suggest that both forms of iron had mixed during cooking and then been reabsorbed randomly into the rice grains. However, in the second meal it was apparent that iron derived from fortified rice was not significantly affected by absorption enhancers operating on the nonheme common pool. It seems unlikely that this was the result of incomplete dissolution of the iron coating since percentage absorption values for the two sources of iron were so similar in the first meal; however, this possibility cannot be excluded completely. It is more likely that the fortification iron was indeed released into the cooking water. If this is so, the results suggest that iron reabsorbed with water into the cooked rice grains is separated from the nonheme common pool and thus unaffected by enhancing ligands; absorption would then be poor irrespective of meal composition. Such a conclusion would have important implications for the use of rice as a fortification vehicle. We are planning a further study to examine this postulate.

Study 34. Absorption from Iron-Fortified Rice.II.

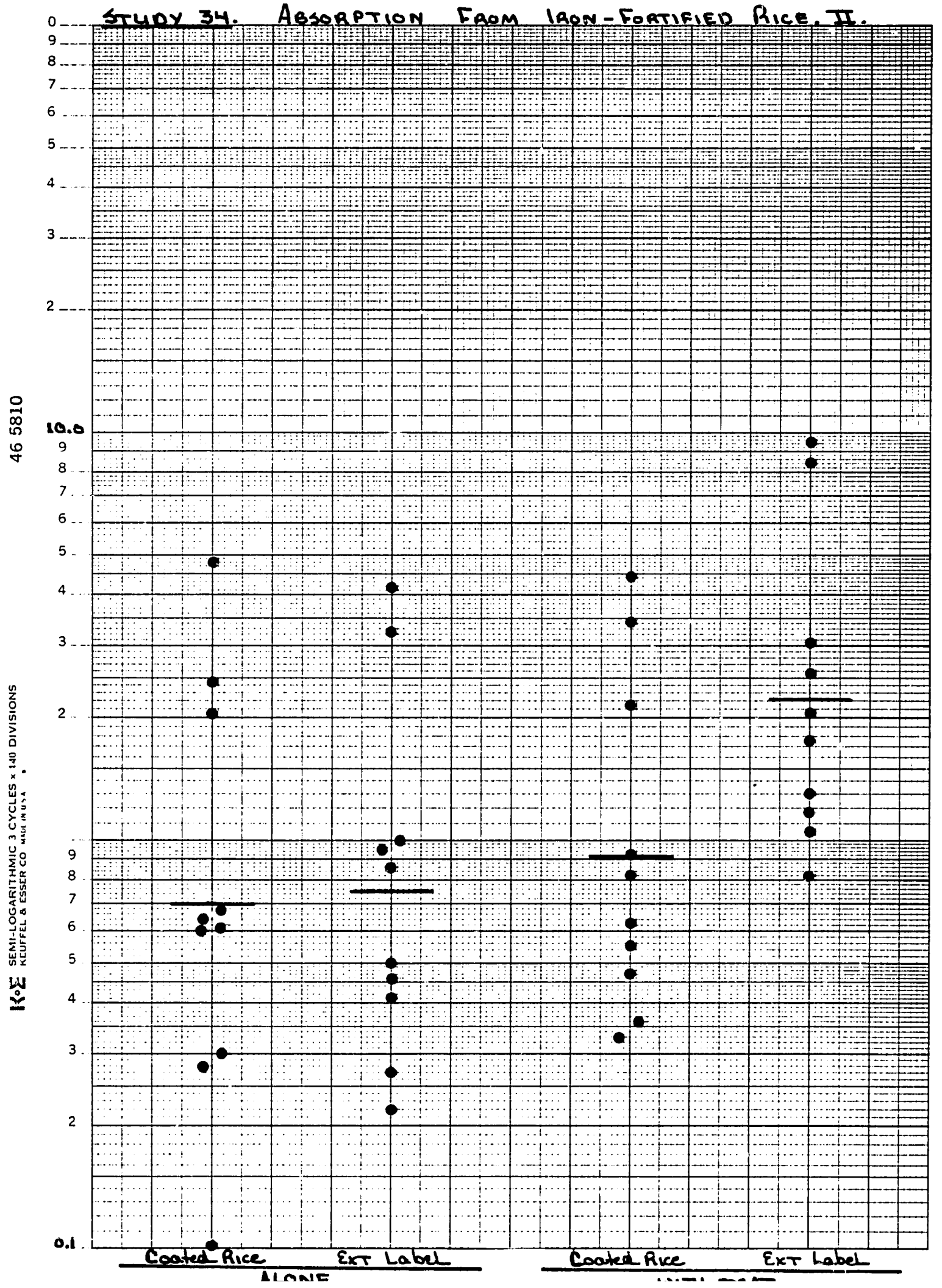
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption (% of dose): Alone, Coated Rice (A)	Alone, Extrinsic Label (B)	With Meat, Coated Rice (C)	With Meat, Extrinsic Label (D)	Absorption Ratio A/B	C/D	C/A	D/B
1	21	46	70	0.10	0.22	0.33	0.82	0.45	0.40	3.30	3.72
2	30	46	59	0.28	0.27	0.47	1.05	1.03	0.44	1.67	3.88
3	21	46	89	0.30	0.46	0.63	1.30	0.65	0.48	2.10	2.82
4	23	44	102	0.60	0.50	0.36	1.17	1.20	0.30	0.60	2.34
5	19	45	46	0.61	0.41	0.55	1.73	1.48	0.31	0.90	4.21
6	23	42	18	0.65	0.95	0.92	2.57	0.68	0.35	1.41	2.70
7	21	47	68	0.68	0.86	0.82	2.05	0.79	0.40	1.20	2.38
8	24	43	65	2.05	1.01	3.45	9.51	2.02	0.36	1.68	9.41
9	18	45	47	2.47	3.25	2.16	3.05	0.76	0.70	0.87	0.93
10	19	45	15	4.83	4.26	4.43	8.43	1.13	0.52	0.91	1.97
Mean*	22	45	50	0.71	0.75	0.92	2.22	0.94	0.42	1.31	2.92
			-1 SE	0.49	0.55	0.69	1.70	0.81	0.39	1.12	2.42
			+1 SE	1.02	1.03	1.24	2.89	1.08	0.45	1.53	3.53

*Geometric means except Age and Packed Cell Volume.

Study 34. Absorption from Iron-Fortified Rice. II.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	0.94	0.77	0.32	0.82	0.34	0.42
-1 SE	0.81	0.65	0.27	0.70	0.28	0.39
+1 SE	1.08	0.90	0.38	0.95	0.41	0.45
1/ Ratio	1.07	1.31	3.12	1.22	2.92	2.39
Student's *t*	0.47	1.69	6.55	.34	5.70	10.91
p value	0.325	0.063	$<10^{-4}$	0.106	0.0001	$<10^{-5}$
Correlation* (r)	0.93	0.91	0.89	0.88	0.80	0.96
p value	$<10^{-4}$	0.0002	0.0003	0.0004	0.003	$<10^{-5}$

* log scale


STUDY 34. ABSORPTION FROM IRON-FORTIFIED RICE. II.
0
9
8
7
6
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0.1
Coated Rice
Ext Label
ALONE
Coated Rice
Ext Label
46 5810
K+E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

STUDY 35
EFFECT OF FIBER ON IRON ABSORPTION. II.

SUMMARY

The effect of three different fiber sources was measured in a meal comprising two muffins and a glass of apple juice. Incorporation of pectin or cellulose into the muffins did not affect absorption whereas the use of bran reduced percentage iron absorption by almost 60%.

RATIONALE

The inhibitory effect of fiber on iron absorption was first examined in Study 29. Unfortunately, absorption from the control meal (two plain muffins) in that study was very low leading to reduced sensitivity for detecting inhibition. For this study, two modifications were made in the composition of the meal in an attempt to elevate basal absorption. Milk was replaced by water in the batter recipe and apple juice containing added ascorbic acid was given with the meal to augment iron absorption. The same fiber sources, bran, pectin, and cellulose, were used again.

DESIGN

Nine volunteers participated in this study. Each of the four test meals consisted of two muffins, two pats of butter, and 180 ml apple juice reconstituted from frozen concentrate. Ascorbic acid (50 mg) and iron (3 mg) in the form of $FeCl_3 \cdot 6H_2O$ containing either ^{55}Fe or ^{59}Fe was added to the apple juice. Meal A consisted of two plain flour muffins. For meals B, C, and D, the following fiber sources were added to the batter (10 g/2 muffins): bran from red spring wheat (standardized bran sample from the American Association of Cereal Chemists, St. Paul MN), citrus pectin (polygalacturonic acid methyl ester, United States Biochemical Corp., Cleveland OH) and α-cellulose, (BW-200 food grade, Solka-floc, Brown Company, Berlin NH) respectively. All muffins were baked at 200°C for 40 minutes the day before they were eaten.

RESULTS

Iron absorption from plain muffins (meal A) was 3.52%. There was no significant change in absorption when pectin or cellulose was

added (3.26% and 3.53% respectively). However, the addition of bran reduced absorption by 57%, to 1.52% (p 0.01).

COMMENT

The results of this study confirm the observations made in Study 29 although the higher basal absorption from plain muffins clearly improved the sensitivity of the test. As in Study 29, iron absorption was inhibited only by bran, the results reaching statistical significance in this study. The purified fiber sources pectin and cellulose had no effect. These results indicate that not all sources of fiber inhibit iron absorption. Thus, the inhibitory effect seen with some high-fiber foods may be a property of the particular type of fiber in those foods. It is also possible that reduced iron availability may not be a direct effect of the fiber but rather the result of an association between the fiber component and some other inhibitory factor.

Study 35. Effect of Fiber on Iron Absorption.II.

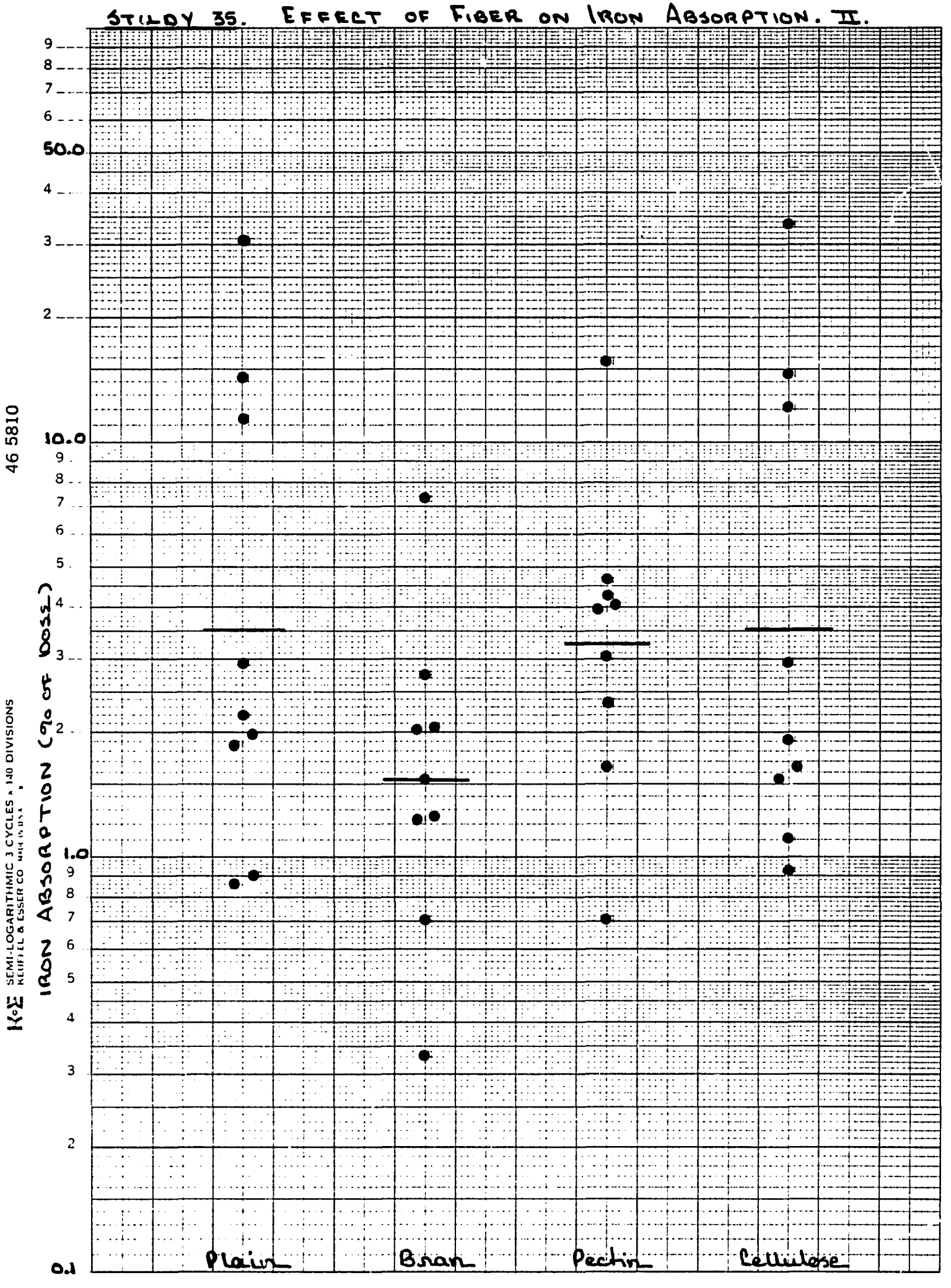
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption (% of dose) Plain (A)	Bran (B)	Pectin (C)	Cellulose (D)	Absorption Ratio B/A	C/A	D/A	B/C	B/D	C/D
1	48	46	135	0.86	0.33	0.71	0.92	0.38	0.82	1.06	0.46	0.35	0.77
2	23	43	50	0.90	1.23	2.35	1.11	1.36	2.61	1.23	0.52	1.10	2.11
3	18	44	45	1.77	1.53	3.93	1.65	0.86	2.22	0.93	0.38	0.92	2.38
4	31	45	73	1.98	2.03	3.03	1.52	1.02	1.53	0.76	0.66	1.33	1.99
5	21	49	76	2.20	0.70	1.66	1.91	0.31	0.75	0.86	0.42	0.36	0.86
6	24	49	104	2.92	1.26	4.08	2.96	0.43	1.39	1.01	0.30	0.42	1.37
7	18	45	36	11.15	2.02	4.66	14.76	0.18	0.41	1.32	0.43	0.13	0.31
8	25	42	33	14.03	2.73	4.28	12.08	0.19	0.30	0.86	0.63	0.22	0.35
9	27	46	7	31.06	7.35	15.80	33.81	0.23	0.50	1.08	0.46	0.21	0.46
Mean*	26	45	49	3.52	1.52	3.26	3.53	0.43	0.93	1.00	0.47	0.43	0.92
			-1 SE	1.14	2.46	2.29	2.31	0.34	0.72	0.95	0.43	0.33	0.71
			+1 SE	2.03	4.32	5.45	5.38	0.55	1.19	1.06	0.51	0.56	1.20

*Geometric means except Age and Packed Cell Volume.

Study 35. Effect of Fiber on Iron Absorption. II.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	2.31	1.08	1.00	0.47	0.43	0.92
-1 SE	1.80	0.84	0.94	0.43	0.33	0.71
+1 SE	2.96	1.39	1.06	0.51	0.56	1.20
1/ Ratio	0.43	0.93	1.00	2.14	2.32	1.08
Student's *t*	3.38	0.31	0.06	9.51	3.19	0.31
p value	0.005	0.38	0.48	$<10^{-5}$	0.006	0.38
Correlation* (r)	0.82	0.82	0.99	0.96	0.80	0.82
p value	0.003	0.003	$<10^{-6}$	$<10^{-4}$	0.004	0.004

* log scale


STUDY 35. EFFECT OF FIBER ON IRON ABSORPTION. II.
IRON ABSORPTION (% OF DOSE)
50.0
10.0
1.0
0.1
Plain
Bran
Pectin
Cellulose
46 5810
K&E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

STUDY 36

EFFECT OF BEEF ON IRON ABSORPTION FROM $FeCl_3$ and NaFeEDTA

SUMMARY

When beef was substituted for egg albumen in the SS meal, mean percentage iron absorption increased from 1.96% to 6.61% (mean ratio 3.35). When the iron in these meals was in the form of NaFeEDTA rather than $FeCl_3$, a similar increase occurred, from 2.09% to 6.79% (mean ratio 3.24). Thus, the enhancing effect of beef was not influenced by the form of the added iron.

RATIONALE

We have previously demonstrated that the enhancing effect of ascorbic acid is largely ablated when iron in a meal is present in the form of NaFeEDTA. In Study 23 we investigated the interaction of NaFeEDTA and another enhancing factor, beef. Percentage iron absorption from a semisynthetic meal (SS) containing beef was 14% less when NaFeEDTA was used instead of $FeCl_3$. When the protein in each of the meals was egg albumen (EA), absorption was significantly better with NaFeEDTA. It was concluded that beef had a smaller enhancing effect on iron absorption in the presence of EDTA. However, values obtained from the two meals were not directly comparable because of differences in the amount of EDTA present. In the present study, we examined the effect of beef on iron absorption from $FeCl_3$ and NaFeEDTA. A constant ratio of EDTA:nonheme iron was maintained in each of the meals.

DESIGN

Four test meals were given to each of 12 male subjects. Meals A and B were the SS meal with full protein (EA) and added inorganic calcium and phosphate. Meals C and D were of similar nutrient composition, but the protein source was beef (Table 36-1). The total nonheme iron content of each meal was adjusted to 4.1 mg by the addition of $FeCl_3$ to meals A, C, and D, and NaFeEDTA to meal B. Thus, meal B contained 21.4 mg EDTA. A similar quantity of EDTA was added as Na_2EDTA to meal D. In Study 14 preformed NaFeEDTA was shown to be absorbed to the same extent as a mixture of Na_2EDTA and $FeCl_3$.

RESULTS

Iron absorption from the SS meal was 1.96%, a typical value for this meal when administered to subjects with normal iron stores. No difference in iron absorption was seen when NaFeEDTA replaced $FeCl_3$ in meal B (2.09%). In meals C and D, the substitution of beef for EA led to a threefold improvement in iron absorption (6.61 and 6.79% respectively). There was no significant difference in percentage iron absorption from these two compounds, and the enhancing effect of beef was similar.

COMMENT

Iron absorption from the SS meal with EA was not affected by the presence of NaFeEDTA in this study, and the value of 2.09% is very similar to the level of 2.05% observed in Study 23. Furthermore, when beef was included, iron absorption from meals with and without EDTA was almost identical and similar to the values obtained for this meal in Study 23 (5.41 and 6.24%). Therefore, it would seem that the apparent decrease in the enhancing effect of beef previously attributed to the chelate in Study 23 may have been artifactual, i.e., the ratio beef meal:EA meal may have been greater with $FeCl_3$ because of a spuriously low absorption value in the EA meal containing $FeCl_3$. The current observations suggest that the enhancing effect of beef is not altered in the presence of EDTA. Thus, while EDTA is deleterious to nonheme iron absorption in a meal containing appreciable quantities of ascorbic acid, no similar disadvantage would exist in meat-containing meals.

Table 36-1 Meal Composition.

Meals A and B

Ingredient	Serving	Prot	Energy	Fat	Ca	P	Fe
	g	g	KCal	g	mg	mg	mg
Egg albumen	36.7	29.4	13.6	0.1	24	40	0.4
Dextrimaltose (Corn Syrup Solids)	66.8		254				
Corn Oil	34.8	30.8	34.8				
$CaHPO_4$ (Anh)	0.60				177	137	
K_2HPO_4	1.33					236	
Water	200						
$FeCl_3 \cdot 6H_2O$	.0178						3.7
NaFeEDTA Meal (B)	(.243)						(3.7)
Total	340	29.4	698	34.9	201	413	4.1

Meals C and D

Lean ground round, boiled liquid protein	94	29.4	178	5.7	12	252	0.61
Corn Syrup Solids	69.0		262				
Corn Oil	29.2		258	29.2			
$CaHPO_4$(Anh)	.645				190	147	
K_2HPO_4(Anh)	.084					15	
Water	100						
$FeCl_3 \cdot 6H_2O$ Meal C and D	.0169						3.5
Na_2EDTA Meal D	.0273						
Total	200	29.4	698	34.9	202	414	4.1

Study 36. Effect of Beef on Iron Absorption from $FeCl_3$ and NaFeEDTA.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption (% of dose) SS/EA $FeCl_3$(A)	SS/EA NaFeEDTA(B)	SS/Beef $FeCl_3$(C)	SS/Beef NaFeEDTA(D)	Absorption Ratio A/B	C/D	C/A	D/B
1	28	45	65	0.16	0.82	5.46	3.31	0.19	1.64	34.12	4.03
2	23	50	67	0.37	1.08	1.06	1.62	0.34	0.65	2.86	1.50
3	31	44	60	0.56	0.67	2.88	4.37	0.83	0.65	5.14	6.52
4	22	48	31	0.95	1.18	9.60	7.23	0.80	1.32	10.10	6.12
5	21	42	83	1.53	1.30	2.81	3.17	1.17	0.88	1.83	2.43
6	23	46	28	1.95	2.35	2.36	16.82	0.82	0.14	1.21	7.15
7	21	48	42	2.13	3.80	4.86	3.61	0.56	1.34	2.28	0.95
8	23	50	82	2.95	1.65	9.20	5.33	1.78	1.72	3.11	3.23
9	24	47	80	6.82	3.57	8.35	4.47	1.91	1.86	1.22	1.25
10	18	47	25	6.85	10.38	14.12	10.67	0.65	1.32	2.06	1.02
11	28	48	17	8.02	1.15	37.13	16.75	6.97	2.21	4.62	14.56
12	18	45	26	15.00	12.55	33.62	70.16	1.19	0.47	2.24	5.59
Mean*	23	47	44	1.96	2.09	6.61	6.79	0.94	0.97	3.36	3.24
			-1 SE	1.32	1.59	4.86	5.07	0.72	0.78	2.56	2.52
			+1 SE	2 91	2.75	8.99	9.10	1.22	1.22	4.42	4.18

*Geometric means except Age and Packed Cell Volume.

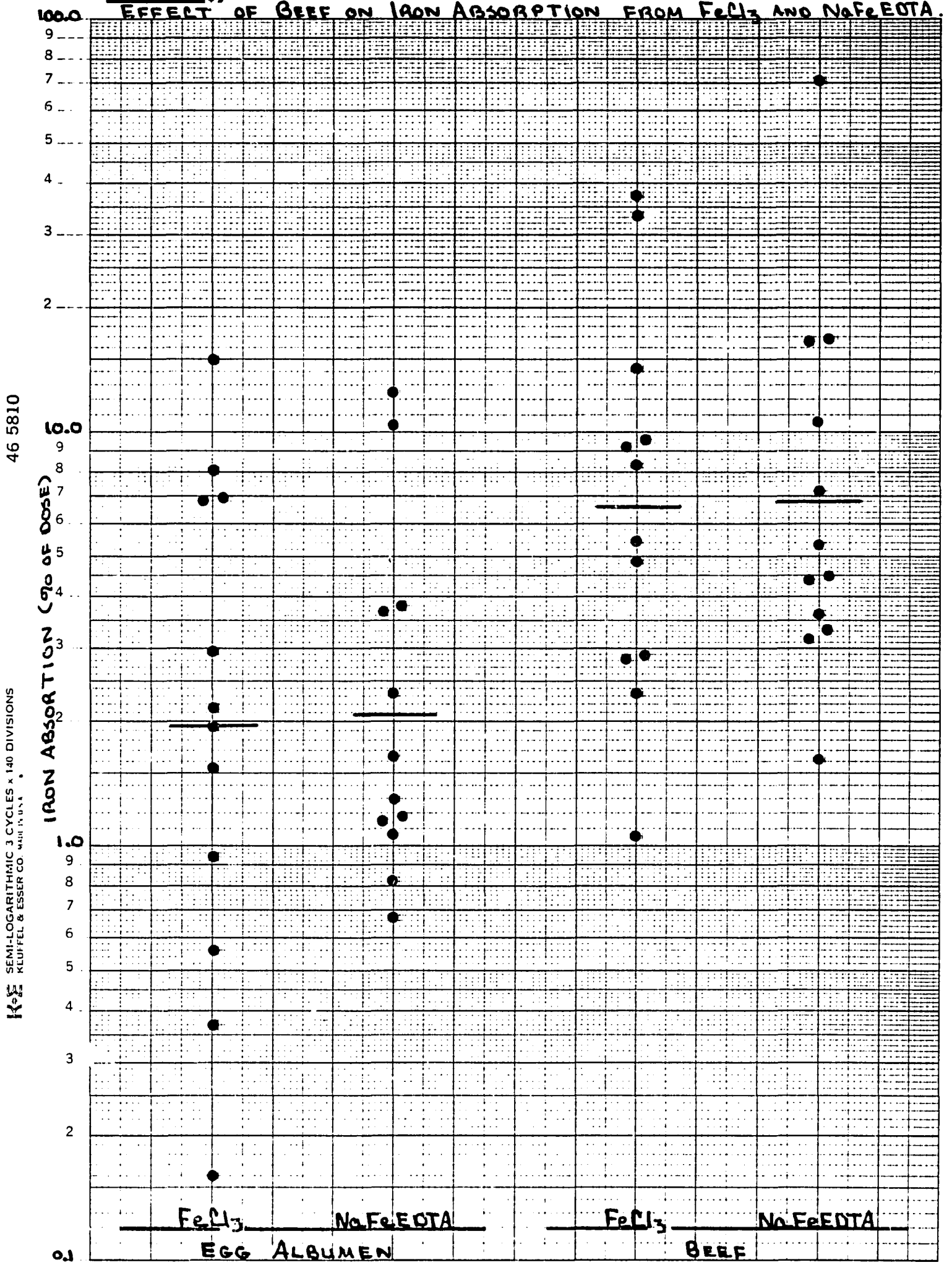
Study 36. Effect of Beef on Iron Absorption from $FeCl_3$ and NaFeEDTA

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	0.94	0.30	0.29	0.32	0.31	0.97
-1 SE	0.72	0.23	0.22	0.24	0.24	0.78
+1 SE	1.22	0.39	0.38	0.43	0.40	1.22
1/ Ratio	1.07	3.36	3.46	3.16	3.24	1.03
Student's *t*	0.25	4.43	4.44	3.85	4.63	0.12
p value	0.405	0.0005	0.0005	0.001	0.0004	0.451
Correlation* (r)	0.75	0.72	0.71	0.48	0.60	0.72
p value	0.002	0.004	0.005	0.569	0.020	0.004

* log scale

STUDY 36.

EFFECT OF BEEF ON IRON ABSORPTION FROM $FeCl_3$ AND NaFeEDTA.



STUDY 37

EFFECT OF COMPONENTS OF CSM ON IRON ABSORPTION

SUMMARY

Several studies have demonstrated poor iron availability from the infant food supplement, CSM. Inorganic calcium and phosphorus in this product are responsible in part for this effect. Nevertheless, even when calcium and phosphorous are omitted from these foods, absorption is still relatively low. The effect of selectively omitting the major components of CSM was examined in this study. The omission of soy led to a twofold increase in iron absorption while the deletion of corn or milk had no significant effect.

RATIONALE

Infant food supplements providing a source of calories, high quality protein, and certain vitamins and minerals are widely distributed to developing countries for use in child feeding programs. Several studies carried out under this contract have demonstrated that the iron in several different products is poorly available (studies 2,6,7). Although 100 g dry supplement contains 18 mg iron, most of it as ferrous fumarate, we have estimated that only 0.3 to 0.7 mg iron would be absorbed by iron deficient children (1).

Inorganic calcium and phosphate are responsible for part of the low absorption values (2). However, the results of Study 2 demonstrate that even though the omission of added calcium and phosphorous from the formulation of CSM leads to a twofold increase in iron absorption, the actual percentage absorption remains low (0.9%). In the present study, we examined the effect of the major components of CSM on iron absorption by separately omitting each of them from the mixture.

DESIGN

Four test meals, in the form of either a hot porridge or a drink, were administered to each of 10 volunteers. Meal A consisted of the standard formulation for CSM while corn meal (CM), dried skim milk (DSM), and soy flour (soy) were omitted from meals B, C, and D respectively (Table 37-1). Water, sugar, salt, and soy oil were added to all meals during cooking. The vitamin and mineral mixes normally included in the complete product were omitted. Meals A, C, and D were prepared as a porridge by boiling the ingredients for 5 minutes; the porridge was then weighed out

into individual servings, allowed to cool overnight, and reheated in a microwave oven before serving. Meal B was prepared one day ahead and served as a cold drink. All meals were extrinsically labeled with 4.0 mg iron ($FeCl_3 \cdot 6H_2O$), and either ^{55}Fe or ^{59}Fe in 1 ml 0.01 N HCl was added immediately prior to serving and mixed thoroughly in the meal.

RESULTS

Iron absorption from CSM (meal A) was 0.57%. No significant change occurred when CM or DSM were left out of the formulation (meal B, 0.59%, meal C 0.45, $p > 0.1$). However, when soy flour was omitted, absorption doubled to 1.18% (D/A = 2.05, $p > 0.001$).

COMMENT

The significant inhibitory effect of soy products was again demonstrated in this study. Of the three protein sources, soy had the greatest inhibitory effect on iron absorption. However, it is noteworthy that only 1.18% of the iron was absorbed in the absence of soy, indicating relatively low availability from the other two components as well. It is important to point out that the omission of soy from CSM would be of little practical value since it is an essential source of protein in this product. Other means of improving iron availability should be sought. It is possible that absorption would have increased if the vitamin and mineral mix containing 20 mg ascorbic acid had been included in the 50 g CSM serving.

REFERENCES

1. Morck TA, Lynch SR, Skikne BS, Cook JD. Iron availability from infant food supplements. Am J Clin Nutr 1981 (in press).

2. Monsen ER, Cook JD. Food iron absorption in human subjects. IV. The effects of calcium and phosphate salts on the absorption of nonheme iron. Am J Clin Nutr 1976;29:1142-8.

Table 37-1

COMPOSITION OF TEST MEALS

Ingredient (g/serving)	MEAL A(All)	B(-CM)	C(-DSM)	D(-soy)
Corn meal, gelatinized (CM)	30.45	-	30.45	30.45
Defatted, toasted soy flour (soy)	9.0	9.0	9.0	-
Dried skim milk (DSM)	7.7	7.7	-	7.7
Soy oil	2.85	2.85	2.85	2.85
Sugar	20	20	20	20
Salt	0.5	0.5	0.5	0.5
Water	225	100	175	175
Total Meal	295.5	139.5	237.8	236.5
Total protein content, (g)	9.5	6.8	6.8	5.4

Study 37. Effect of Components of CSM on Iron Absorption.

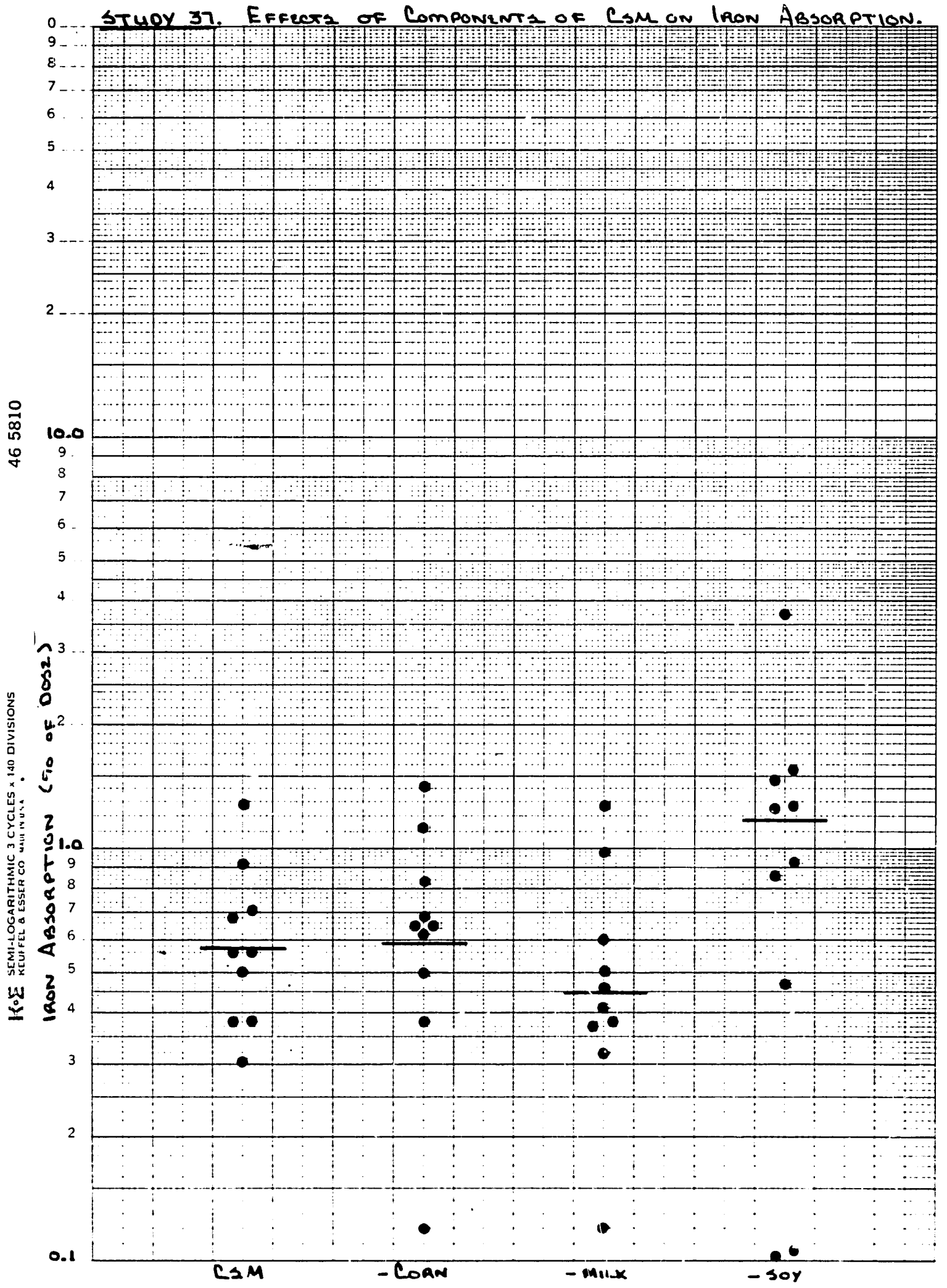
Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption: Corn-Soy-Milk (CSM) Total(A) (% of dose)	-Corn(B)	-Milk(C)	-Soy(D)	Absorption Ratio B/A	C/A	D/A
1	22	50	98	0.31	0.68	0.12	0.47	2.19	0.38	1.51
2	23	47	15	0.[illegible]	0.38	0.50	1.27	1.00	1.31	3.34
3	21	45	20	0.[illegible]	0.12	0.60	1.05	0.31	1.57	2.76
4	23	46	14	0.50	0.65	0.37	0.86	1.30	0.74	1.72
5	24	48	30	0.56	0.65	0.97	1.53	1.16	1.73	2.[illegible]3
6	18	46	107	0.56	0.83	0.41	1.47	1.48	0.73	2.62
7	21	45	38	0.68	0.50	0.32	0.92	0.73	0.47	1.35
8	25	48	74	0.71	0.62	0.38	1.01	0.87	0.53	1.42
9	24	45	8	0.92	1.41	1.27	3.73	1.53	1.38	4.05
10	18	43	26	1.27	1.12	0.46	1.26	0.88	0.36	0.99
Mean*	22	46	31	0.57	0.59	0.45	1.18	1.03	0.79	2.05
			-1 SE	0.50	0.47	0.37	1.00	0.87	0.65	1.77
			+1 SE	0.65	0.73	0.55	1.40	1.22	0.95	2.37

*Geometric means except Age and Packed Cell Volume.

Study 37. Effect of Components of CSM on Iron Absorption.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	0.97	1.27	0.49	1.30	0.50	0.38
-1 SE	0.82	1.05	0.42	0.98	0.40	0.36
+1 SE	1.15	1.53	0.56	1.73	0.62	0.42
1/ Ratio	1.03	0.79	2.05	0.77	1.99	2.60
Student's *t*	0.18	1.25	4.97	0.94	3.18	10.69
p value	0.431	0.122	0.0004	0.187	0.006	$<10^{-5}$
Correlation* (r)	0.62	0.43	0.56	0.76	0.37	0.90
p value	0.269	0.106	0.047	0.417	0.149	0.0002

* log scale


STUDY 37. EFFECTS OF COMPONENTS OF CSM ON IRON ABSORPTION.
IRON ABSORPTION (% OF DOSE)
0
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8
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6
5
4
3
2
10.0
9
8
7
6
5
4
3
2
1.0
9
8
7
6
5
4
3
2
0.1
CSM
- CORN
- MILK
- SOY
46 5810
K+E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

STUDY 38
IRON AVAILABILITY OF THAI MEALS

SUMMARY

Iron absorption from three typical Thai meals was measured in six volunteers who had an average reference absorption value of 21.6% which is typical for men with normal iron stores. Absorption values were 4.95% for rice and soup, 2.41% for a beef-noodle dish, and 6.22% for a meal containing rice and curried vegetables. These values are considerably higher than those obtained for typical Filipino meals (studies 15 and 16) and Egyptian meals (Study 30). However, because of low iron content (1.4-2.7 mg/meal), the quantity of absorbed iron is insufficient to maintain normal iron balance.

RATIONALE

An accurate knowledge of the amount and bioavailability of iron in typical meals is necessary for the development of effective fortification strategies for combatting iron deficiency anemia in developing countries. The purpose of this study was twofold; first, to define bioavailability of iron in three meals commonly eaten in Thailand, and second, to examine the validity of performing iron absorption measurements in normal American voluteers rather than in subjects in a developing country by repeating the study in Thai subjects using identical meals prepared from indigenous ingredients. Theoretically, any difference in iron status will be eliminated by expressing food iron absorption as a ratio to the absorption from the reference dose of inorganic iron. It is hoped that comparable studies will be done in Thailand using foods purchased locally.

DESIGN

Six volunteers ate three Thai meals each prepared by Ms. Malee Sritongkul from Bangkok, Thailand, who spent a year in our laboratory learning various techniques for studying iron nutrition. Meal A was a soup made with ground pork boiled in water and served over rice with green onions as a garnish. A dish composed of rice noodles, chopped collard greens, and strips of beef round steak were stir fried together in a skillet and served as meal B. For meal C, cabbage, cauliflower, and young corn were boiled in a curry sauce and served over rice. Nutrient composition of the three meals is given in Table 38-1. A trace amount of iron (0.1 mg iron as $FeCl_3 \cdot 6H_2O$) together with the radioiron tag was added to each meal. In meal A, this was added to the soup. For meals B and C, the iron was sprinkled over each serving and mixed into the meal. Bowls were rinsed twice with water which was then drunk by each subject for a quantitative transfer. The fourth meal, was the REF dose of 3 mg ferrous iron and 18.9 mg freshly prepared ascorbic acid.

RESULTS

Relatively high percentage absorption values were obtained for all the meals, 4.95% for rice soup in meal A, 2.41% for iron in noodles with beef (meal B), and 6.22% for rice and curried vegetables in meal C. The mean value for the REF meal of 21.6% is comparable to the values for this meal in other studies.

COMMENT

Percentage iron absorption for the Thai meals was higher than the majority of meals tested from other developing countries. In studies 15 and 16, Filipino meals gave percentage absorption values between 1.13 and 1.71% with the exception of one meal where the figure was 3.49%. In Study 30, percentage absorption from all three Egyptian meals was between 1.41 and 1.49%.

The reason for the relatively high availability of iron in these meals has not been precisely defined. However, it seems likely the meat may have enhanced the absorption from meals A and B while the ascorbic acid content may have been an important factor in meal C since the vegetables are not extensively cooked. The significantly lower absorption in meal B is striking. One important difference between this meal and the others is the use of noodles made from rice flour rather than the presence of cooked rice grains. Rice flour has been shown to be inhibitory to iron absorption with a mean value of 0.4% being obtained with rice flour and 6-9% with intact rice in Thai meals (Hallberg et al, 1976).

The iron content of these meals is quite low, 1.4-2.3 mg. Their caloric content is also low, but the additional calories in Thai meals would probably be derived from a greater quantity of rice

which would provide little iron. (The iron content shown in Table 38-1 is for rice fortified with iron; unfortified rice contains only 0.2 mg iron/100 g cooked rice). Therefore, despite the relatively high percentage absorption, only 0.24 mg iron would be absorbed from the total of 5.2 mg in all three meals. This is well below the amount required to maintain normal iron balance.

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Table 38-1. Major Components of Thai Meals and Nutrient Composition per Serving.

Ingredient	Amt (g)	Energy (kcal)	Prot (g)	Fat (g)	Total CHO (g)	Fiber (g)	Iron Total (mg)	Iron Non-heme (mg)	Vit C
Meal A									
Pork, ground	40	123	6.3	11	0	0	0.9	0.5	0
Rice, long grain, enriched	200	218	4.0	0	48	0.2	1.8	1.8	0
	240	341	10.3	11	48	0.2	2.7	2.3	0
Meal B									
Beef, round steak, fried	30	57	9.4	2	0	0	1.1	0.5	0
Collard Greens, cooked	75	22	2.0	0	4	0.6	0.4	0.4	34
Noodles, rice flour	70	108	2.0	0	24	0	0.6	0.6	0
	175	187	13.4	2	28	0.6	2.1	1.5	34
Meal C									
Rice, long grain enriched	100	109	2.0	0	24	0.1	0.9	0.9	0
Young corn, boiled	25	5	0.7	0	1	0.2	0.3	0.3	4
Cauliflower, boiled	17	4	0.4	0	1	0.2	0.1	0.1	13
Cabbage, boiled	33	7	0.4	0	2	0.3	0.1	0.1	14
	175	125	3.5	0	28	0.8	1.4	1.4	31

Study 38. Iron Availability from Thai Meals.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ug/l)	Iron Absorption (% of dose) Meal A*	Meal B*	Meal C*	REF D	Absorption Ratio A/D	B/C	C/D	A/B	A/C	C/B
1	25	46	98	0.85	0.42	1.71	11.20	0.07	0.03	0.15	2.02	0.49	4.07
2	19	44	90	4.90	2.26	4.91	10.80	0.45	0.20	0.45	2.16	0.99	2.17
3	21	46	29	5.33	1.41	9.33	37.28	0.14	0.03	0.25	3.78	0.57	6.61
4	18	47	28	5.98	5.36	13.96	30.32	0.19	0.17	0.46	1.11	0.42	2.60
5	26	44	39	9.83	8.85	8.76	28.40	0.34	0.31	0.30	1.11	1.12	0.98
6	20	48	35	11.28	3.13	6.05	26.26	0.42	0.11	0.23	3.60	1.86	1.93
Mean+	26	46	46	4.95	2.41	6.22	21.60	0.23	0.11	0.29	2.05	0.80	2.58
			-1 SE	3.40	1.55	4.61	17.36	0.11	0.08	2.92	1.65	0.63	1.97
			+1 SE	7.22	3.74	8.37	26.90	0.31	0.16	4.13	2.55	1.00	3.36

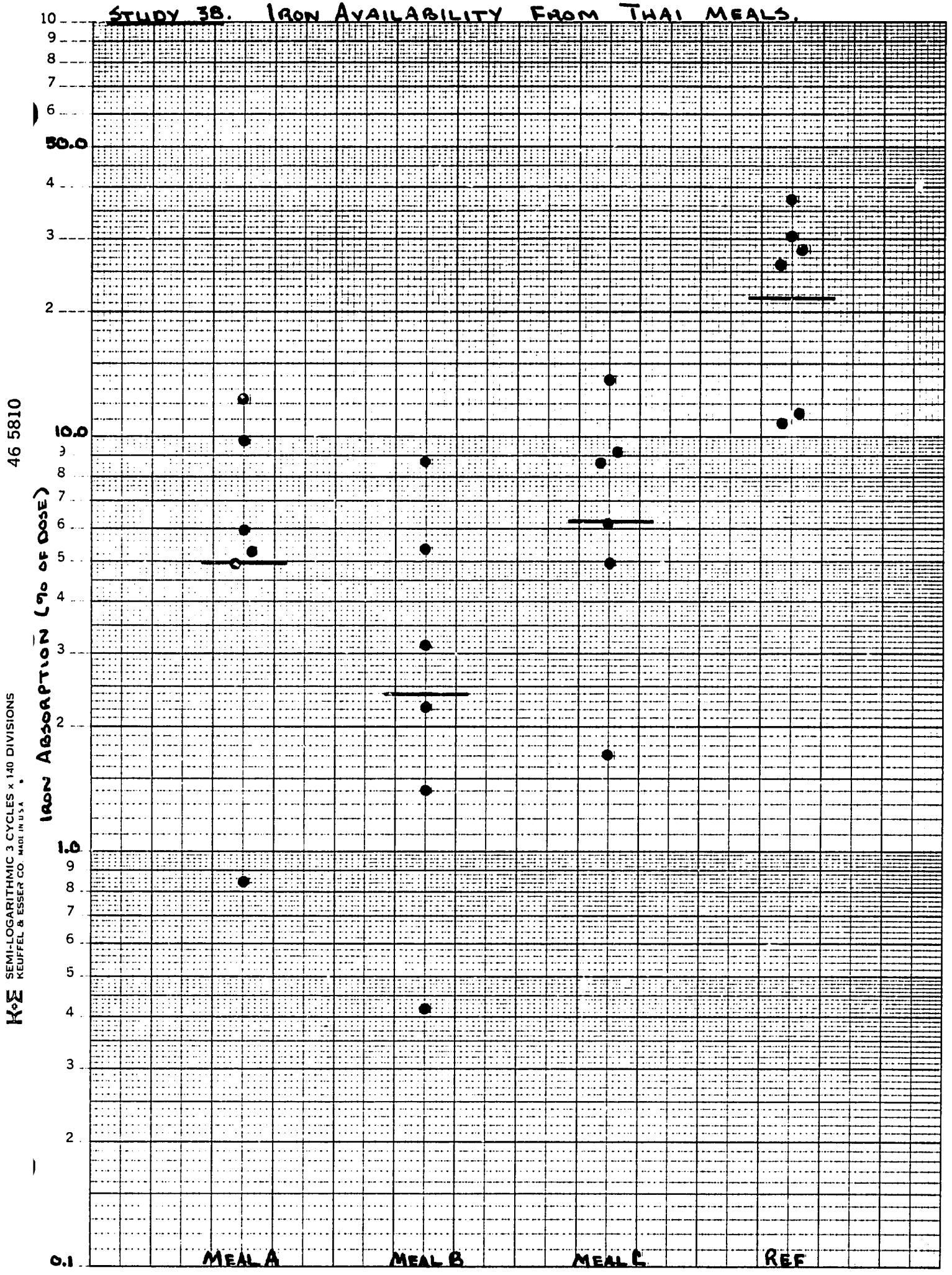
*Meal contents are described in Summary.

+Geometric values except Age and Packed Cell Volume.

Study 38. Iron Availability from Thai Meals.

	ABSORPTION RATIOS					
	A/B	A/C	A/D	B/C	B/D	C/D
Mean Ratio	2.05	0.80	0.23	0.39	0.11	0.29
-1 SE	1.65	0.63	0.17	0.30	0.08	0.24
+1 SE	2.55	1.00	0.31	0.51	0.16	0.34
1/ Ratio	0.49	1.26	4.37	2.58	8.95	3.47
Student's *t*	3.27	0.99	5.11	3.55	5.98	7.17
p value	0.011	0.185	0.002	0.008	0.0009	0.0004
Correlation* (r)	0.87	0.79	0.65	0.80	0.55	0.82
p value	0.013	0.031	0.081	0.027	0.130	0.024

* log scale


STUDY 38. IRON AVAILABILITY FROM THAI MEALS.
IRON ABSORPTION (% OF DOSE)
50.0
10.0
1.0
0.1
MEAL A
MEAL B
MEAL C
REF
46 5810
K+E SEMI-LOGARITHMIC 3 CYCLES x 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

APPENDIX B

- PUBLICATIONS -

DIETARY INHIBITORS OF IRON ABSORPTION IN MAN

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It is now well known that when several food items are ingested in the same meal, a common pool of nonheme iron is formed, the absorption of which is measured by extrinsic radioiron tagging (Cook et al, 1972; Bjorn-Rasmussen et al, 1972). Studies of food iron absorption with this technic have focused primarily on dietary constituents that enhance the absorption of nonheme iron. Of these, ascorbic acid and animal tissue including fish and poultry are of overriding importance. In fact, a method has recently been proposed for predicting iron absorption from a meal based on its content of ascorbic acid and animal tissue (Monsen et al, 1978). This model is an important advance because it focuses on iron availability rather than total iron content. However, the model is oversimplified because many dietary factors, especially substances that inhibit iron absorption, are not taken into account. These inhibitory factors are the subject of the present review.

In evaluating the effect of an inhibitor or an enhancer of iron absorption, it is necessary to choose a basal meal that is presumably 'neutral' with respect to iron absorption. The degree of inhibition/enhancement will undoubtedly depend on the choice of this reference meal. For example, the effect of an inhibitor may be much less dramatic in a meal that also contains an enhancer such as ascorbic acid or animal protein. The use of baseline

meals with different levels of availability undoubtedly explains some of the conflicting reports on the potency of dietary inhibitors. The major known inhibitors of food iron absorption are listed in the following table.

Dietary Inhibitors of Iron Absorption

	potency
Egg	++
Bran	+++
Phytate	±
EDTA	±
Ca/PO_4	±
Tea	+++
Coffee	++
Protein	
Ovalbumin	+
Soy	+++

Eggs. Early studies employing biosynthetic tagging indicated that iron in egg, especially in yolk, is poorly assimilated. For example, the addition of an egg to a meal consisting of bread reduced percentage absorption from 5.1 to 0.9% (Elwood et al, 1968). Peters et al (1971) reported that the major inhibitory effect was in egg yolk. The addition of egg white to ferrous ascorbate reduced percentage absorption from 33 to 14% whereas the addition of yolk caused a decrease to 3.9%. The inhibiting effect is attributed to vitellin, the major phosphoprotein complex in egg yolk. A less dramatic inhibiting effect has been observed in more recent studies. When a boiled egg was added to a continental breakfast, Rossander et al (1979) observed a de-

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crease in absorption that was not statistically significant. In a study by Cook and Monsen (1976a), a powdered egg was found to be no more inhibitory than whole milk or egg albumin. These inconsistencies may relate to the nature of the meal used to examine the effect of egg

Bran. In the classic chemical balance studies performed by Widdowson and McCance (1942), it was noted that less iron is absorbed from a diet containing brown bread than one containing white bread. In later studies using extrinsic radioiron tagging, the degree of inhibition in nonheme iron absorption was proportional to the amount of bran added. Bjorn-Rasmussen (1974) reported that the addition of 1.7, 3.3, 6.7, and 10% bran produced a decrease in absorption of 18, 31, 57, and 54% respectively. In studies done in our laboratory, we observed a 74% decrease in percentage absorption of nonheme iron, from 2.4 to 0.6%, when 12 g bran was added to a meal containing a milkshake and muffins. When the meal contained an absorption enhancer the inhibiting effect was less pronounced. For example, when meat was added to the meal containing 12 g bran, absorption decreased from 3.5 to 1.7% (51%). When 100 mg ascorbic acid was added to the meals, absorption with bran fell from 9.3 to 3.9%, a 58% decrease (unpublished results).

Most workers have attributed the inhibiting effect of bran to its phytate content. We attempted to assess the role of phytate by incubating bran at 37°C for 16 hours to allow destruction of phytate by endogenous enzymatic hydrolysis. Although the phytate content was reduced from 139 to 9 mg, the inhibiting effect was very similar to that of whole bran. In another study, dephytinized bran was separated into soluble, phosphate-rich and insoluble, high fiber fractions. Studies with these two components demonstrated that the major inhibitory effect resided with the soluble fraction.

EDTA. Interest in the effect of EDTA on iron absorption has been stimulated by its suggested use for iron fortification. A significant inhibition in absorption was observed when Na_2EDTA was added to a

meal containing either common food items or semi-purified ingredients. Nonheme iron absorption decreased by 20 and 50% with molar ratios for EDTA:iron of 1:1 and 2:1 respectively (Cook and Monsen, 1976b).

More recent studies, however, have suggested that EDTA may sometimes enhance iron absorption. Layrisse and Martinez-Torres (1977) observed that the absorption of 5-50 mg iron as NaFeEDTA was twofold higher than when iron was added as ferrous sulphate. Viteri et al (1978) reported 0.6% absorption when 5 mg NaFeEDTA was added to a milk-rice-sugar formula, compared to 0.3% when ferrous sulfate was added. The effect of EDTA on nonheme iron absorption is apparently related to both the total content of EDTA and its molar ratio to iron. When the molar ratio of EDTA:iron is less than 1, absorption from the meal may be enhanced, particularly when basal availability is low.

Calcium and Phosphate. There are conflicting reports on the effect of inorganic calcium and/or phosphate on iron absorption. Peters et al (1971) suggested that phosphate compounds strongly inhibit iron absorption. More recently, Cook and Monsen (1976) observed a 50% decrease in iron absorption when 178 mg calcium and 138 mg phosphorous were added to a meal containing semipurified ingredients. However, separate addition of soluble calcium or phosphates had little effect on iron absorption. The formation of an insoluble complex of calcium and phosphate may have bound and insolubilized the iron. Additional studies in this area are necessary because of the extensive use of phosphate compounds as food additives.

Tea and Coffee. Tea is one of the most potent inhibitors of food iron absorption. So striking is its inhibitory effect that regular consumption of the beverage has been proposed as a means of decreasing the absorption of dietary iron in patients with iron loading disorders (Alarcon et al, 1979). In the original description of its inhibiting effect, the addition of a cup of tea to a meal containing radiolabeled bread decreased percentage absorption from 10 to 2% (Disler et al, 1975a). In a

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subsequent study in rats, no inhibiting effect was seen with tannate-free tea or with caffeine whereas tannins extracted from tea and tannic acid produced a marked inhibition of iron absorption (Disler et al, 1975b). This effect is due to the formation of a black insoluble precipitate of iron tannate. Tannins are widely distributed throughout the vegetable kingdom and may be responsible, at least in part for the low availability of iron in these foods

We have observed in our laboratory a similar inhibiting effect of coffee. When a cup of coffee was taken with a meal containing meat, a 40% decrease in absorption was observed, compared to a 65% decrease when taken with tea. These observations are relevant to the possible use of sugar as an iron fortification vehicle because a large quantity of sugar is consumed in these beverages.

Protein. Because animal tissue enhances iron absorption, protein is generally regarded as having an enhancing effect on iron absorption. However, studies with a semipurified meal containing dextrimaltose, corn oil, and egg albumin, have demonstrated that of these, egg albumin is the only significant inhibitor (Monsen and Cook, 1979). When the protein source was omitted from the meal, absorption increased from 3.8 to 9.6%; when the protein level was doubled, absorption decreased significantly from 2.3 to .4%. These results indicate that if protein *per se* has an effect on iron absorption, it is an inhibitory one.

We have recently studied the effect of other semipurified proteins and have found an even more striking inhibition by soy protein. When various soy products (including fat-free soy flour, textured soy protein, and isolated soy protein) were substituted for ovalbumin, a 70-80% inhibition in absorption was observed. Similarly, a 50-60% decrease was seen when soy protein was substituted for 30% of the meat protein. These findings have implications for iron fortification programs because infant protein supplements contain high concentrations of soy protein.

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Summary

Whereas the effect of certain dietary enhancers of iron absorption such as ascorbic acid and animal tissue are well defined, the effects of inhibiting substances in our diet have not yet been fully characterized. The most potent inhibitors are egg, bran, tea, coffee, and soy products. The effect of these substances should be included in models to estimate food iron availability.

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IN VITRO MEASUREMENT OF FOOD IRON AVAILABILITY

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1.14

Dietary iron content cannot be used as the sole measure of nutritional adequacy for this element. Heme in the hemoglobin and myoglobin of meat is the only form of iron in food that is virtually always well absorbed. However, heme constitutes only a small fraction of dietary iron even in Western nations and little or no meat is eaten in many developing countries. All other forms of iron (collectively called nonheme iron) are considerably less well absorbed and their absorption can be altered markedly by changes in the composition of the meal in which they are eaten (from -80% to + 500%, Bothwell et al, 1979). These observations have focused the attention of nutritionists on the importance of the bioavailability of nonheme iron.

The most reliable method for determining bioavailability is the measurement of absorption in human volunteers using a radioisotopic technic. However, the method is too costly and time consuming for the screening of large numbers of food items. Therefore, other methods have been sought. Animal models have been used extensively, but they are also expensive. Moreover, physiological differences between animals and man with respect to iron absorption have cast doubt on some of the results obtained (Amine et al. 1972, Narasinga Rao et al. 1977, Finch et al. 1978). The importance of the chemical nature of food iron to its absorption has long been appreciated (Elvehjem et al. 1933, Shackleton and McCance 1936), but early attempts at defining bioavailability in vitro were hampered by an incomplete understanding of dietary factors governing food iron absorption. The application of radioisotopic technics has demonstrated that nonheme iron is absorbed from a common pool and has led us to a better appreciation of the paramount importance of the interaction of different meal components with this pool (Bothwell et al, 1979). Such studies have provided the appropriate physiological background for a re-examination of the nature of bioavailable iron.

We used this information to study the potential value of an in vitro technic for predicting the effect of known enhancers and inhibitors of iron absorption. Two enhancers (ascorbic acid and beef) and five inhibitors (egg albumen, calcium and phosphate salts, tea, coffee, and isolated soya bean protein) for which human absorption data were available were studied. In each case absorption from the same meal eaten with and without the relevant factor had been measured in a single group of volunteers. We used identical ingredients to prepare similar meals. After the addition of an extrinsic radioiron tag the meals were homogenized. An aliquot was then mixed with 0.17 N hydrochloric acid containing 1% porcine pepsin. Following incubation at 37° for 30 min, the pH was adjusted to approximately 6 with an acetate buffer. After centrifugation, the supernate was incubated with bathophenanthrolene dissolved in 40% ethanol. The proportion of radioiron which became bound to bathophenanthrolene was measured after extraction of the complex into isoamyl alcohol and expressed as a percentage of the radioiron in the original aliquot. This was assumed to represent the percentage of "bioavailable iron".

A comparison between the ratio of percentage available iron in meals with:without the specified factor with similar ratios derived from human absorption studies showed good agreement in almost all cases demonstrating that the in vitro test was sensitive to known enhancers and inhibitors of food iron absorption and that the information had quantitative value. The most significant discrepancy was seen with coffee which had a greater inhibitory effect on absorption than on predicted bioavailability in vitro. We believe that this technic represents a promising approach to the development of an assay for predicting food iron availability.

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REDUCTION OF THE SOY-INDUCED INHIBITION OF NONHEME IRON ABSORPTION[1,2,3]

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ABSTRACT

Recent studies in normal men have demonstrated that soy products have a pronouced inhibitory effect on the absorption of nonheme dietary iron. The purpose of the present investigation was to explore approaches to overcoming this inhibition. A modest but significant increase in iron absorption was observed when test meals containing either isolated soy protein or whole soybeans were baked at 200°C. The addition of 100 mg ascorbic acid to a meal containing isolated soy protein had a more dramatic effect, increasing mean absorption from 0.6 to 3.2%. A similar but less pronounced increase was observed when meat was added to the isolated soy protein meal. These results indicate that the inhibitory effect of soy can be reversed to some extent by substances known to enhance nonheme iron absorption.

INTRODUCTION

In a recent study comparing the effects of semipurified proteins, we observed that several soy products inhibited nonheme iron absorption (1). When protein equivalent amounts were substituted for egg albumen (EA) in a meal containing no meat products, decreases in iron absorption ranged from 65% to 92%. Similarly, when 30 g dry textured soy flour was added to a meal containing either 100 g or 70 g ground beef, absorption was reduced by 61 and 53% respectively. Since soy products are a good source of high quality protein, the feasibility of counteracting their inhibitory effect on iron absorption by dietary manipulation merits careful consideration. Two approaches were examined in this report: 1) extensive heating of soy products to destroy possible inhibitory compounds, and 2) adding enhancers of iron absorption to meals containing soy products to improve iron availability.

METHODS

Subjects

Twenty-six subjects ranging in age from 18 to 50 years participated in three separate iron absorption studies. All were in good health and denied a history of gastrointestinal disorders that might affect iron absorption. Iron status was uniformly normal with the exception of one volunteer who had a serum ferritin level of 2 ug/l, indicative of markedly reduced iron

stores. Written informed consent was obtained from each volunteer before the study. Experimental procedures were in accordance with the standards of the Human Subjects Committee at the University of Kansas Medical Center.

In study I (9 subjects), iron availability from whole soybeans and from a meal containing isolated soy protein (SP) was determined both before and after extensive baking of the soy component. The other two studies, each with 7 subjects, examined the effect of adding the known iron absorption enhancers ascorbic acid (study II) and beef (study III) to semi-purified meals containing either EA or SP as the protein source.

Absorption Measurements

Double sequential radioiron labels were used to measure iron absorption from four separate test meals in each subject. All meals were administered between 7 and 9 AM following an overnight fast, and only water was allowed for the subsequent 3 hours. The absorption of nonheme iron was measured by adding variable quantities of $FeCl_3$ in 1 ml 0.01 N HCl labeled with either 2 uCi ^{59}Fe or 5 uCi ^{55}Fe to the test meals. Percentage absorption was determined from incorporated red cell radioactivity as described previously (1,2).

Test Meals

With the exception of meals C and D in study I which contained whole soybeans, all test meals consisted of a mixture of semi-purified ingredients which were thoroughly mixed and served as a drink (SS meal). This meal contained corn syrup solids (Dri-Sweet 36, Hubinger Co., Keokuk, IA) and corn oil which provided 66.8 g carbohydrate and 34.8 g fat respectively. The protein component, a variable under study, consisted of either 18.4 g spray-dried EA (Monark Egg Corporation, Kansas City, MO) or 16.9 g SP (Supro 710, Ralston Purina Co., St. Louis MO). Both of these products provided 14.7 g protein per meal.

In the first part of study I (meals A and B) the effect of baking SP on iron absorption from the SS meal was examined. A slurry of SP in water was prepared (1:3 by weight). It was spread onto a lightly greased cookie sheet and baked for one hour at 200°C. The dried residue was finely ground with a mortar and pestle and added to the other meal ingredients. Baking destroyed the water dispersibility of SP resulting in a gritty precipitate that settled out of the liquid meal upon standing. Taste was not appreciably altered. Ferric chloride providing 2.0 mg iron and the radioiron label were added to both meals bringing the total iron content to 4.0 mg per serving.

The effect of heating whole soybeans was studied in the second part of study I (meals C and D). Dried soybeans (Arrowhead Mills, Inc., Herford TX) were first soaked in water overnight. This water was discarded and fresh water added together with diced onions, salt, monosodium glutamate and bay leaves. After boiling for two hours, the liquid was decanted and 100 g portions of boiled soybeans were weighed out. Additional 100 g portions were placed on greased cookie sheets and baked at 200°C for 45 min with periodic turning. Average weight per serving of these light brown, crunchy, toasted soybeans was 38 g, indicating a 62% loss of moisture. Individual portions of boiled or baked beans were served with 100 ml deionized water containing 3.0 mg iron.

Erythrocyte radioiron concentration was measured by the method of Eakins and Brown (3) and serum ferritin by the 2-site radioimmunometric technic (4). The frequency distribution of iron absorption measurements when expressed as a percentage of the administered dose is logarithmic (5). Absorption data were therefore converted to logarithms for statistical analysis and the results reconverted to recover the original units. When comparing absorption between any pair of test meals in the same subjects, a paired *t*-test was used to determine whether mean log of the meal ratios differed significantly from zero. This is equivalent to testing whether the mean ratio in percentage absorption differed from 1.0.

RESULTS

Effect of Baking (Study I). Mean absorption from the SS meal containing uncooked SP (meal A) averaged 0.64% (Table 1). When SP was baked (meal B), mean absorption increased to 1.28%. The absorption ratio B:A (1.99) was highly significant ($p < 0.001$). A similar but less pronounced increase in absorption was observed when boiled soybeans (meal C) were further baked (meal D). Mean absorption from meal C averaged 1.06% and increased to 1.60% after baking (meal D), (ratio D:C = 1.50, $p < 0.05$).

Effect of Ascorbic Acid (Study II). The effect of adding 100 mg ascorbic acid to the SS meal containing either SP (meals A and B) or EA (meals C and D) was examined. Absorption from the SP meal without ascorbic acid was very low, averaging 0.56% (Table 2). When ascorbic acid was added, absorption increased significantly to 3.20% (meal ratio B:A = 5.69, $p < 0.001$). Absorption of 5.05% iron from meal C containing EA was much higher than from meal A containing SP. With the addition of ascorbic acid (meal D), absorption increased over 2-fold to 10.19% (D:C = 2.2, $p < 0.001$).

The value of 0.11 for mean absorption ratio A:C indicates a 90% inhibition of iron absorption when SP was substituted for EA in the SS meal. The degree to which 100 mg ascorbic acid reduced this inhibitory effect is indicated by the absorption ratio B:C

= 0.63. Although iron absorption from meal B with SP and ascorbic acid remained 37% lower than from Meal C with EA alone, this difference was not statistically significant.

Effect of Beef (Study III). A 100 g ground beef patty was broiled and served with the SS meal containing SP (meal B) or EA (meal D). Meals A and B contained 2.1 mg nonheme iron derived from SP while EA provided 0.2 mg iron in meals C and D. In meals B and D, the beef contributed 1.5 mg total iron and 0.5 mg nonheme iron. Sufficient $FeCl_3$ was added to all the meals to raise the iron content to 5.6 mg.

When beef was added to the EA meal, absorption increased by only 26% (5.94% to 7.47%; $p < 0.05$) (Table 3). On the other hand, absorption increased nearly fourfold, from 0.36% to 1.44% ($p < 0.001$) when beef was added to the meal containing SP. Absorption from meal B (SP + beef) expressed as a ratio to that of meal C (EA alone) equals 0.25 showing a highly significant difference ($p < 0.001$). Therefore, the enhancing effect of beef failed to completely reverse the inhibiting effect of SP in this meal.

DISCUSSION

We recently reported an inhibitory effect of soy products on nonheme iron absorption (1). The observations made in that

report, although similar to findings described here, are at variance with some earlier studies in which much better absorption from soy-based meals was recorded (6,7). It seemed possible that the discrepancy might have resulted from differences in the methods used to prepare test meals containing soybeans. In the earlier investigations, ground soybeans were boiled in water to a thick paste and then baked at 150-200°C for 45-60 min. Iron absorption from these baked soybean meals was approximately 2.6 times greater than absorption from boiled beans in a later study performed in Jamaican children (8). In the present study, baking soybeans produced only a modest 50-100% increase in iron absorption and the iron was still considerably less available than it was in the SS meals containing EA. It should also be noted that extensive heating is not a practical solution for preparations like SP since unacceptable changes such as reduced water dispersibility occur in the physical properties of the product.

Another approach to counteracting the inhibitory effect of soy is to add substances that enhance the absorption of nonheme iron. The two that are most effective are ascorbic acid and meat (2). When 100 mg ascorbic acid was added (study II), iron absorption from the SP meal was only slightly lower than from the meal with

EA and no ascorbic acid (3.20% vs. 5.05% or 0.13 mg vs. 0.21 mg iron), a difference that is not statistically significant. On the other hand, when beef was added to the meal with SP (study III), only 1.44% (0.07 mg) nonheme iron was absorbed compared to 5.94% (0.33 mg) from the meal containing EA alone. It is apparent from this study that the small enhancing effect of beef would be of little practical value for counteracting the inhibition caused by SP.

Our observations indicate that ascorbic acid partially reverses the soy-induced inhibition of nonheme iron absorption although the increase in amount of iron absorbed may be considerably less than when the same amount of ascorbic acid is added to a less inhibitory meal. For example, the addition of ascorbic acid to the soy-based meal in study II resulted in a more than 5-fold increase in percentage absorption compared to only a 2-fold increase when it was added to the meal with EA. However, the absolute increase in absorbed iron was only 0.11 mg in the case of the soy meal as compared to 0.2 mg with EA.

There are several practical limitations to the use of ascorbic acid for enhancing iron absorption. It is expensive and unstable in oxidizing environments. Sayers et al. (6) reported no improvement in iron absorption when 100 mg ascorbic acid was

added to soy flour before it was boiled and baked; 85% of the reduced form of the vitamin was lost during cooking. The enhancing effect of ascorbic acid is better preserved when the food is only boiled (6). Furthermore, much of the vitamin would probably be lost during storage.

Another approach to overcoming the low availability of iron in soy-containing meals that should be considered is the addition of fortification iron to the meal. This would supply additional iron if percentage absorption remained constant. Because of the low availability of iron in these meals, relatively large amounts of fortification iron would be needed. The use of iron in a form such as sodium iron EDTA merits consideration since absorption of iron from this compound is less affected by inhibitory ligands in cereals than more conventional forms of fortification iron (9).

It is clear from our studies that neither extensive heating nor the addition of the two major enhancers of iron absorption can completely circumvent the inhibitory effect of soy products on iron absorption. Our studies emphasize the need for a better understanding of the factor or factors responsible for this effect in order to develop effective strategies for combatting it.

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ble 1. Effect of cooked isolated protein and whole soybean on iron absorption (Study I)

				IRON ABSORPTION							
				Isolated Soy Protein		Whole Soybean		Absorption Ratio			
bject	Age	Packed Cell Volume (%)	Serum Ferritin (ng/ml)	Uncooked (A)	Cooked (B)	Boiled (C)	Baked (D)	B/A	D/C	C/A	D/B
				(% of dose)							
1	22	41	14	0.26	0.51	0.63	0.96	1.96	1.52	2.42	1.88
2	27	46	172	0.37	1.16	1.37	0.72	3.13	0.52	3.70	0.62
3	20	45	55	0.52	1.08	1.38	2.63	2.07	1.90	2.65	2.43
4	29	46	132	0.53	1.48	0.85	0.97	2.79	1.14	1.60	0.65
5	19	45	61	0.68	2.45	0.18	0.92	3.60	5.11	0.26	0.37
6	35	44	35	0.70	1.15	4.06	7.07	1.64	1.74	5.80	6.14
7	18	46	32	0.85	1.67	1.65	1.65	1.96	1.00	1.94	0.98
8	25	46	139	0.87	0.83	1.02	1.82	0.95	1.78	1.17	2.19
9	20	46	56	2.10	2.61	1.43	2.02	1.24	1.41	0.68	0.77
MEAN	24	45	46*	0.64*	1.28*	1.06*	1.60*	1.99*	1.50*	1.65*	1.25*
			-1 SE	0.53	1.08	0.80	1.26	1.73	1.23	1.21	0.93
			+1 SE	0.78	1.52	1.41	2.02	2.30	1.84	2.25	1.67

* geometric mean

Table 2. Effect on iron absorption of 100 mg ascorbic acid added to semisynthetic meals made with isolated soy protein and with egg albumen.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ng/ml)	IRON ABSORPTION (% of dose) Isolated Soy Protein Alone (A)	Isolated Soy Protein +AA (B)	Egg Albumen Alone (C)	Egg Albumen +AA (D)	Absorption Ratio B/A	D/C	A/C	B/D
1	24	41	2	0.23	4.91	5.77	11.53	21.34	1.99	0.03	0.85
2	26	42	38	0.33	1.93	6.37	8.67	5.84	1.36	0.05	0.30
3	33	42	49	0.36	1.25	8.93	11.75	3.47	1.31	0.04	0.13
4	50	43	72	0.43	1.56	3.36	7.65	3.62	2.27	0.12	0.46
5	24	45	15	0.46	11.01	3.53	10.33	23.93	2.92	0.13	3.11
6	19	47	29	0.48	3.68	2.06	5.17	7.66	2.50	0.23	1.78
7	24	47	130	0.80	3.40	4.90	12.43	4.25	2.53	0.16	0.69
8	25	44	72	0.82	2.47	3.20	8.15	3.01	2.54	0.25	0.77
9	26	42	24	0.82	2.97	13.38	19.82	3.62	1.48	0.06	0.22
10	20	44	32	0.90	4.32	4.90	14.86	4.80	3.03	0.18	0.88
11	23	45	36	1.45	4.53	6.63	8.68	3.12	1.30	0.21	0.68
MEAN	27	44	31*	0.56*	3.20*	5.05*	10.19*	5.69*	2.02*	0.11*	0.63*
			-1 SE	0.47	2.66	4.31	9.14	4.55	1.83	0.09	0.49
			+1 SE	0.66	3.84	5.91	11.37	7.10	2.23	0.14	0.83

* geometric mean

Table 3. Effect on iron absorption of 100 g beef added to semisynthetic meals made with isolated soy protein and with egg albumen.

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ng/ml)	IRON ABSORPTION (% of dose) Isolated Soy Protein Alone (A)	Isolated Soy Protein +Beef (B)	Egg Albumen Alone (C)	Egg Albumen +Beef (D)	Absorption Ratio B/A	D/C	A/C	B/D
1	23	45	89	0.11	0.28	2.05	4.12	2.54	2.00	0.05	0.06
2	28	44	76	0.12	1.01	3.90	6.68	8.41	1.71	0.03	0.15
3	23	50	39	0.18	0.75	4.02	8.67	4.16	2.15	0.04	0.08
4	21	44	80	0.35	0.88	1.65	1.27	2.51	0.76	0.21	0.69
5	24	46	94	0.75	3.45	6.22	4.07	4.60	0.65	0.12	0.84
6	19	44	45	0.77	2.36	17.48	20.71	3.06	1.18	0.04	0.11
7	19	45	18	1.95	8.48	45.61	51.03	4.34	1.11	0.04	0.16
MEAN	22	45	55*	0.36*	1.44*	5.94*	7.47*	3.90*	1.26*	0.06*	0.19*
			-1 SE	0.24	0.94	3.79	4.75	3.33	1.05	0.05	0.13
			+1 SE	0.55	2.20	9.30	11.76	4.58	1.50	0.08	0.28

* geometric mean

IRON AVAILABILITY FROM INFANT FOOD SUPPLEMENTS[1-3]

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Key Words: Iron, CSM (or Corn-Soya-Milk), Infant Foods

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Abstract

Radioiron absorption tests were performed in human volunteer subjects to measure iron absorption from five infant food supplements. These products included corn-soya-milk, corn-soy blend, wheat-soy blend, wheat protein concentrate blend, and whey-soy drink mix. In iron replete adult males, mean percentage absorption ranged from 0.6 to 1.4%. By relating these results to absorption from a reference dose of inorganic iron, it was estimated that iron deficient infants would absorb between 1.7 and 4.1% of the iron contained in the infant foods. These results indicate that this fortification iron is poorly absorbed, and probably supplies only about half of the daily iron requirement for iron deficient children between 6 months and 3 years of age.

Introduction

Food supplements are distributed to undernourished infants and children in developing countries as part of the Food for Peace Program of the U.S. Department of Agriculture. Corn meal, wheat flour or protein concentrate, soy flour, non-fat dried milk, and sweet cheese whey are combined in varying proportions. The foods are distributed in dry form and are either diluted in water and consumed as a drink or porridge, or mixed with other foods to enhance protein and caloric intake.

Iron, as ferrous fumarate, is added at a level of 7.8 mg iron/50 g. Ferrous fumarate is well absorbed in the fasting state (1), but its assimilation when added to food has not been adequately studied. In the present study, extrinsic radioiron tagging was employed to measure iron absorption from the following five infant food supplements: corn-soya-milk (CSM) (2), corn-soy blend (CSB) (3), wheat-soy blend (WSB) (4), wheat protein concentrate blend (WPC) (5), and whey-soy drink mix (WSD) (6). The protein content of these foods is listed in Table 1.

Methods

Studies were performed in 27 adult male volunteers ranging in age from 18 to 45 years. All subjects gave written informed consent prior to their participation in the study and procedures followed were in accord with the ethical standards of the Human Subjects Committee of the University of Kansas Medical Center. None had a

history of hematologic abnormalities or disorders known to influence gastrointestinal absorption. Iron status was judged to be normal in 25 of the subjects on the basis of hematocrit, transferrin saturation, and serum ferritin. Two had reduced iron stores as determined by serum ferritin levels of 12 and 13 ug/l.

The subjects were divided into two groups of 13 and 14 individuals each. Four separate iron absorption tests were performed in each subject using dual radioiron labels. In both groups, one of the test meals consisted of CSM, the most widely distributed infant food supplement. The second and third test meals differed in the two studies, consisting of WSB and WPC in Study 1 and CSB and WSD in Study 2. In both, the fourth test meal was a reference dose (REF) of inorganic radioiron containing 3 mg iron as ferrous sulfate and 19 mg freshly dissolved ascorbic acid. The effect of differences in iron status between the two study groups was minimized by expressing iron absorption from the infant foods as a ratio with respect to REF absorption. CSM was administered in both studies to gauge the accuracy of this adjustment.

All test meals were administered between 7 and 9 a.m. following an overnight fast and water alone was allowed for 3 hours. The infant foods were prepared the day before serving, weighed into individual portions, and refrigerated overnight. Each meal contained 50-59 g dry product, 20 g sucrose, 1 g salt, and 170-225 ml water. Mineral and vitamin mixes were included in the product formulation and

brought the total content to 9 mg iron and 20 mg ascorbic acid per serving. The meals were tagged extrinsically by adding 0.1 mg iron as ferric chloride in 1 ml 0.01 N HCl and either 2 uCi ^{59}Fe or 5 uCi ^{55}Fe (7). WSD was served as a cold beverage and the remaining foods were heated in a microwave oven and served as a hot porridge.

Blood was drawn on the first day of the study for measurement of serum iron, iron-binding capacity, serum ferritin, and background radioactivity. Meals A and B, tagged separately with either ^{59}Fe or ^{55}Fe, were then administered on two successive days. Blood was obtained 14 days later to measure incorporated red cell radioactivity and meals C and D were then given on two successive days. A final blood sample was drawn 2 weeks later to measure the rise in circulating red cell radioactivity. All radioiron assays were performed on duplicate 10 ml samples of whole blood using a modification of the method of Eakins and Brown (8). Percentage absorption in each subject was calculated on the basis of a blood volume estimated from the height and weight (9). Red cell incorporation of absorbed radioactivity was assumed to be 80% (10). Serum iron was measured by the ICSH method (11), iron-binding capacity by a magnesium carbonate technic (12), and serum ferritin by a 2-site immunoradiometric assay (13).

Because of the highly skewed distribution of iron absorption data when expressed as a percentage of the administered dose, statistical analysis was performed on logarithms and the results transformed to

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antilogarithms to recover the original units (14). One-way analysis of variance was used to compare absorption from the five infant foods after adjustment for REF absorption.

Results

Iron status measurements and iron absorption values for both studies are listed in Table 2. Iron absorption from all supplements was remarkably low; nearly two-thirds of the absorption values were less than 1%. Geometric mean values ranged from 0.6% for CSM in Study 2 to 1.4% for the same food in Study 1. Absorption from the REF dose averaged 26.8 and 13.4% in studies 1 and 2 respectively. This difference is presumably related to differences in iron status even though mean serum ferritin levels in the two groups do not reflect such a difference.

Absorption data were corrected for differences in iron status by expressing each value as a ratio with respect to REF absorption (Figure 1). The accuracy of this correction can be assessed by comparing the absorption ratios obtained for CSM in study 1 and 2. Uncorrected percentage absorption from CSM differed by 60% in the two studies whereas the absorption ratios of .052 and .043 differed by less than 20% (Table 3). One-way analysis of variance was performed on logarithms of absorption ratios and showed marginal significance ($F=2.70$, $P<.05$). However, Duncan's multiple range test (15) failed

to show a significant difference between any of the infant supplements.

Discussion

An important advantage of expressing absorption in relation to a reference dose is that percentage absorption can be predicted in subjects with differing iron status. The relatively low means for REF absorption of 27 and 13% in the present study are typical values for iron replete individuals. A much higher percentage REF absorption of about 60% would be observed in the iron deficient children who would receive these food supplements. Iron absorption from each of the foods has been calculated for REF absorption of 60% in Table 3. Values range from 1.7% with both WSB and WPC to 4.1% for CSB; intermediate values of 3.1 and 2.6% were obtained with CSM and 2.9% for WSD. The corrected values are remarkably similar despite variations in the composition of each supplement. Thus, children who take 100 g supplement daily might derive only 0.30-0.74 mg iron daily from these infant foods.

The extremely low assimilation of iron contained in infant foods was unexpected. Nevertheless, these results are comparable to those obtained in a previous study in Jamaican infants reported by Ashworth and March (16). Absorption from CSM averaged 6% in that study compared to a mean REF absorption of 64%. When adjusted to 60% REF absorption, CSM absorption was 5.7% in comparison with lower values

of 2.6 and 3.1 observed in the present study. Much of the disparity between the two studies can be explained by the fact that arithmetic means rather than geometric means were calculated in the report by Ashworth and March. Geometric values are invariably lower than arithmetic means, especially when percentage absorption is low. Their data can be corrected to geometric means by assigning values of 0.1% to two subjects reported as absorbing no iron. When adjusted to REF absorption of 60%, absorption from CSM in iron deficient children averages 3.1% as compared to values of 2.6 and 3.1% obtained in iron replete males in the present study. The remarkable similarity in results obtained in subjects with widely different iron status underscores the importance of REF corrections. It also demonstrates the validity of measuring food iron availability in subjects who may differ greatly from the population that will consume the food. Further validation will be difficult because of the concern in administering radioisotopes to children.

Another aspect of methodology relating to the present study is that absorption of the fortification iron, ferrous fumarate, was measured by adding an extrinsic radioiron tag (ferric chloride) rather than using radiolabeled ferrous fumarate. Previous studies have shown that complete exchange occurs between dietary iron and fortification iron when the latter is added as ferric chloride or ferrous sulfate (17,18). However, an extrinsic tag may not exchange completely with fortification iron when the latter is highly insoluble such as ferric

orthophosphate or sodium iron pyrophosphate (17). There is no evidence that an extrinsic tag is less well absorbed than conventional forms of added fortification iron. If the extrinsic tag did not exchange completely with ferrous fumarate in this study, falsely high rather than low absorption values would have been observed.

The recommended intake of iron is 15 mg/day for children 6 months to 3 years of age (19, 20). These values are based on the assumption that 10% of dietary iron is absorbed by the average child although 2-3 times this amount may be absorbed by iron deficient children (21,22). The results of the present study indicate that the iron added to infant food supplements may not fulfill the daily requirement of 0.7 to 1.0 mg absorbed iron (23). Since we estimated that iron deficient children would absorb only 0.3 to 0.7 mg iron from 100 g of product, the WHO/UNICEF recommended quantity of 80-100 g CSM/day might satisfy only about one-half of their iron requirements. It is unlikely that the typical diet in a developing country would furnish the balance of daily iron needs.

There are at least two factors which could account for the low availability of fortification iron added to infant foods. Calcium and phosphorous salts impair the absorption of food iron when combined in the same meal but not when added separately (24). Specifications for the mineral mixes used in each of the five

products tested in this study require the inclusion of either tricalcium phosphate or a combination of calcium carbonate and dicalcium phosphate. We have recently observed a substantial increase in iron absorption from CSM when these minerals are omitted (unpublished observation). A second possibility for low absorption in this study is the presence of soy protein. We have noted a striking inhibition of food iron absorption with a variety of soy products ranging from whole soybean to isolated soy protein (unpublished observation). The importance of this inhibitory effect of soy on iron absorption from infant foods cannot be assessed from the data contained in this report because soy protein contributed between 45-75% of the total protein in each of the five infant foods examined.

Poor availability of fortification iron is not in itself adequate justification for eliminating calcium and phosphate or soy protein from infant foods. Availability could be improved by increasing the level of ascorbic acid in the vitamin mix (25). Alternatively, it may be possible to add a form of iron, the absorption of which is less inhibited by food. Recent studies suggest that sodium iron EDTA may be less inhibited than other iron salts in certain diets and may therefore enhance iron absorption from infant foods (26,27).

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Table 1. Percentage of total protein contributed by major ingredients.

	CSM	CSB	WSB	WPC	WSD
Soy	45	63	55	61	74
Cornmea	27	37			
Milk	28				
Wheat			45	39	
Whey					26
Total %	100	100	100	100	100

TABLE 2. Iron bioavailability from infant food supplements.

Subject	Age	Packed Cell Volume (%)	Serum Iron (μg/dl)	TS[a] (%)	Serum Ferritin (ng/ml)	Iron Absorption (% of dose)			
STUDY 1						CSM	WSB	WPC	REF
1	18	48	164	41	56	0.58	0.72	0.71	10.86
2	18	47	104	23	58	0.62	0.70	1.58	32.33
3	24	51	113	31	113	0.65	0.28	0.42	18.96
4	20	48	172	46	105	0.70	0.40	0.45	27.18
5	18	46	84	21	37	0.71	0.47	1.51	42.88
6	31	45	142	39	107	1.00	0.61	0.33	11.46
7	19	50	106	29	39	1.03	0.35	0.67	23.40
8	19	46	119	34	44	1.28	1.22	0.47	19.66
9	20	46	100	25	54	2.16	0.91	1.20	51.46
10	20	49	123	34	40	2.17	0.58	0.40	23.00
11	21	49	89	24	67	2.22	0.45	0.31	33.25
12	18	50	134	40	41	4.10	1.93	0.97	46.83
13	20	45	150	39	13	12.51	9.68	7.08	49.35
MEAN[b]	21	48	123	33	52	1.40	0.76	0.77	26.82
STUDY 2						CSM	CSB	WSD	REF
1	26	44	144	35	126	0.03	0.58	0.37	11.35
2	45	41	136	49	86	0.12	0.28	0.13	3.11
3	27	41	156	46	62	0.27	0.45	0.38	11.37
4	24	48	118	28	24	0.33	0.50	0.62	12.87
5	27	44	99	25	81	0.41	0.25	0.23	1.22
6	22	42	118	36	79	0.47	0.28	0.17	5.70
7	27	42	69	21	26	0.66	0.46	0.28	17.00
8	25	46	173	44	28	0.71	1.28	0.80	14.71
9	25	44	140	38	30	0.76	0.75	0.68	23.78
10	24	47	86	23	72	1.13	1.86	1.25	18.42
11	29	40	108	30	43	1.32	1.77	1.50	32.82
12	24	42	112	32	65	1.56	1.80	5.67	41.53
13	42	46	168	57	56	2.17	3.73	0.82	17.27
14	31	45	94	25	12	4.22	10.40	4.63	62.47
MEAN[b]	28	44	123	35	48	0.57	0.92	0.65	13.36

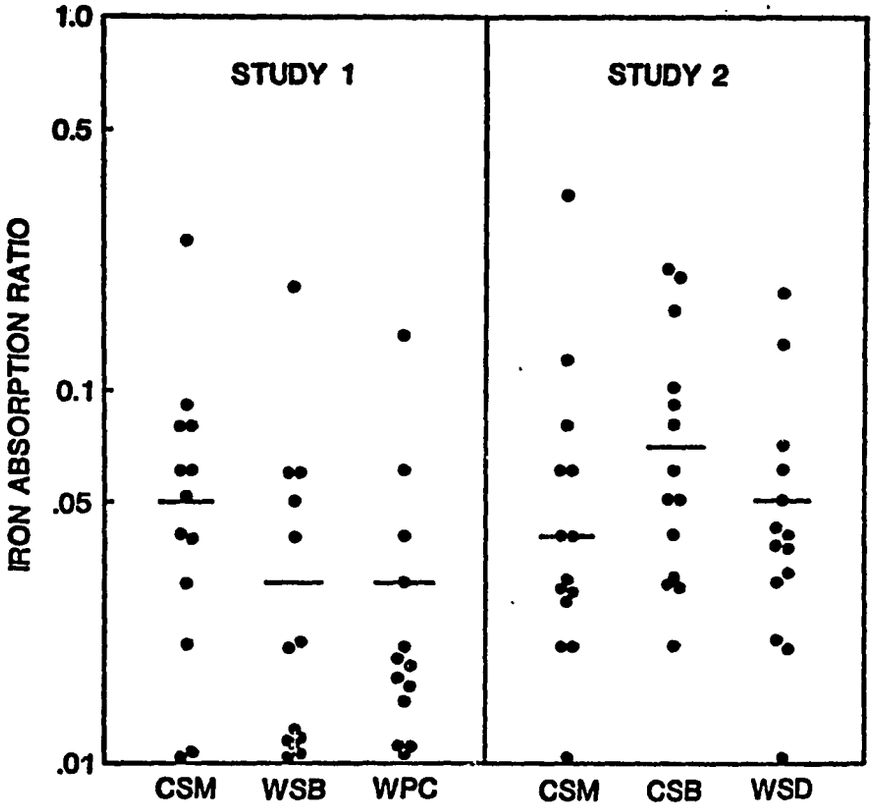
Table 3. Iron absorption from infant food supplements adjusted for iron status.

Study	Test Meal	Iron Absorption (% of dose)	Meal:REF	Adjusted Absorption* Percent	Adjusted Absorption* mg Iron
1	CSM	1.40	0.052	3.12	0.56
	WSB	0.76	0.028	1.68	0.30
	WPC	0.77	0.028	1.68	0.30
2	CSM	0.57	0.043	2.58	0.46
	CSB	0.92	0.069	4.14	0.74
	WSD	1.96	0.049	2.94	0.52

* corrected to 60% REF absorption and 18 mg Fe/100 g dry product.

FIGURE LEGEND

Absorption of iron from infant food supplements expressed as a ratio with respect to absorption from a standard reference dose of inorganic iron. Ratios are plotted on a logarithmic scale. The horizontal bars depict geometric means.


1.0
0.5
0.1
.05
.01
IRON ABSORPTION RATIO
STUDY 1
STUDY 2
CSM
WSB
WPC
CSM
CSB
WSD

THE INHIBITORY EFFECT OF SOY PRODUCTS ON NONHEME IRON ABSORPTION IN MAN[1,2,3]

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ABSTRACT

Radioiron absorption studies were performed in male volunteer subjects to determine the effect on nonheme iron absorption of various semipurified proteins. When egg albumen and casein were substituted in protein-equivalent quantities in a semisynthetic meal, similar mean absorptions of 2.5 and 2.7% were observed. In contrast, isolated soy protein reduced absorption sharply, to an average of 0.5%. When egg albumen in the semisynthetic meal was replaced with full fat soy flour, textured soy flour, and isolated soy protein, absorption fell from 5.5 to 1.0, 1.9, and 0.4% respectively, indicating an inhibitory effect by a wide range of soy products. The effect of substituting textured soy flour for meat in a meal containing a hamburger, french fries, and a milkshake was also evaluated. With 3:1 and 2:1 ratios of meat to unhydrated textured soy flour, absorption decreased by 61 and 53% respectively. The soy products tested in this study have a pronounced inhibitory effect on the absorption of nonheme iron.

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INTRODUCTION

The majority of clinical studies have shown that protein facilitates the absorption of dietary nonheme iron. Animal tissue including fish and poultry are especially important not only because they contain assimilable heme iron (1-3) but also because they enhance absorption of nonheme iron from foods eaten in the same meal (4-7). However, not all sources of protein enhance iron absorption. For example, egg yolk has been shown to impair iron absorption (8,9) and dairy products in general have a relatively inhibitory effect on nonheme iron absorption (5).

We recently reported the results of iron absorption studies in which the effect of doubling or deleting the sources of carbohydrate, fat, and protein in a semisynthetic meal were examined (10). Egg albumen, the protein source, had an inhibiting effect on iron absorption whereas the content of fat or carbohydrate had little influence. To determine whether this inhibition was unique to egg albumen, we examined in this study the effect of other semipurified proteins including casein and isolated soy protein. Several soy products were found to markedly inhibit the absorption of nonheme iron in human subjects.

METHODS

Subjects. Three separate studies were performed in 36 men ranging in age from 18 to 50 years. Study I (15 subjects) compared the effect of different semipurified proteins; Study II (10 subjects) examined the influence of various soy products on iron absorption; Study III (11 subjects) evaluated the effect on iron absorption of adding soy protein to meat. All of the volunteers were in good health and denied a history of gastrointestinal disorders that might affect iron absorption. With the exception of one subject who had a serum ferritin of 12 ng/ml indicating depleted iron stores (11), iron status was normal. Written consent was obtained from each volunteer and all procedures were carried out in accordance with the standards of the Human Subjects Committee of the University of Kansas Medical Center.

Absorption measurements. Three or four separate iron absorption tests were performed in each subject using double sequential radioiron labels. Test meals were administered between 7 and 9 A.M. following an overnight fast, and only water was allowed for the subsequent 3 hours. All meals were labeled extrinsically by adding varying quantities of $FeCl_3$ in 1.0 ml 0.01 N HCl containing either 2 uCi ^{59}Fe or 5 uCi ^{55}Fe(12,13). On the first day of each study, blood was drawn for measurements of serum ferritin (14) and background blood radioactivity. The first pair of test meals were tagged separately with either ^{59}Fe or ^{55}Fe and administered on two consecutive days. Blood was obtained 14 days later to measure incorporated red cell radio-

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activity and a second pair of separately tagged meals were again given on successive days. Subjects in Study I were given only one additional test meal after the initial pair of measurements. A final blood sample was drawn 2 weeks after the last test meal to measure the increase in circulating red cell radioactivity. All radioiron measurements were performed on duplicate 10 ml samples of whole blood using a modification of the method of Eakins and Brown (15). Percentage absorption was calculated on the basis of blood volume estimated from height and weight (16) and an assumed red cell incorporation for absorbed radioactivity of 80% (17).

Test meals. All meals in studies I and II were comprised of semipurified ingredients (Table 1). They represent modifications of the semisynthetic meal used in previous studies (5,18) except that no inorganic calcium or phosphate was added. In Study 1, each meal contained 68 g carbohydrate derived from dextrimaltose, 35 g fat from corn oil, and 29.4 g protein from various sources. The protein in meal A was supplied as egg albumen[a], in meal B as sodium caseinate[b], and in meal C as isolated soy protein (ISP)[c]. ISP is the spray-dried washed precipitate that is recovered after the water extract of defatted soybean flour has been adjusted to pH 4.5, the isoelectric point of the soy protein.

[a]Monark Egg Corp., Kansas City, Mo.
[b]National Casein Sales, Chicago, Il.
[c]Supro 710, Ralston Purina Co., St. Louis, Mo.

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In Study II, the carbohydrate and fat components of the meals were the same as for Study I, but each meal contained only 14.7 g protein. This was supplied in meals A through D as albumen, full fat soy flour[a] (FSF), textured soy flour[b] (TSF), and ISP respectively. TSF is obtained by the extrusion of defatted soy flour. Sufficient $FeCl_3$ was added to each meal in studies I and II to provide a final iron content of 4.1 and 4.0 mg respectively.

The components of each of the meals in studies I and II were thoroughly mixed and served as a drink with the exception of the TSF meal (meal C, Study II). In the latter, the dry TSF granules were hydrated in a styrofoam bowl with twice their weight of water and warmed for 45 seconds in a microwave oven. The fat and carbohydrate components were mixed and a portion poured over the TSF granules which were then eaten with a spoon. The remaining carbohydrate and oil mixture which contained the radioiron was taken as a drink.

The effect of partial substitution of meat with TSF was examined in Study III. Meals A, B, and C each contained 55 g french fried potatoes, 180 ml vanilla milkshake, a meat patty containing varying amounts of TSF, and a bun. Meal A contained 100 g lean ground beef (pre-cooked weight). In meal B, 30 g dry TSF granules were added to 100 g beef while meal C contained the same quantity of TSF but only 70 g beef. This resulted in weight ratios of meat to unhydrated TSF of approximately 3:1 in meal

[a]El Molino Mills, City of Industry, CA
[b]Supro 50A, Ralston Purina Co., St. Louis, MO

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B and 2:1 in meal C and protein ratios of 1.2 and 0.8 respectively. In meals B and C, TSF was moistened with an equal quantity of water and then mixed with the raw ground beef. All patties were broiled for 5 to 7 minutes per side, refrigerated overnight, and heated in a microwave oven immediately prior to serving. The hamburger meals were tagged extrinsically by adding 0.1 mg iron as labeled $FeCl_3$ to the milkshake. The final test (meal D) in Study III consisted of a reference dose of 3 mg iron as $FeSO_4$ and sufficient freshly prepared ascorbic acid solution to give a 2:1 molar ratio of ascorbate to iron (6,19). The purpose of this measurement, which has been used extensively in prior iron absorption studies in human subjects, is to standardize measurements of nonheme iron absorption in subjects with varying iron status.

Statistical analysis. Because of the highly skewed distribution of iron absorption measurements when expressed as a percentage of the administered dose, absorption data were converted to logarithms for statistical analysis and the results were reconverted to antilogarithms to recover the original units (20). When comparing any two test meals in the same study, a paired t-test was used to determine whether mean log absorption values differ significantly from 0 which is equivalent to testing whether the mean ratio in percentage absorption differ from 1.

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RESULTS

The effect of three semipurified protein sources on iron absorption (Study I) is shown in Table 2. Geometric mean absorption in meal A containing egg albumen averaged 2.49%. This is somewhat higher than previously observed values for this meal, which presumably reflects the omission of inorganic calcium and phosphate (21). Mean absorption from meal B containing sodium caseinate was nearly identical to that with egg albumen, averaging 2.74% (mean ratio B/A = 1.10). In contrast, absorption from meal C containing ISP was much lower, averaging only 0.46% (mean ratio C/A = 0.19, p 0.001).

A comparison of the effect of different soy products on iron absorption (Study II) is summarized in Table 3. Mean iron absorption from meal A containing egg albumen averaged 5.5%. This value is higher than that from the similar meal in Study I, reflecting the lower albumen content in this study (10). In all three meals containing soy products in Study II, iron availability was markedly reduced and in all instances the differences with meal A were highly significant. With FSF (meal B) absorption averaged 0.97% (mean ratio B/A = 0.18). Inhibition of iron absorption from TSF (meal C) was somewhat less pronounced, averaging 1.91% (mean ratio C/A = 0.35). The lowest iron absorption occurred in meal D containing ISP with a mean ab-

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sorption of 0.41% (mean ratio D/A = 0.08). Thus, when soy products were substituted for albumen in equivalent protein quantities, percentage nonheme iron absorption was reduced by 82%, 65%, and 92% with FSF, TSF, and ISP respectively. In Study II, the difference in absorption between the 3 meals containing soy products were also statistically significant. Absorption with TSF was significantly higher than with either FSF (mean ratio C/B = 1.95, p 0.01) or ISP (mean ratio C/D = 4.62, p .001). Absorption with FSF was also significantly higher than with ISP (mean ratio B/D = 2.37, p 0.01).

The effect of partial substitution of TSF for meat was examined in Study III (Table 4). With meat alone (meal A), absorption averaged 3.2% with a range of 0.48% to 14.61%. When 30 g TSF was added to the same quantity of meat (meal B), absorption fell to 1.24%, a highly significant decrease (mean ratio B/A = 0.39, p .001). When the meat content was reduced in proportion to the quantity of added soy (meal C), absorption was slightly higher, averaging 1.51%. The difference in absorption from this meal and meal A containing no soy was also highly significant (mean ratio C/A = 0.47, p .001). Thus, in meals containing a meat to soy ratio of 3:1 and 2:1, the decrease in absorption due to TSF averaged 61% and 53% respectively. In these iron replete men,

reference absorption (meal D) averaged 19.88%. Absorption of nonheme iron from meals A, B, and C therefore averaged 16%, 6%, and 8% of the reference dose absorption, respectively.

DISCUSSION

The inclusion of soy products caused a significant inhibition of iron absorption from all meals in this study (Figure 1). The immediate question posed by these results is the contrast with earlier investigations that indicated a relatively high bioavailability for soybean iron. For example, animal studies using the hemoglobin-repletion method in rats have shown a relative biological value of 60-80% for soy products (22). However, it is known that iron absorption measured in small laboratory animals does not always reflect bioavailability for human beings. For example, Fitch et al (23) observed that rhesus monkeys developed iron deficiency anemia when placed on diets containing ISP but not when fed casein-based diets with comparable levels of protein and iron. These workers also demonstrated that radioiron absorption from ISP was less than half that from casein.

A number of human studies have also shown relatively high iron availability from meals containing soybean that had been hydroponically labeled with radioiron. For example, Moore reported a mean absorption of 18% in 7 normal subjects and values of 20-

25% in patients with iron deficiency anemia (24). In a study comparing iron absorption from a variety of food items, a mean absorption of 11% was observed in 17 subjects given intrinsically labeled soy (19). Sayers et al later reported a mean absorption of 20% from intrinsically labeled soybean meals given to 5 iron deficient subjects (25).

However, not all human studies have shown this relatively high absorption of intrinsically labeled soybean. Ashworth et al (26), in a study of 10 infants, observed a geometric mean absorption of 2.6% for boiled soybean; when the soybean was baked prior to administration to 16 infants, mean absorption was 6.7%. Bjorn-Rasmussen et al recorded an absorption of 2.62% when soybeans were given to 15 normal subjects, 3 of whom were women (27). Geometric mean absorption in this study was 1.53%, a value well within the range of absorption observed in the present study.

The cause of this wide difference in iron absorption from hydroponically labeled soybean is unknown. In four separate studies with different batches of the same variety of tagged soybean, absorption ranged from 2.7 to 11.0% (28) (Table 5). This broad range is not explained by differences in iron status because the absorption ratio of soybean to reference varied from

0.11 to 0.46.

Other workers have not demonstrated diminished absorption from soy-based diets. In a study by Rios et al, in 4-7 month old infants, mean absorption of fortification iron added to a soy-based formula was 5.4% as compared to 3.4% from a milk-based formula (29). The relatively high proportion of fortification iron (ferrous sulfate) in this study may have masked an inhibiting effect. Young et al recently measured iron absorption from a soy-based diet using a fecal balance technic and ^{58}Fe as an extrinsic tag (30). An exceptionally high absorption of 21 $\pm$ 6% was observed which the investigators attributed to a generous but unspecified ascorbic acid intake with each meal. Van Stratum and Rudrum found no effect on serum iron levels, iron binding capacity, or transferrin saturation in 92 healthy volunteers fed a diet containing 25% protein derived from soy protein foods (31). Since these iron parameters do not become abnormal until iron stores are exhausted (32), and since iron deficiency develops slowly in the absence of blood loss, one would not expect these measurements to show a difference.

This variability in iron absorption from soybean meals is perplexing. One difference between previous studies and ours is that our meals were extrinsically tagged whereas most earlier

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studies used intrinsically tagged soybean. However, this observation does not explain the low absorption in this study because in each instance where a discrepancy has been noted between extrinsic and intrinsic tagging, the extrinsic label was better absorbed. In 11 subjects given intrinsically tagged soybean, absorption averaged 6.3% from the intrinsic label as compared to 7.8% from an extrinsic label in the same meal (12). The 27% higher absorption in that study may be explained by the fact that the extrinsic tag was taken as a drink and not mixed with the food; other workers have observed extrinsic to intrinsic tag ratios of 1.04 with soybean meals (27). It therefore seems unlikely that the lower absorption values for soy products observed in the present study relate to the extrinsic tag method.

Other differences between earlier studies and this report lie in the nature of the meals and the methods used for preparing them. The semisynthetic meal contained both fat and carbohydrate whereas soybean was administered alone in many prior studies. Moreover, in some of the latter the soybeans were baked; it is noteworthy that in the studies by Ashworth et al (26), baking resulted in a more than twofold increase in percentage iron absorption from soybean. We have also found a significant

increase in iron absorption from baked soy products (Morck, Lynch, Cook, unpublished observations). Processing soybean to obtain higher protein-containing products cannot be excluded as a factor in the inhibition of iron absorption. Although TSF rather than FSF gave the highest iron absorption in Study II, (1.91%), the product with the highest protein content (ISP) gave the lowest iron absorption (0.41%). Since all three meals in Study II were prepared on an equal protein basis, the results suggest that the processing of whole beans to obtain the three products altered their influence on food iron absorption.

While our results demonstrate that soy products may, under some circumstances, depress iron absorption significantly, the mechanism of the inhibition is unknown. Phytate is believed to have a strong inhibiting effect on iron absorption (33). We did not measure phytate levels of the soy fractions but they are known to contain relatively high amounts of phytate; ISP has been reported to contain 1.86% phytic acid (22). However, in a recent study with whole wheat bran, we found that the inhibiting effect of bran was not lessened when it had been dephytinized (34). Moreover, while fully iron saturated phytate is insoluble at neutral pH, it has been shown that more than half of the iron in wheat bran is present in the form of monoferric phytate, an iron complex soluble at neutral pH (35). Monoferric phytate is absorbed by dogs equally as well as food iron (36). Further studies will be necessary before phytate can be implicated in the inhibition of nonheme iron absorption caused by soy products.

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The implications of our findings in relation to iron status in the population are uncertain because of the complexity of dietary factors that influence nonheme iron absorption. In this country, soy is increasingly used as a meat extender and in meals containing meat, nonheme iron is readily available (3). In addition, the heme portion of meat is absorbed independently, and is not impaired by factors that reduce nonheme iron absorption (2). Therefore, one would expect the inhibiting effect of soy in people consuming a relatively large proportion of animal tissue to be quite limited.

Inhibition of iron absorption by soybean derivatives is more likely to be an important problem in areas where soy constitutes the major source of protein, especially if the diet contains little meat or ascorbic acid. We have recently reported that infant protein supplements such as corn-soya-milk, which contain a high proportion of soy protein, may not supply sufficient iron for normal nutritional needs of children (37). There is little doubt that soy is a valuable and relatively inexpensive source of dietary protein. The inhibitory effect on iron absorption reported here does not detract from this important property but does emphasize the need to evaluate the adequacy of iron in diets containing significant amounts of soy products. It seems likely that the deleterious effects with respect to iron nutrition could be circumvented by the addition of fortification iron to these diets or perhaps the addition of an enhancer of iron absorption such as ascorbic acid.

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Table 1. Protein and iron content of semipurified meals

	Meal	PROTEIN SUBSTITUTE			IRON		
		Source	Weight (g)	Protein Content (g)	Protein Substitute (mg)	$FeCl_3$ (mg)	Total (mg)
STUDY I							
	A	Albumen	36.7	29.4	0.4	3.7	4.1
	B	Casein	32.6	29.4	0.3	3.8	4.1
	C	Soy (ISP)	33.9	29.4	4.0	0.1	4.1
STUDY II							
	A	Albumen	18.4	14.7	0.1	3.9	4.0
	B	FSF	40.4	14.7	3.4	0.6	4.0
	C	TSF	28.6	14.7	3.3	0.7	4.0
	D	ISP	17.0	14.7	2.0	2.0	4.0

Table 2. Effect of protein concentrates on iron absorption (Study I)

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ng/ml)	IRON ABSORPTION (% of dose) ALB* (A)	Casein (B)	Soy (C)	Absorption Ratio B/A	C/A
1	29	47	61	0.97	1.00	0.16	1.03	0.17
2	26	50	34	1.26	1.11	0.07	0.88	0.06
3	27	46	59	1.40	1.40	1.70	1.00	1.22
4	25	45	164	1.41	1.21	0.56	0.86	0.40
5	26	43	31	1.43	4.12	0.36	2.94	0.25
6	25	46	46	1.55	1.83	0.18	1.19	0.12
7	25	44	94	1.63	6.43	0.30	4.00	0.18
8	25	43	51	1.80	1.06	0.27	0.59	0.15
9	25	49	88	2.26	2.41	0.95	1.08	0.42
10	28	50	48	3.78	1.26	0.52	0.33	0.14
11	30	43	55	4.51	6.03	0.56	1.35	0.12
12	24	44	37	4.82	8.37	1.16	1.75	0.24
13	25	46	45	6.25	4.43	0.12	0.71	0.02
14	26	48	114	6.50	2.78	1.03	0.43	0.16
15	26	44	12	7.51	20.45	4.97	2.78	0.66
MEAN†	26	46	53	2.49	2.74	0.46	1.10	0.19+
			-1 SE	2.08	2.16	0.35	0.92	0.15
			+1 SE	2.98	3.47	0.62	1.31	0.24

* ALB = egg albumen

† All values are geometric means except age and packed cell volume.

+ $p < .001$

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Table 3. Effect of various soy products on iron absorption (Study II)

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ng/ml)	IRON ABSORPTION (% of dose) ALB*(A)	FSF (B)	TSF (C)	ISP (D)	Absorption Ratio B/A	C/A	D/A
1	24	44	83	2.01	0.81	1.00	0.16	0.40	0.49	0.07
2	21	46	105	3.28	0.91	0.90	0.52	0.27	0.27	0.15
3	35	40	149	3.46	0.40	1.71	0.28	0.11	0.49	0.08
4	24	46	59	3.75	1.52	3.30	0.61	0.40	0.88	0.16
5	42	42	94	4.11	0.46	1.47	0.13	0.11	0.35	0.03
6	25	47	30	5.53	0.57	2.40	0.17	0.10	0.43	0.03
7	24	44	112	6.08	1.21	2.82	0.33	0.19	0.46	0.05
8	24	46	46	8.37	4.67	2.73	1.27	0.55	0.32	0.15
9	34	46	86	14.03	0.66	1.06	1.55	0.04	0.07	0.11
10	25	42	26	18.36	1.85	4.43	0.72	0.10	0.24	0.03
MEAN†	28	44	69	5.50	0.97	1.91	0.41	0.18+	0.35+	0.08+
			-1 SE	4.42	0.77	1.60	0.31	0.14	0.28	0.06
			+1 SE	6.83	1.23	2.27	0.54	0.23	0.43	0.09

* ALB = egg albumen

† All values are geometric means except age and packed cell volume.

+ $p < .001$

Table 4. Effect of soy protein on iron absorption from a meal containing beef (Study III)

Subject	Age	Packed Cell Volume (%)	Serum Ferritin (ng/ml)		IRON ABSORPTION (% of dose) 100g beef(A)	Beef : Soy 3:1 (B)	Beef : Soy 2:1 (C)	REF*(D)	Absorption Ratio B/A	Absorption Ratio C/A	Absorption Ratio B/C
1	36	44	60		0.48	0.13	0.25	17.80	0.27	0.52	0.52
2	22	44	70		1.43	1.16	0.18	14.05	0.81	0.75	1.07
3	18	39	33		2.30	1.16	1.22	17.91	0.50	0.53	0.95
4	19	44	59		2.41	0.78	0.60	7.73	0.32	0.24	1.30
5	18	48	41		3.10	1.06	0.85	31.95	0.34	0.27	1.24
6	18	46	61		3.47	1.55	3.25	23.43	0.44	0.93	0.47
7	19	49	60		3.76	1.31	1.75	13.71	0.34	0.46	0.74
8	37	41	51		3.81	0.88	0.91	7.88	0.23	0.23	0.96
9	18	48	61		3.91	0.82	0.91	19.46	0.20	0.23	0.90
10	20	46	31		10.76	6.47	8.08	36.85	0.60	0.75	0.80
11	29	49	27		14.61	7.87	15.62	95.83	0.53	1.06	0.50
MEAN†	23	45	48		3.20	1.24	1.51	19.88	0.39+	0.47+	0.82+
				-1 SE	2.43	0.89	1.06	16.02	0.34	0.40	0.73
				+1 SE	4.21	1.72	2.17	24.68	0.44	0.56	0.91

* REF = reference dose

† All values are geometric means except age and packed cell volume.

Table 5. Absorption of hydroponically-tagged soybean

Number of Subjects		IRON ABSORPTION* Soybean	IRON ABSORPTION* Reference	Absorption Ratio
17		11.0	25.8	.46
15		2.7	25.5	.11
7		9.8	44.3	.22
11		5.7	38.0	.15
	MEAN	6.1	29.4	.21

* All values are geometric means.

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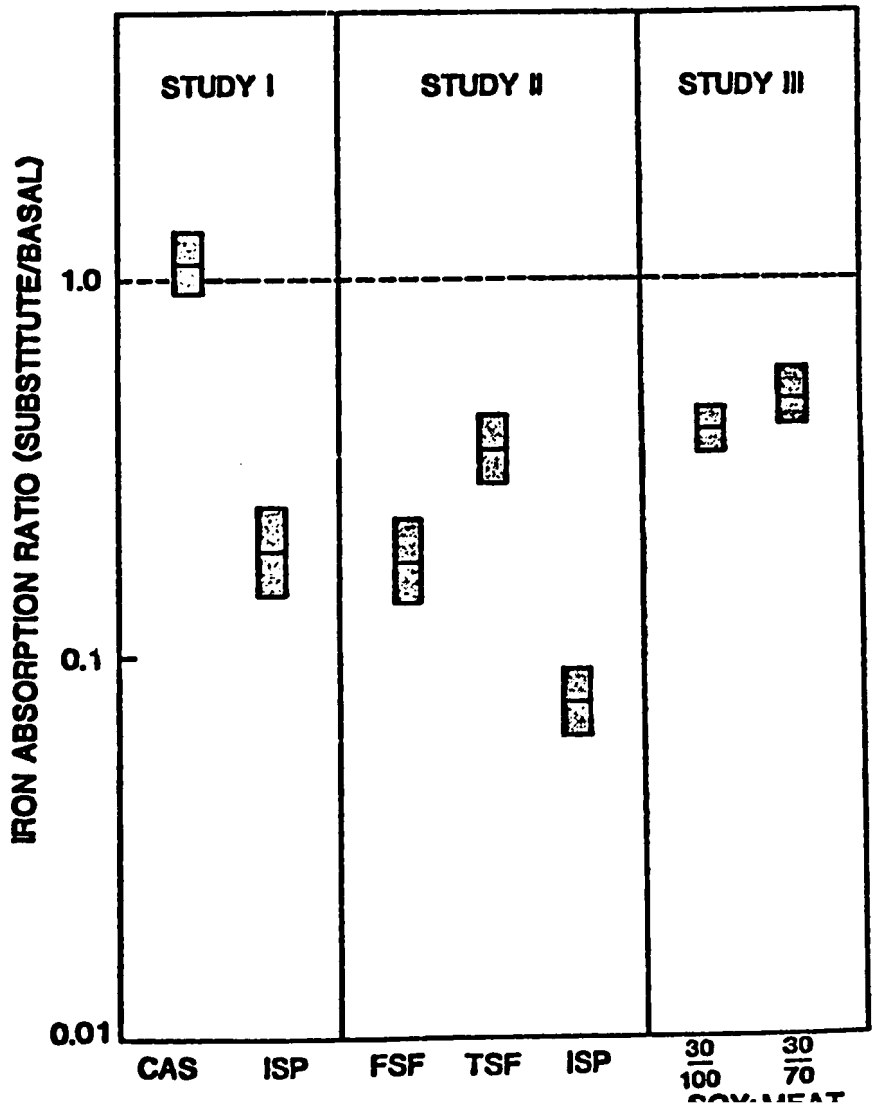
FIGURE LEGEND

Figure 1. The effect of semipurified proteins on nonheme iron absorption. Absorption ratios of soy product:albumen are plotted for studies I and II. For study III, absorption ratios are plotted for meals containing varying weight ratios of soy to meat. The height of each bar represents ±1 SE.



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BIOCHEMICAL DETERMINANTS OF IRON ABSORPTION

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Poor assimilation of dietary iron is both the major cause of nutritional iron deficiency and the major obstacle in designing food fortification programs to alleviate this deficiency. Studies in the past decade have shown that when several food items are ingested in the same meal, a common pool of nonheme iron is formed. In contrast to earlier studies with single food items, this approach has provided some insight into the specific biochemical determinants of iron availability. Nevertheless, many foods influence iron absorption by mechanisms that remain undefined. The factors that have been identified are shown in the following table:

Biochemical Determinants of Food Iron Absorption

	Potency
Enhancers	
Heme*	+++
Ascorbic Acid	+++
Amino Acids	+
Inhibitors	
Tannins	+++
Ca/PO_4	++
Protein	+
Phytate	+
Fiber	+
Ferritin*	+

*Intrinsic availability

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ENHANCERS

Heme Iron. The most important biochemical distinction in regard to dietary iron is the proportion that is in the form of hemoglobin and myoglobin. Although this heme iron constitutes only 5-10% of dietary iron, it accounts for nearly one-third of the iron absorbed each day because of its high availability. Since heme iron enters the mucosal cell as an intact porphyrin complex, its absorption is little affected by the composition of the meal. In a normal individual with iron stores of 500 mg, absorption of heme iron is about 25% as compared to 5% of nonheme iron (Monsen et al, 1978). About 40% of the iron in animal foods (including poultry and fish) is in the form of heme, giving a total intake of about 1 mg per day (Bjorn-Rasmussen et al, 1974). Heme is considered an enhancer in this discussion not because of its effect on the absorption of iron ingested in the same meal, but because of its high intrinsic availability.

Ascorbic Acid. In contrast to heme iron, absorption of nonheme iron is profoundly influenced by the composition of the meal. Ascorbic acid is the most potent enhancer of nonheme iron absorption known and has no ceiling to its enhancing effect, even at megadose levels (Cook and Monsen, 1977). When the relative increase in iron absorption from a meal is plotted against the molar ratio of ascorbic acid/dietary iron, the dose response curve has two components (Lynch and Cook, 1980). The break occurs at a molar ratio of ascorbate/iron of about 7.5 which corresponds to about 100 mg ascorbic acid in an average meal. Above this level, the enhancing effect of ascorbic acid is less pronounced. Ascorbic acid facilitates iron absorption by forming a soluble iron chelate at low pH which remains soluble at the higher pH of the small intestine (Conrad and Schade, 1968).

So marked is the enhancing effect of ascorbic acid on iron absorption that it has been proposed as a dietary fortificant to render food iron more available. While this approach is attractive in theory, there are a number of practical problems. About one-third of added ascorbic acid is inac-

tivated by boiling and almost all is lost by baking. Moreover, when both ascorbic acid and fortification iron are added to a vehicle such as salt, discoloration occurs. The relatively high cost of ascorbic acid as compared to iron is an additional constraint in developing countries. Even if ascorbic acid is not suitable for fortification, its value should be stressed in educational programs designed to improve iron status. The absorption of iron in a meal can be increased three times by including 100 ml orange juice (Callender et al, 1970) and up to seven times by including fresh fruit such as papaya (Layrisse et al, 1974).

Amino Acids. Animal tissue is an important constituent of the diet, not only because of its heme iron content but also because of its ability to enhance absorption of iron in foods ingested in the same meal. Substitution of animal protein in a meal containing only nonheme iron produces a two- to four-fold increase in iron absorption (Cook and Monsen, 1976). This is true for a variety of animal foods but does not include dairy products such as milk, cheese, and eggs. One gram meat is roughly equivalent in its enhancing effect to 1 mg ascorbic acid (Monsen et al, 1978).

Despite many years of investigation, the biochemical factor responsible for the enhancing effect of animal tissue has not been identified. In early studies, cysteine was believed to be responsible for the enhancing effect of fish (Martinez-Torres and Layrisse, 1970), but this has not been confirmed in later studies. Bjorn-Rasmussen and Hallberg (1979) have recently shown that the enhancing effect of meat is not mediated by an increase in gastric secretion and that the enhancing effect resides with the insoluble residue rather than with a water extract of meat. Since amino acids are known to enhance iron absorption (Forth and Rummel, 1973), the meat effect may relate to the ease with which animal foods are digested in the intestinal tract to polypeptides or amino acids.

INHIBITORS

Until recently, little attention was given to

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factors that inhibit the absorption of dietary iron. Moreover, as with animal foods, the biochemical mechanism by which certain foods reduce iron assimilation has not been identified. The following are the most important inhibitors.

Tannins. The pronounced inhibitory effect of tea on iron absorption was first reported by Disler et al (1975a; 1975b). These workers showed that a single cup of tea reduces the absorption of ferric chloride from 22 to 6% and in a meal with several ingredients from 11 to 2.5%. In animal studies, this inhibiting effect did not exist with tannate-free tea or with caffeine but it could be reproduced exactly with either tannic acid or with tannins extracted from the tea. The South African workers concluded that the inhibiting effect results from the formation of a black, insoluble precipitate of iron tannate. We have observed a similar but less pronounced inhibiting effect of coffee which is apparently due to tannins as well (Morck et al, unpublished observations). Tannins are widely distributed in vegetable foods and may be responsible for the low iron assimilation from these foods. Tea has such a marked inhibitory effect that its use has been proposed as a means of reducing the absorption of dietary iron in patients with thalassemia major or intermedia (de Alarcon et al, 1979).

Calcium/Phosphate. Animal studies have led to conflicting views on the effect of these minerals on iron absorption. In human studies, egg yolk has been shown to markedly inhibit iron absorption, an effect that is attributed to vitellin, the major phosphoprotein complex in egg yolk. Peters et al (1971) concluded that phosphate compounds, either as inorganic phosphate or in phosphate-containing meals, are among the most important inhibitors of iron absorption. The effect is presumed to be due to the formation of highly insoluble ferric phosphate.

In studies with a semipurified meal (Monsen and Cook, 1976), the separate addition of a soluble calcium salt and a soluble phosphate salt had little effect on iron absorption. However, pronounced in-

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hibition was observed when both were added simultaneously, an effect that probably resulted from co-precipitation of iron by an insoluble complex of calcium phosphate.

Protein. The effects on iron absorption of protein, carbohydrate, and fat remain controversial. The bulk of information is derived from animal studies in which the proportion of these macronutrients is altered while maintaining a constant caloric intake. It has been difficult to determine in these studies whether the observed effect is due to an increase in one constituent or the reduction of another.

Data on the effect of protein have been inconsistent. The enhancing effect of meat has often been ascribed to protein per se but without direct evidence. In fact, recent studies have shown that certain semipurified proteins may actually inhibit absorption. For example, when carbohydrate, fat, or protein were either doubled or eliminated from a semipurified meal, a significant effect was observed only with the protein source, egg albumen. When the latter was omitted, absorption increased from 3.8 to 9.6%, but fell from 2.3 to 1.4% when the level was doubled (Monsen and Cook, 1979). More recently, a striking inhibitory effect of soy protein has been observed (Cook et al, 1981). When full-fat soy flour, textured soy protein, or isolated soy protein were substituted for egg albumen, absorption was inhibited 70-80%. A significant but less dramatic inhibition was also observed when textured soy protein was used as a meat extender. The mechanism for this inhibition has not been defined.

Phytate. In the early 1940's, in a study using a chemical balance technic, it was observed that less iron is absorbed from a diet containing brown bread than one containing white bread (Widdowson and McCance, 1942). Modern studies with extrinsic tagging have confirmed that bran inhibits iron absorption in proportion to the amount added to a meal (Bjorn-Rasmussen, 1974). It has been widely assumed that the effect is due to its phytate

content. However, Sharpe et al (1950) observed that while sodium phytate added to a meal reduced absorption, naturally-occurring phytate did not depress iron absorption significantly. Morris and Ellis (1976) found that more than half the iron in wheat bran is present as monoferric phytate. In contrast to fully saturated iron molecules, monoferric phytate is soluble at neutral pH but insoluble in an acid environment. Studies in dogs (Lipschitz et al, 1979) and in humans (Simpson et al, 1981) have demonstrated that radiolabeled monoferric phytate is relatively well absorbed.

In the study by Simpson et al (1981), examining the inhibitory effect of bran, the phytate content seemed to have little effect on absorption. When 12 g whole bran was added to a meal containing a milkshake and muffins, absorption was reduced from 2.4 to 0.6%. When whole bran was incubated at 37°C to destroy phytate by enzymatic hydrolysis and included in a similar meal, the inhibitory effect of bran was not diminished. Thus, while naturally-occurring phytate may reduce iron absorption, its effect is less pronounced than previously assumed.

Fiber. The effect of fiber on iron absorption has not been extensively studied in man, but there is reason to believe that it is inhibitory. In a recent study in humans (Cook et al, unpublished observations), two separate meals were given that were nearly identical with respect to macronutrient composition but maximally different with respect to fiber content - 5.1 g vs. 0.3 g. Absorption from the low fiber meal averaged 6.1% as compared to 3.0% from the high fiber meal. Thus, at these two extremes, the inhibiting effect of fiber was relatively modest. Furthermore, studies with pectin and hemicellulose have failed to demonstrate an inhibiting effect of these fiber sources (Cook et al, unpublished observations). It would appear that fiber has relatively little effect on iron absorption.

Ferritin. The nonheme iron fraction of animal tissue is well absorbed. However, when purified radiolabeled ferritin was administered to human

subjects, absorption averaged less than 1% as compared to 2% from a maize meal in the same subjects (Layrisse et al, 1975). On the other hand, when 2 mg ferritin iron was administered with veal muscle, percentage absorption increased markedly from 1.3 to 12%. The low availability of purified ferritin therefore appears to be an artifact of protein purification.

SUMMARY

The technic of extrinsic radioiron tagging has greatly assisted in the identification of specific biochemical determinants of food iron absorption. Nevertheless, there remains a large gap between our knowledge of the intrinsic availability of iron in certain foods and the specific biochemical factors that account for these effects. The enhancing effect of ascorbic acid and animal tissue on iron absorption is well established if not well understood. As yet, there is only scant information on the biochemical factors that cause inhibition of iron absorption by tannins, calcium/phosphate, protein, phytate, and fiber.

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APPENDIX C

- LDC COLLABORATIONS -

IRON-59 EXTRINSIC TAG AND WHOLE BODY SCANNING IN HUMAN IRON ABSORPTION STUDIES+

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INTRODUCTION

Nutritional anemia is widely prevalent especially in developing countries like the Philippines. Local studies have shown that prevalence is as high as 48.4% in some population groups on the basis of levels of hemoglobin, hematocrit, serum iron and transferrin saturation(1). Nutritional anemia may be caused by inadequate intake of the blood-forming nutrients such as folic acid, vitamin B_{12} and iron. The anemia is however mainly caused by iron lack.

Although iron deficiency is often associated with low socio-economic status, it is also widely prevalent in the developed and highly industrialized nations. More than 5 million adult women in the United States have iron deficiency anemia and that at least twice this number have no iron reserves to meet the physiologic needs of menstruation and

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+This study is part of collaborative research program on the control of iron deficiency anemia partly supported the WHO and USAID.

pregnancy[2].

The problem in the Philippine is not so much of inadequate amount of dietary intake but rather of poor availability of the iron in the diet.

One methodologic problem in the study of iron absorption is the enormous variability of the results obtained[3]. Most of this variation is not due to errors in determining absorption but rather to actual normal fluctuations in intestinal iron absorption. Physiological variations within the same subjects, called intra subject variation, occur when tests are done on different days. Intra subject differences presumably arise from differences in the gastrointestinal motility or secretion at different times of dosing. Of greater magnitude is the variation in iron absorption among apparently healthy subjects called inter subject variation which can be due to differences in their iron nutritional status. Inter subject differences are of interest since they provide a sensitive index of the iron requirement of different individuals. The information obtained from iron absorption measurements also enables one to assess the iron balance of an individual. When two or more test meals are being evaluated, it is important to eliminate both intra and inter variations among subjects. The most suitable approach to minimize these variations

is to compare the absorption of the iron on the test meals in each subject against a standard reference dose of inorganic iron. The ratios of iron absorption of the test meal to the reference iron dose are then compared between individuals. Appropriate statistical treatment is then applied to the data taking into consideration that the absorption values follow a log normal distribution[4].

The nature of the administered radioiron is of great importance in food iron absorption studies. Biosynthetic labeling or intrinsic labeling has been done extensively since Moore and Dubach[5] introduced it some 20 years ago. Instrinsic labeling of some Philippine vegetables fed to experimental rats have been done locally[6,7]. The most significant limitation of this type of study however, is that none of the single-tagged food item is normally eaten alone by the population. Data obtained with biosynthetic labeling would only be applicable if absorption was the same when the labeled food is given alone as when administered as part of a complete meal. In view of the complexities of a modern diet it became obvious that biosynthetic labeling would provide little practical information about food iron assimilation.

The most important advance in studies of food iron absorption is the use of extrinsic tag to measure iron

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absorption from a complete meal[8-11]. Food iron may be divided into heme-iron and non-heme iron. Studies indicate that the absorption of non-heme iron from different foods ingested in the same meal will be the same. In developing countries where iron practically comes from vegetables or foods of non-animal origin the absorption of non-heme iron is the major concern. Studies indicate that a common pool of non-heme iron is formed by foods ingested in the same meal and that iron absorption from this pool can be measured by extrinsic tagging.

In addition to the major fraction of non-heme iron in food, there is a second pool of dietary iron-heme iron-that must be considered separately. It has been shown in both animals and man that heme iron is not degraded within the intestinal lumen but is absorbed into the mucosal cell as an intact porphyrin complex. The absorption on heme iron differs markedly from non-heme iron in that it is not affected by enhancing substances such as ascorbic acid or by inhibiting substances such as phytate or desferrioxamine, which have a profound influence on the absorption of non-heme iron. Studies of dietary factors affecting iron assimilation has therefore been focused on non-heme iron. Meat, the primary source of heme iron is seldom contained or is

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a minor constituent in the diets of populations where iron deficiency is most prevalent.

The availability of a whole body scanner at the Atomic Research Center made possible the present investigation on dietary iron absorption. The method used to measures the iron retained by subjects given extrinsically iron labeled test meals.

The objectives of the present investigation are as follows:

1. To determine iron absorption from representative or typical Philippine meals among apparently normal Filipino male adults by radioisotope extrinsic labeling technique and whole body scanning.
2. To correlate absorption with the chemical composition of the meals and possibly evaluate the dietary factors which influence iron absorption.
3. To be able to recommend to nutrition workers explicit measures to enhance iron absorption.
4. To obtain quantitative data on iron absorption that will be useful in formulating an iron fortification program to help control nutritional anemia in the country.

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VII. METHODOLOGY

A. Experimental Subjects

Serial iron absorption studies were performed in 16 male volunteers whose ages ranged from 21 to 55 years and who were studied in three groups of six subjects each. Because of personal inability to attend the regimen imposed by the studies two subjects of the third group were disqualified. Informed consent was obtained from all subjects prior to study. The subjects were found to be in fair-to-good health by physical examination by a medical doctor. Measurements of serum iron[12] and total iron binding capacity were performed on a fasting blood sample in all subjects using procedures recommended by the WHO Expert Group on Nutritional Anemia [13]. Serum ferritin was measured by using the (I-125) ferritin Radioimmunoassay Kit abtained from GammaDab Clinical Assays, Cambridge, Massachusetts.

B. Test Meals

Three absorption studies were done on each subject consisting of feeding two test meals and a reference dose of iron. The two meals are typical or commonly consumed Philippine meals

designed on the basis of data obtained from the first nationwide food consumption survey conducted by the FNRI[14]. Since it was important that the preparation of each meal used was reproducible and its composition was relatively constant, foods selected were those which could be purchased in quantity and stored prior to preparation. Thus, the first meal (MO1) consisted of rice, sauteed mongo beans and banana while the second meal (MO2) consisted of rice, "sinigang na bangus", fried pork and banana. The chemical composition of the meals were analyzed and the results are listed in Table 1. The total iron content of MO1 and MO2 were 2.6 and 4.45 mg, respectively.

An extrinsic radioiron tag was administered on the cooked rice using 5 uCi of Fe^{59} as Ferric chloride in 0.1 M HCl (specific activity = 3-20 mCi/mg Fe) obtained from Radiochemical Center, Amersham, England. A carrier of 0.1 mg cold iron as $FeCl_3 \cdot 6 H_2O$ was used. Correction of carrier for isotope iron contribution was unnecessary because of insignificant (< 0.5%) iron contributed by the isotope.

C. <u>Reference Dose</u>

The third test dose consisted of a reference dose (RD) of 3 mg iron as labeled ferrous sulfate

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containing 2 moles of ascorbic acid/mole of iron and tagged with 3 uCi of Fe^{59}. The reference dose gives a measure of optimum absorption. By relating absorption of the other test meals to the absorption of this standard dose of inorganic iron, variability in absorption due to differences in iron status of the subjects can be reduced(15)

D. Radioiron Absorption Tests

A total of three serial absorption test were performed in each subject using Fe^{59} making a total of 48 absorption tests. The first test meal was administered on the fasting subject after obtaining background radioactivity of the whole body scanner. No other food or drink was consumed for three hours following each test meal. Retention of iron was measured after fourteen days in a shadow-shield type whole body scanner at an energy range of 0.400 - 1.328 MeV. After the retention measurement of the first meal the second meal was administered; the reference dose was then given after the retention measurement of MO2 and completed after the required lapse time of 14 days.

The sequence of administration of the meals was varied for statistical validity of results

according to the following design:

MO1 MO2 RD

MO1 RD MO2

RD MO1 MO2

where MO1 = the first meal, MO2 = the second meal, RD = reference dose of iron.

Percent absorption was calculated using:

$$\frac{R_2^{1}\ R_2''}{R_1^{1}\ R_1''} \times \frac{ST_1 - BP_1}{ST_2 - BP_2} \times 100 = \%\ \text{absorption}$$

where:

$R_2^{1}\ R_2''$ are retention measurements after 14 days (supine and prone, 7 minutes each).

$R_1^{1}\ R_1''$ are retention measurements which give total dose of radioactivity or "100% value" measured within 30 minutes after ingestion of "hot" meals; (supine and prone, 7 minutes each).

ST_2, ST_1 are standard measurements at day 14; day 0.

BP_2, BP_1 are patient background measurements at day 14 and day 0.

The second ratio in the formula gives the correction for radioactive decay.

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The calculated total radiation dose averaged over a period of 8 weeks, assuming that the whole of each test dose had been retained, would have been approximately 28% of the permissible whole body burden for continuous exposure in the case of Fe^{59}. The computations were based on guidelines issued by IAEA on basic safety standards for radiation protection[16].

E. Calibration/Standardization of the Whole Body Scanner

Initial tests undertaken on the whole body scanner included calibration using Cs-137, energy resolution, linearity, sensitivity check, background check, 60-cycle test counts and Chi-square test of goodness of fit. A number of these tests were routinely done for the duration of the study. (Appendix A).

F. Statistical Analysis

Iron absorption data have been shown to follow a skewed distribution[17]. Thus, the only statistical model in which proportionality between food and inorganic iron absorption is retained and in which criteria for common methods of statistical analysis could be satisfied was one in which the logarithms of the ratio of food to inorganic iron were employed . Statistical tests including calculation of means and standard deviation

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for each meal were performed on the logarithmic scale and the results retransformed as anti-logarithms to recover the original units. To express the absorption of a particular meal as a percentage of the reference dose of inorganic iron, the mean and confidence limits are retransformed by taking antilogarithms.

VIII. Results and Discussion of Results Obtained:

The nutrient composition of the meals as determined by chemical analyses are summarized in Table 1. MO2, composed of rice, sinigang na bangus, fried pork and banana, contained more protein, iron and vitamin C than MO1 (27.8 g vs. 18.9 g proteins; 4.45 mg vs. 2.6 mg Fe; and 10.6 mg vs. 15.4 mg Vit. C). MO1 provided 31% of the Recommended Dietary Allowance (RDA) for protein and iron; MO2 provided 45% of protein and 49% of iron of the Recommended Dietary Allowances for Filipinos.

Hematologic and iron absorption data on the 16 volunteer subjects are shown in Table 2. Hematocrit levels ranged from 39-51 vol.%; serum iron from 27 - 291 ug/100 ml while serum ferritin from 11.5 ng/ml - 500 ng/ml. Iron deficiency and lack of iron stores is shown by subject V.O. as indicated by serum iron and ferritin values.

Arithmetic means were computed for age and hematocrit. Because of the skewed distribution of iron absorption data and serum ferritin levels, mean and standard error values were calculated on the logarithmic scale and the results retransformed as antilogarithms to recover the original units.

Substantial variations in iron absorption were noted with both MO1 and MO2 meals. (Table 2). Absorption from MO1 ranged from 1.2 to 26.6% while that from MO2, 3.1 - 37.5%. The variability was less when absorption was expressed as a ratio of the reference dose and thereby allowing each subject to serve as his own control (Fig. 1). The geometric mean ratio for MO1 was 0.35 ($\pm$ ISE, 0.29 - 0.41) as compared with that of 0.49($\pm$ ISE, 0.42 - 0.57) for MO2. The mean absorption values obtained were 5.0%, 7.4% and 12.7% for MO1, MO2 and RD respectively.

Absorption ratios were likewise studied statistically by transforming values into their logarithms and getting the antilogarithms to recover original units. Student's t-test of difference between the two ratios indicate no significant differences ($p > .05$). Correlation analyses performed on these ratios indicate significant positive linear correlations between these thwo sets of parameter data ($r = 0.66$ $p < .001$ for MO1/RD; $r = 0.88$ $p < 0.001$ for MO2/RD) indicating a high degree of consistency of

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the method used for measuring iron absorption for particular individuals. A regression equation to show the relationship of both test meals and the reference dose is shown in Fig. 2.

To test whether the sequence would exert significant influence on iron absorption data, Friedman's Two-Way Analyses of Variance by Rank were used on the three sets of data as separate samples and on the total group. The application of this non-parametric statistical technique was chosen because of the small sample size and the wide disparity in the variances. The test on the three samples show significant variation in essentially the same direction, i.e., RD effects greater absorption than either MO1 or MO2. Since the trend is the same, it can be inferred that the effects were independent of sequence. This independence is shown in Figure 3). If at all, interaction is very minimal, once sequence is held constant.

IX. Highlights of Results

The test meals employed in these studies contained the major components of typical Philippine meals with appropriate levels of calories, proteins and carbohydrates, as indicated in the first nationwide nutrition survey. The availability of the non-heme iron of these

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meals was evaluated by the addition of an extrinsic tag of Fe^{59}. It has been previously shown that common pool of non-heme iron is formed by items ingested simultaneously.

MO2 contains a small amount of pork and is thus expected to enhance absorption of the non-heme iron. Vitamin C, a known enhancer of iron absorption is also greater in amount in MO2 than MO1.

The most significant finding in these studies is the low absorption obtained in both rice-based meals commonly consumed by Filipinos. This lend support to the findings of very poor absorption of iron in rice containing diets. Bjorn-Rasmussen and co-workers[20] showed 4.6 + 7.0% absorption of iron in both polished and unpolished rice.

In a similar study conducted among 17 normal American males fed diets approximating Philippine dishes mean absorption ranged from 1.13% to 3.49% while absorption from the RD averaged 12.58% and 19.24%[21]. In this earlier study there was an inverse relationship between serum ferritin and percent iron absorption while dietary iron and dietary vitamin C content correlated positively with percent iron absorption. Crude fiber and percent iron absorption were inversely correlated. The variation in iron stores as a major cause of variation in absorption as reflected by the inverse correlation between serum ferritin and absorption was underscored by this earlier investigation.

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The quantity of iron which must be absorbed from a normal diet to maintain iron balance can be accurately predicted from measurements of body iron loss. Basal losses in man average about 10% of body iron per year or about 0.9 mg iron/day[22]. Taking the upper level of 7.4% as the average iron absorption of this set of subjects consuming 13.35 (4.45 x 3) mg Fe per day would absorb 9.9 mg which barely meets daily requirements. The ratio of iron to calorie intake i.e., 6 mg/1000 calories, varies relatively little in different segments of the population[14]. Therefore, in women of child-bearing age with an additional requirements of 0.4 mg iron/day to replace menstrual losses, daily ingestion of a diet containing 1800 calories and 11 mg Fe/day[14] necessitates about 12% absorption of dietary iron to maintain iron balance. Assuming that the percent of dietary iron absorption in Filipino women of child - bearing age is similar to those in this study, a mean absorption of 7.4% of non-heme iron is way below this 12% estimate. The deficiency could possibly be met by increased heme iron intake. For economic reasons, however, heme iron intake in the typical Filipino diet is likely to remain a small portion of total dietary iron. In most developing countries the contribution of heme iron is expected to be low if not negligible. Moreover, the absorption of heme iron is relatively fixed in

contrast to that of non-heme iron. Whereas ascorbic acid and desferrioxamine have profound effects on the description of non-heme iron, there is little or no influence on the absorption of heme iron. Thus, in evaluating dietary factors which influence iron availability, heme iron absorption can be ignored. It becomes obvious that a consistent consumption of a diet devoid of meat or fish will exacerbate the already precarious state of iron balance of Filipino women specially those of child-bearing age.

X. Significance/Impact of the Study

XI. Conclusions and Recommendation

The results of this study now confirm the low absorption of dietary iron which has been heretofore suspected as the primary cause of the high prevalence of iron deficiency and anemia observed in particular segments of the Philippine population. For the first time in the country, a direct method of assessing iron absorption was undertaken. This was made possible through the use of extrinsic labeling and the availability of the whole body scanner. This method has been found to be accurate and direct although replete with significant methodological details. This method finds application in studying

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many important and pressing public health nutrition problems. Through this technique, the effect on iron absorption of various components of a meal, e.g. by changing its composition may be studied. The amount of iron to be added in the diet by supplementation and/or fortification that should effectively prevent iron deficiency can also be quantitatively determined. Such information is essential before effective nutrition intervention strategies aimed at eradicating iron deficiency anemia can be worked out.

The amount of meat and fish contained in a diet seems to be of great importance for the nutritive enhancement of the diet with respect to iron. Recent economic trends point to the vulnerability of third world countries to meet this nutritional demand. In the Philippines and more so in the rural areas the diets of especially the low socio-economic groups are poor in meat and fish. It is therefore improbable that an increase in the amounts of meat and fish products in the diets is a realistic immediate approach to solve our iron nutrition problem. Other alternatives must be worked out by nutrition workers through manipulation of dietary factors that will enhance iron absorption. Some reports have shown that vitamin C enhances iron absorption and apparently works with

the same biochemical mechanism as that of meat[23]. Because it is relatively low in cost, vitamin C supplementation of the local diets may be of value in attacking the iron deficiency problem that continually confronts us. However, the possible beneficial effects of increasing the vitamin C content of Philippine diets needs verification and quantitative evaluation by actual studies on iron absorption similar to those done in the present study.

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Table 1. Nutrient composition of test meals used for absorption studies.

	Food Energy (cal)	Protein (gm)	Iron (mg)	Vit. C (mg)
MO1				
Rice	194	3.88	0.97	-
Mongo Guisado	309.5	15.02	1.63	-
Banana	70.8	-	-	5.4
	574.3	18.9	2.6	5.4
MO2				
Rice	318	6.36	1.59	-
Sinigang na bangus	90.9	16.8	2.40	6.0
Pork	67.1	4.6	0.46	-
Banana	61.3	-	-	4.6
	537.3	27.76	4.45	10.6

Fig. 2. Absorption of test meals (MO1 and MO2) expressed as a ratio of the absorption from a reference dose of inorganic iron. Ratios are plotted on a logarithmic scale. The horizontal bars depict geometric mean.

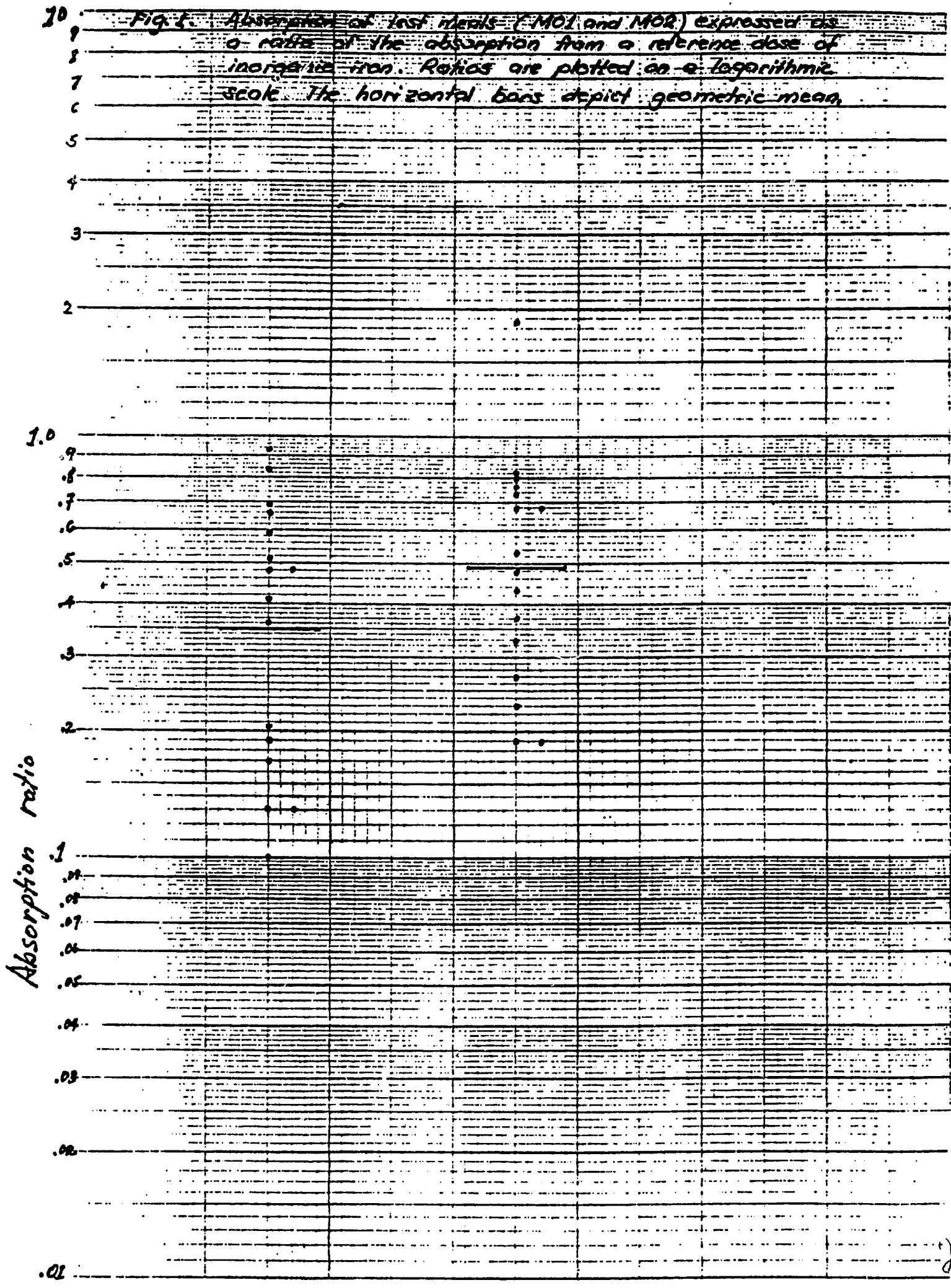

Table 2. Hematologic and iron absorption data on Filipino volunteers consuming typical Philippine meals.

SUBJECTS	Sex Age	% Hematocrit Vol.%	Serum Iron ug/100 ml	Serum Ferritin ng/ml	Fe Absorption % MO1	MO2	RD	Absorption Ratio* MO1/RD	MO2/RD
1. A.A.	M21	40.5	81	96	3.2	9.2	6.3	0.51	0.68
2. R.L.	M29	46	(421)	135	6.7	6.0	13.9	0.48	0.43
3. D.C.	M24	44	72	173	2.0	7.0	9.4	0.21	0.74
4. B.B.	M31	39	(303)	110	1.9	6.0	3.2	0.59	1.88
5. G.L.	M48	46	(231)	500	1.2	3.1	1.5	0.10	0.27
6. R.D	M31	46	221	300	2.0	3.6	15.3	0.13	0.23
7. A.B.	M34	41	104	133	2.0	4.0	4.9	0.41	0.82
8. V.H.	M39	50	(421)	130	4.5	4.5	9.3	0.48	0.48
9. V.O.	M28	41	27	11.5	26.6	10.6	31.7	0.84	0.33
10. A.G.	M42	44	113	260	2.7	5.7	15.5	0.17	0.37
11. M.C.	M37	51	91	170	11.5	3.3	17.5	0.66	0.19
12. D.M.	M48	41	291	280	24.7	6.7	36.0	0.69	0.19
13. J.S.	M55	44.5	110	120	6.1	13.9	17.1	0.36	0.81
14. T.M.	M28	49	166	200	8.6	7.0	9.2	0.93	0.76
15. M.D.	M28	50	(287)	64	9.3	35.4	48.0	0.19	0.74
16. D.C.	M22	46.5	123	64	9.2	37.5	70.4	0.13	0.53
Geometric Mean	33**	45**	127.2	132.4	5.02	7.4	12.7	0.35	0.49
± ISEM		±0.944		142.1-201.25	3.96-6.35	6.14-8.92	9.84-16.26	0.334 - 0.467	0.485 - 0.702

* Logarithmic transformations on absorption ratios were made.

** Arithmetic mean

( ) Total iron binding capacity, ug/100 ml.

Fig. 2. Regression Equation between test meals (MO1 and MO2) and Reference Dose (RD).

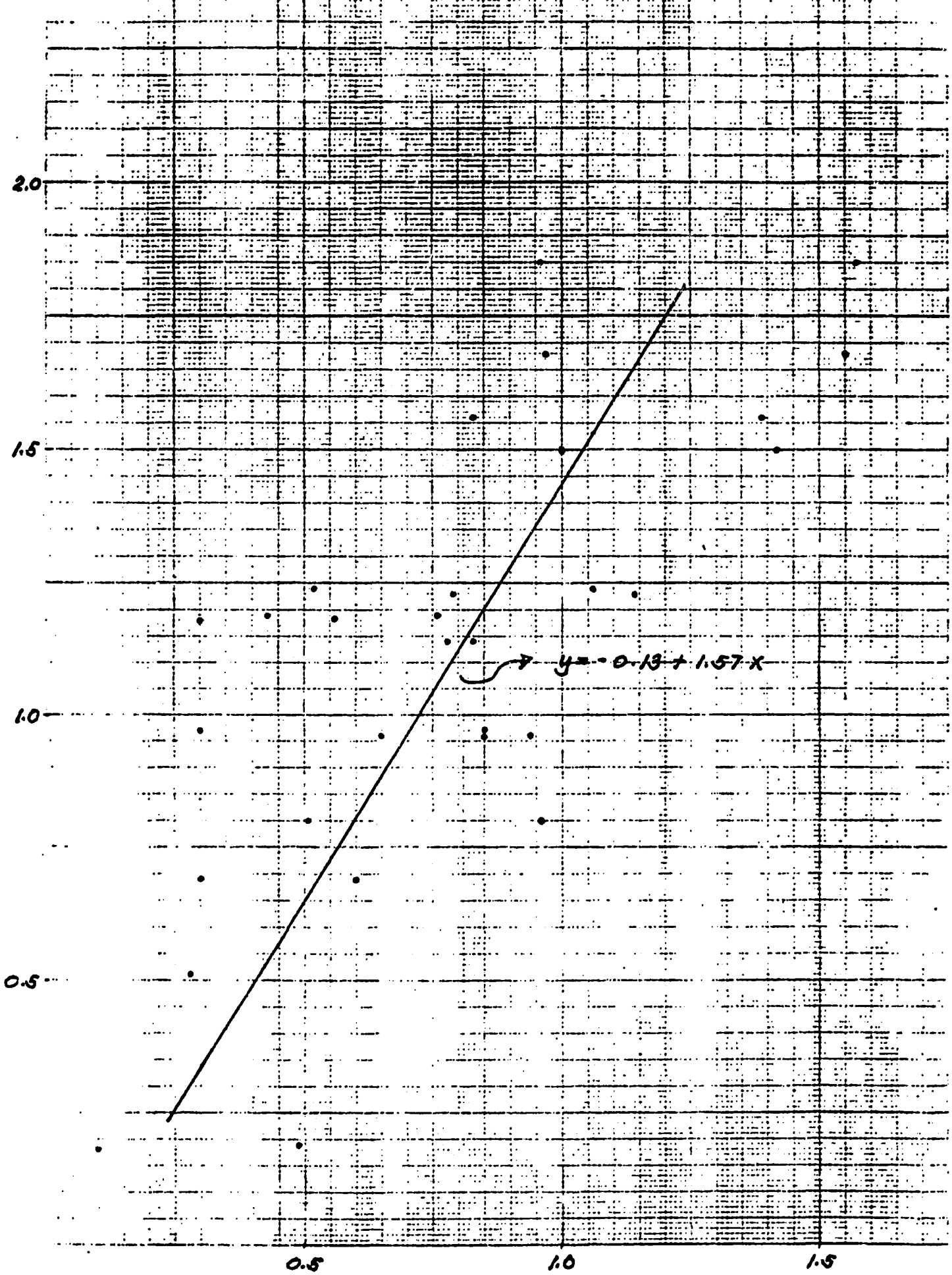

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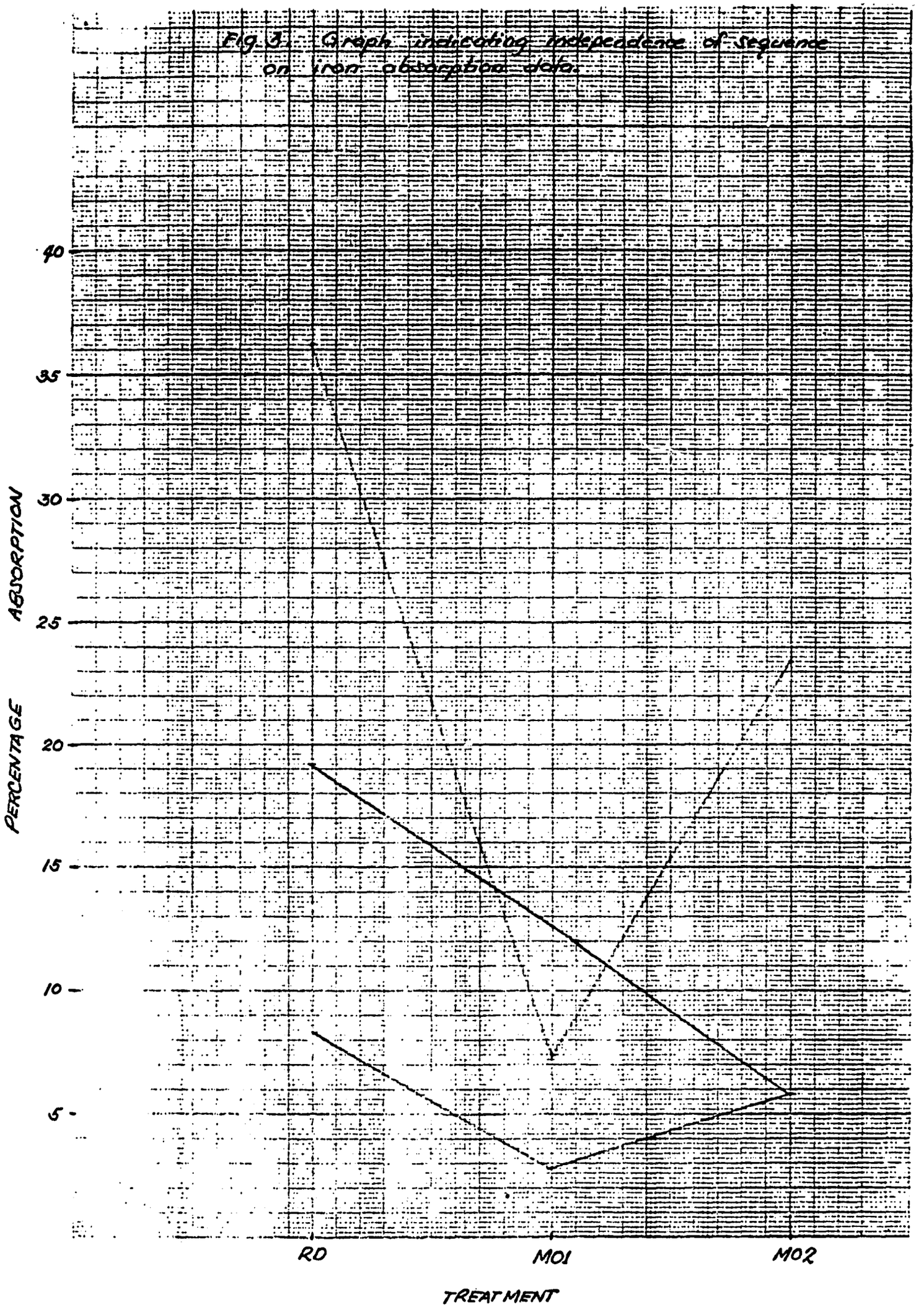
Fig. 3. Graph indicating independence of sequence on iron absorption data.



Appendix A. <u>Quality Assurance Tests on Whole Body Scanner During Human Iron Absorption Studies (July - August, 1980)</u>.

Quality assurance tests were made regularly on the Whole Body Scanner to insure proper operation and reliability of data obtained from the instrument. Daily sensitivity tests were performed which show if the detector, protomultiplier and pre-amplifier is working properly from day to day. Background tests on the instrument were also done daily, to determine contamination, if any, on the bed. An increase of background when there is no contamination may be an indication of some electronic "noise". There was none indicated by the tests for the month of July. Calibration (setting of gain) was performed daily using a cesium 137 standard source.

Weekly tests included the linearity test which is used to determine whether the amplifier is working properly. X^2 tests were done to assure reliability of counts on the instrument.

At the end of the month, the energy resolution tests were conducted to assure that the detector was still in proper working order. Mechanical check-ups, like scanning time of the bed and minor checks and repairs were performed as needed for the duration of the study.

A C K N O W L E D G E M E N T

It is a pleasure to acknowledge the technical expertise and assistance of Dr. Helmar Bergman, IAEA Visiting Expert who guided us in the calibration and standardization of the whole body scanner; Ms. Felicidad Velandria, FNRI dietician who assisted us in the preparation of test meals; Dr. Fatima Banaag who undertook the physical and medical examination of each subject and Ms. Norma Pablo for her involvement during blood extraction activities; Dr. Manuel C. Macapinlac and Dr. Milagros D. Ibe for the various consultation services rendered and Dr. Benjamin de la Cruz, Dr. Manolito G. Natera and Commissioner Zoilo M. Bartolome for their well-sustained support for the study.

We sincerely thank the sixteen volunteers who conscientiously and patiently cooperated in meeting the requirements of the study.

The clerical assistance of Ms. Julieta G. Juson and Mr. Gerardo M. Santos is hereby gratefully acknowledged.

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CHI-SQUARE TEST

Aug. 12, 1980

1. 33548
2. 33454
3. 33122
4. 33174
5. 33572
6. 33510
7. 33198
8. 33190
9. 33223
10. 33289

$x_1 = 17371049$

$\bar{x} = 33328$

$$\frac{(x_1 - \bar{x})^2}{\bar{x}} \quad \text{or} \quad \chi^2 = \frac{x_2 - n\,(\bar{x})^2}{\bar{x}}$$

$$= \frac{3332800 - 10\,(3328)^2}{33328}$$

$$= 8.1 \quad \text{acceptable}$$

Aug. 18, 1980

1.	28066	6.	27977
2.	28208	7.	28110
3.	27933	8.	28091
4.	28095	9.	27955
5.	28200	10.	28198

$x_1 = 10210022$

$\bar{x} = 28082.3$

$$\chi^2 = \frac{x_2 - n\,(\bar{x})}{\bar{x}} = 3.34 \quad \text{acceptable}$$

CHI-SQUARE TEST

Aug. 25, 1980

1. 41363
2. 40968
3. 41222
4. 41159
5. 40873
6. 41089
7. 41272
8. 40968
9. 41240
10. 41072

$\Sigma_1 = 15386466$

$\bar{X} = 41124.6$

$$\chi^2 = \frac{x_2 - n\,(\bar{x})^2}{\bar{X}}$$

$= 5.18$ acceptable

Chi-Square Test July 13, 1980

X_i	$(X_i - \bar{X}$	$(X_i - \bar{X})^2$
1. 33548	220	48400
2. 33454	126	15876
3. 33122	-206	42436
4. 33174	-154	23716
5. 33572	244	33124
6. 33510	182	16900
7. 33198	-130	16900
8. 33190	-138	19044
9. 33223	-105	11025
10. 33189	- 39	1521

$\Sigma X_i = 333280$

$\bar{X} = 33328$

$$\frac{(X_i - \bar{X})^2}{\bar{X}} = \frac{271581}{3328} = 8.14$$

LINEARITY TEST JULY 18, 1980

Sources	KeV	Window	LL	Midpoint
AM	060	40	33	35
57 Co	124	40	44	46
131 I	364	100	184	189
137 Cs	662	100	330	335
22 Na	1274	350	614	632
60 Co	1332	350	656	674

$X = 1911$ $\bar{X} = 318.5$

$X^2 = 1004987$ $\bar{Y} = 656.8$

$Y = 3916$

$XY = 1997996$

$$b = \frac{6\ (1997996) - (1911)\ (3916)}{6\ (1.004.987)}$$

$$= \frac{4695600}{2375001}$$

$= 1.97$ or 2 KeV/Step

$a = \bar{Y} - b\bar{X}$

$= 656.8 - 2(318.5)$

$= -0.2$

$$L\ (\text{steps}) = \frac{Y + .2}{1.97}$$

Best Available Document

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LINEARITY TESTS July 2, 1980

Sources	KeV (y)	window	LL	Midpoint (x)
Am	060	40	34	36
57 Co	124	40	50	52
131I	364	100	187	192
137 Cs	662	100	326	331
22 Na	1274	350	602	620
60 Co	1332	350	635	652

$$b = \frac{n \quad XY - x \quad Y}{N \quad x^2 - (X)^2} \qquad a = \bar{y} - b\,\bar{x}$$

$X = 1883$ $\qquad \bar{X} = 313.8$

$X^2 = 959929$ $\qquad \bar{Y} = 636.8$

$Y = 3821$

$XY = 1955962$

$N = 6$

$$b = \frac{6\ (1955962) - (1883 \times 3821)}{(6 \times 959929) - (1883)^2}$$

$$= \frac{4540829}{2213685} = 2.0511 \text{ KeV/Step}$$

$$a = 636.8 - 2.05 \times 313.8 = -6.84$$

$$x \text{ (steps)} = \frac{y \text{ (KeV)} + 6.84}{2.0511}$$

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Aug. 19, 1950

SENSITIVITY CHECK

1.	41394	11.	41256
2.	41556	12.	41152
3.	41319	13.	41588
4.	41202	14.	41083
5.	41631	15.	41440
6.	41285	16.	41654
7.	41614	17.	41468
8.	41625	18.	41594
9.	41255	19.	41585
10.	41491	20.	41580

$$\bar{X} = 41462.1$$

$$\sigma^2 = 19.2$$

$$= \sqrt{\sigma^2}$$

$$2\sigma = 407.2$$

$$(UP)\ \bar{X} + 2\sigma = 41869.3$$

$$(LP)\ \bar{X} - 2\sigma = 41054.9$$

$$At = Aoe - \frac{1.02}{T^{1/2}} \times T$$

$$= 41462.1\ x^{0} - \frac{.693}{27} \times \frac{50}{365}$$

$$= 41462.1 \times .996$$

$$= 41316$$

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Quality Assurance Test Dose for the Month of August

Aug. 18, 1980

ENERGY LINEARITY TEST

Sources	MeV	Window	Lower Level	Midpoints
Al	.060	40	29	31
57 Co	.124	60	45	48
131 I	.364	100	175	180
137 Cs	.662	100	326	331
^{22}Na	1.274	350	606	624
^{60}Co	1.332	350	620	657

$$b = n\sum xy - \sum x \sum y / n\sum x^2 - (\sum x)^2$$

$$= \frac{6\ (1962554) - (1871)\ (3816)}{6\ (566251) - 3500641}$$

$$= \frac{11775324 - 7139736}{2296865}$$

$$= \frac{4635588}{2296865}$$

$$= 2.02$$

$$a = \bar{y} - b\bar{x}$$

$$= 636 - 311.3\ (2.02)$$

$$= 6.2$$

$$\Delta = (\text{steps}) = \frac{Y + 6.2}{2.02}$$

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PAEC WHOLE BODY COUNTER

QUALITY CONTROL FORM

	DATE	GAIN	SENS.	BKGD	INIT.		DATE	GAIN	SENS.	BKGD	INIT.
1	27.6.80	6.8 (MCA)	42150	587.5	Re	12	7/21/80	5	—		
2	30-6-80	"	42639	572.5		13	7/22/80	5	—	720	
	1-7-80	8.1	42498			14					
4	1-7-80	6.8				15	7/17/80				
5	2-7-80	6.5	42410	662.4		16	7/23/80	5	42034	715	
6	3-7-80	7.8	42517	640		17	7/24/80	5	42318	745.4	
7	7-7-80	8	42502	665.7		18	7/25/80	5	42372	716	
8	7-8-80	6.	42010	656..		19	7/28/80	5	42030	720.4	
9	7-9-80	6, 5.2	42118	607		20	7/29/80	5	42238	685.0	
10	7-10-80	6.5	42380	604		21	7/30/80	5	42123	693.8	
11	7-11-80		42872	3350 1580		22	7/31/80	5	41897		
12	7/17/80	4.9	42185	682.2	Re	23	8/1/80	5	42071	712.7	
	7/18/80	4.9	42073	647.8			8/4/80	5	42020	668.0	

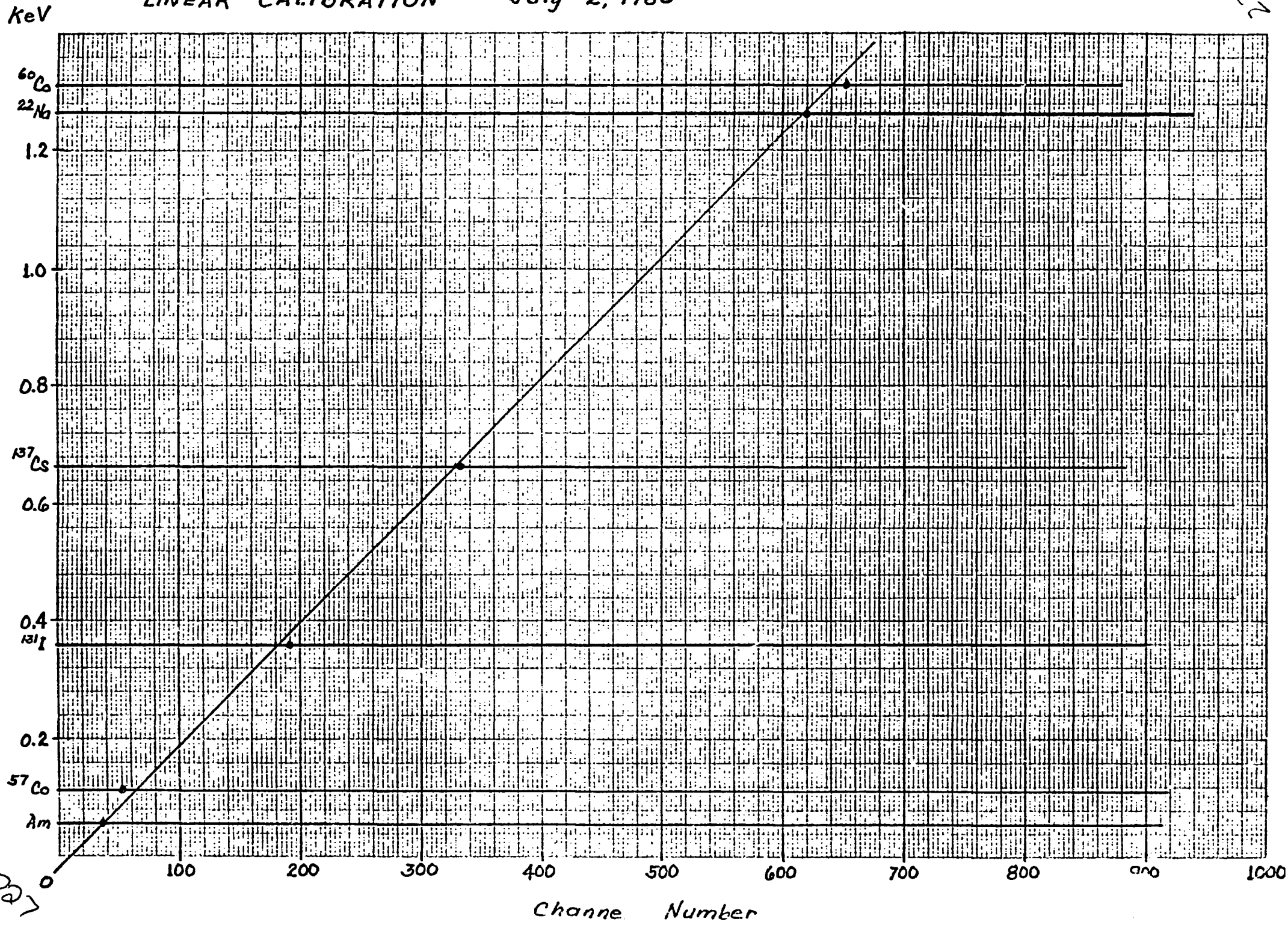
PAEC WHOLE BODY COUNTER

QUALITY CONTROL FORM

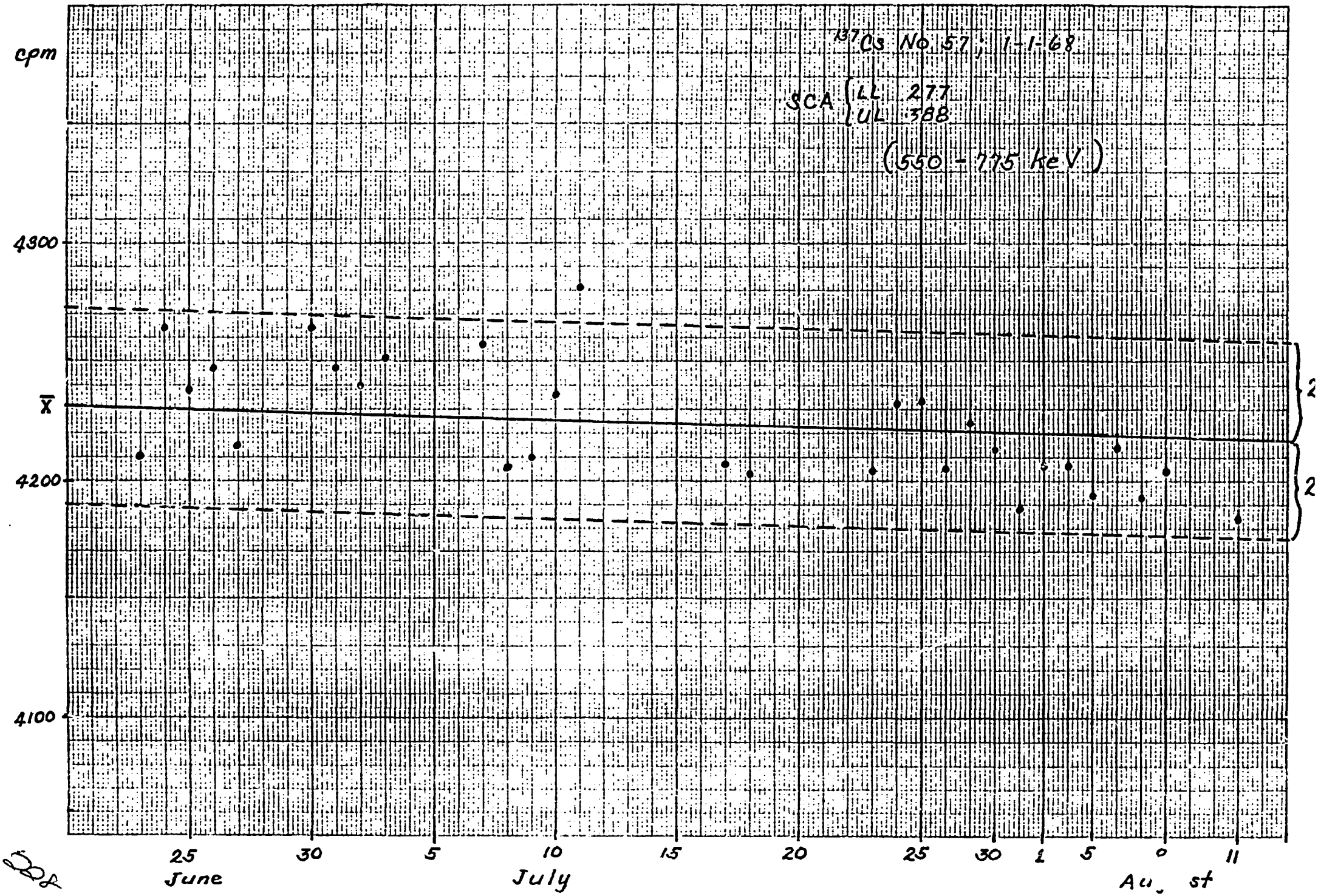
DATE	GAIN	SENS.	BKGD	INIT.	DATE	GAIN	SENS.	BKGD	INIT.
8/5/80	5	41918	792.2		8/26/80	5	41241	634	
8/6/80	5	42102	679.8						
8/7/80	5	42153	802.2		8/29/80	5	41264	610	
8/8/80	5	42050	690.0		9/1/80	5	41471	590.4	
8/11/80	5	42013	678.5		9/2/80	5	41410	602.8	
8/12/80	5	42035	667		9/3/80	5	41282	605.2	
8/13/80	5	41989	684.6		9/4/80	5	41150	608	
8/14/80	5	41925	703.9		9/5/80	5	41241	587	
8/15/80	5	42019	686.4		*9/8/80	5	*40175*	—	
8/18/80	5	41845	679.2		9/9/80	5	40137		
8/19/80	5	41[illegible]	622		9/10/80	5	40250	613	
8/19/80	5	41250			9/11/80	5	40288	608	
8/20/80	5	41294	636.8		*9/12/80	5	27214	651	
8/21/80	5.2	41504	632.7		9/15/80	5	27023	624.4	
8/22/80	5	41293	646.4		9/16/80	5	27058	662	
8/25/80	5	41293	621		9/17/80	5	27117	954	

Continuing Calibration

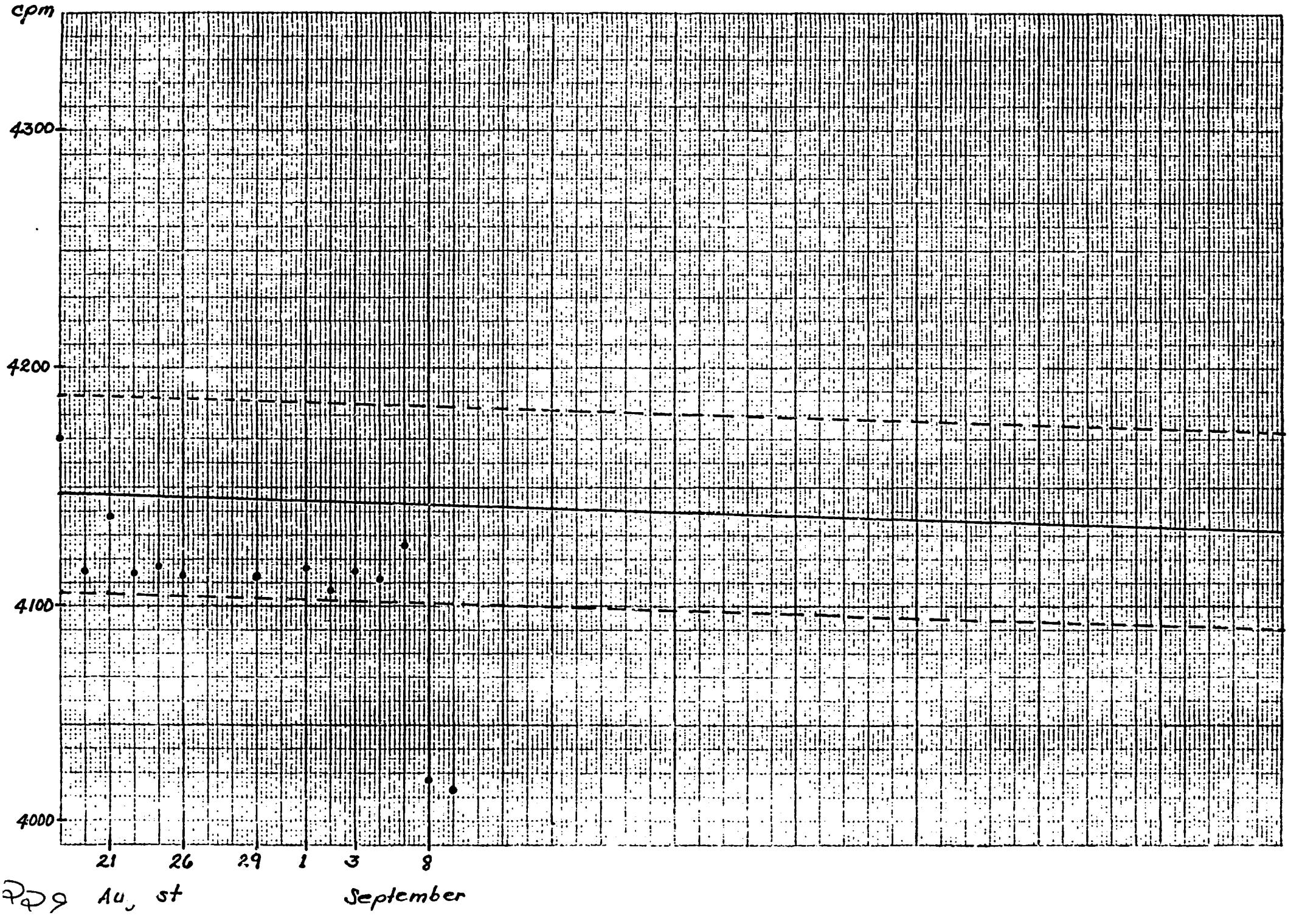
LINEAR CALIBRATION July 2, 1980



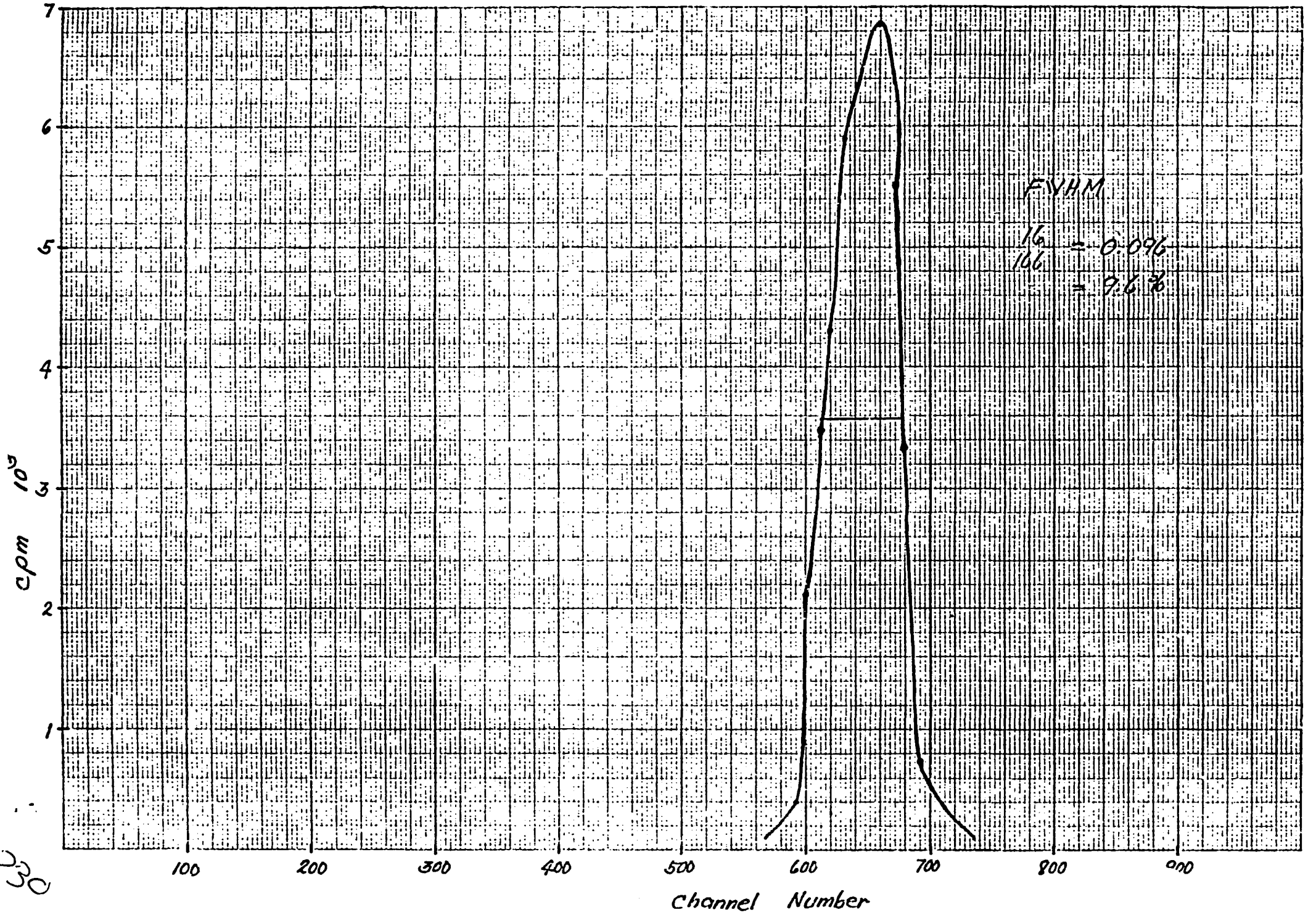
DAILY SENTIVITY CHECK



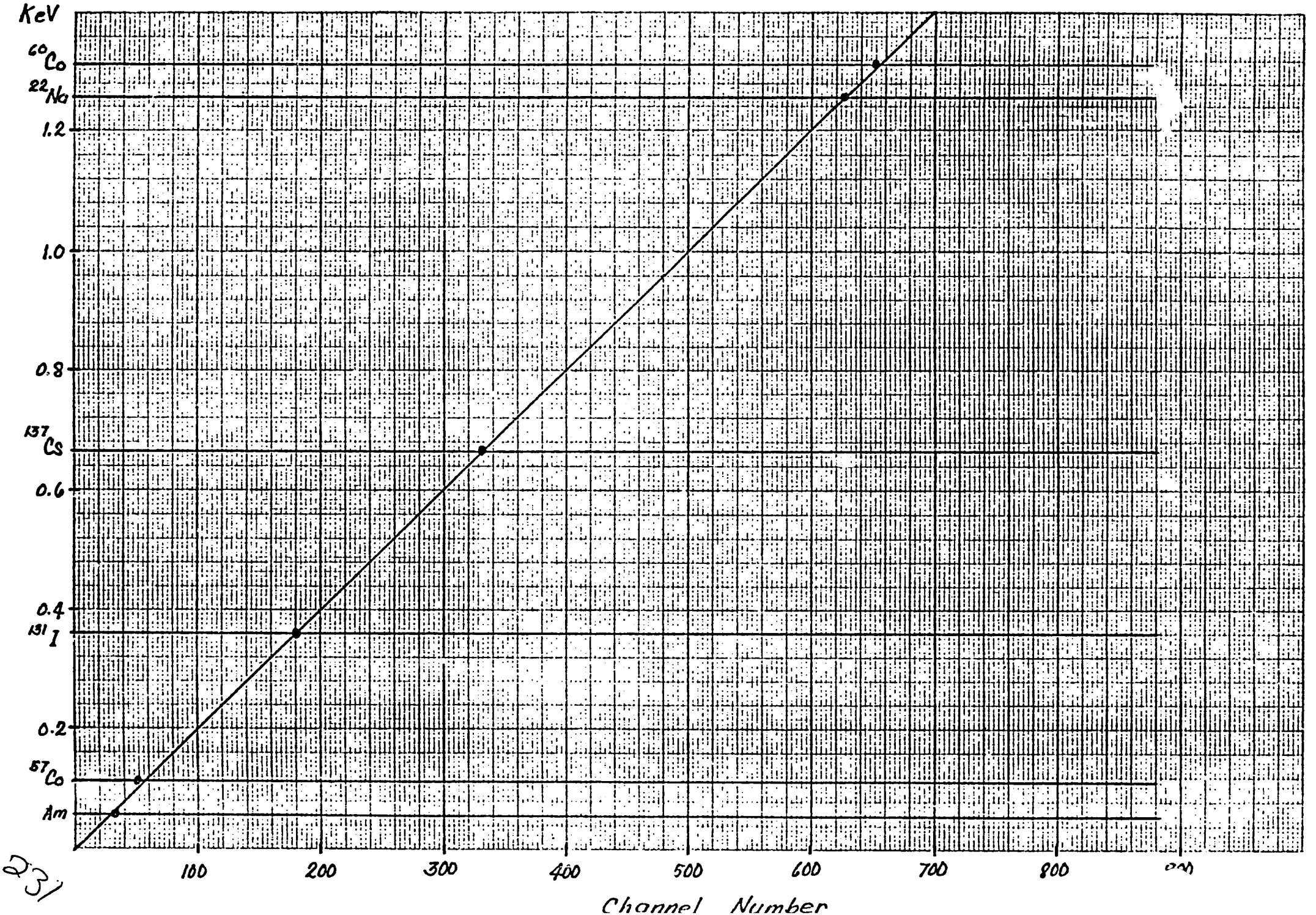
DAILY SENSITIVITY CHECK



ENERGY RESOLUTION Aug. 7, 1980



ENERGY LINEARITY TEST Aug. 18, 1980



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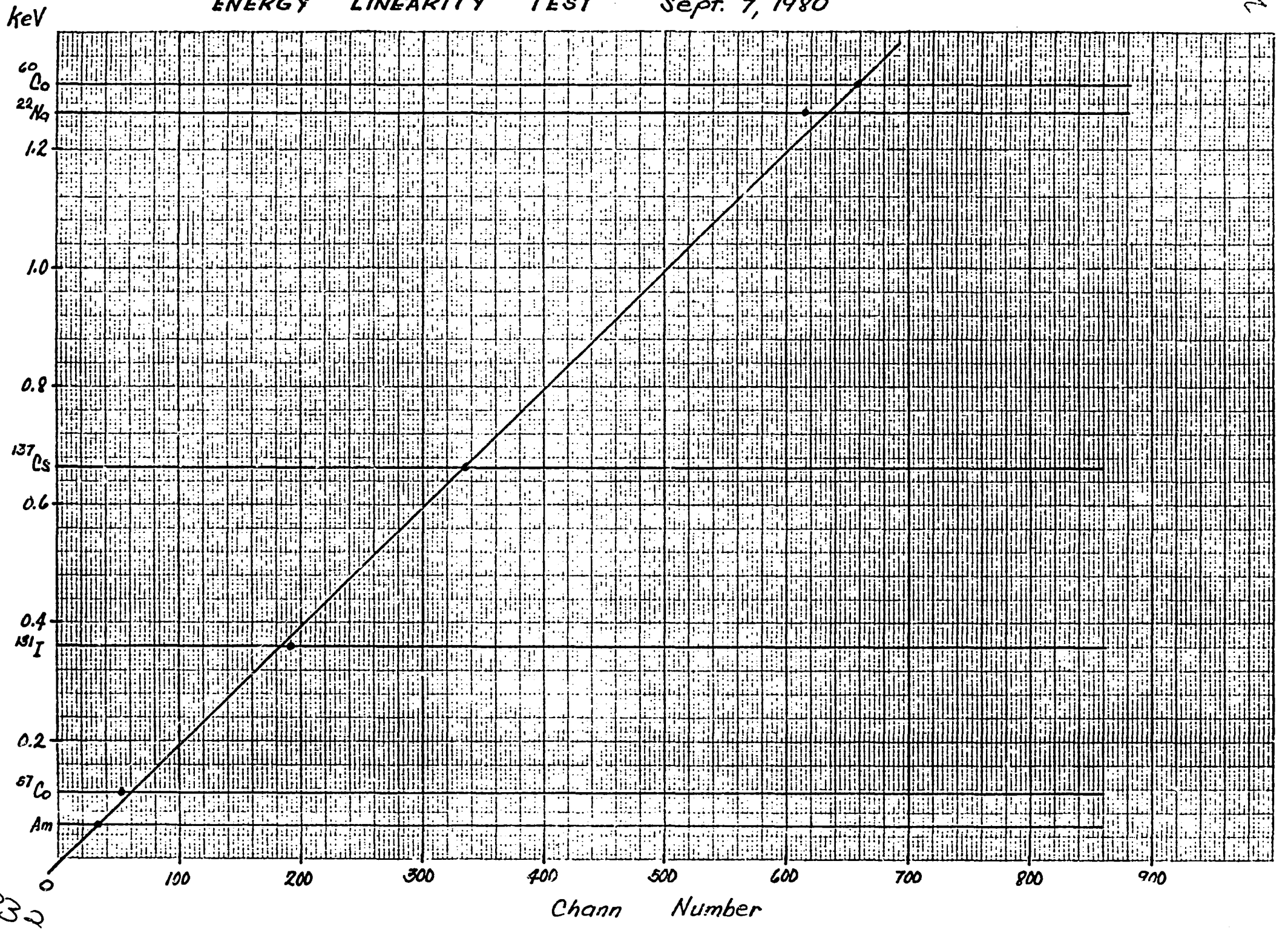

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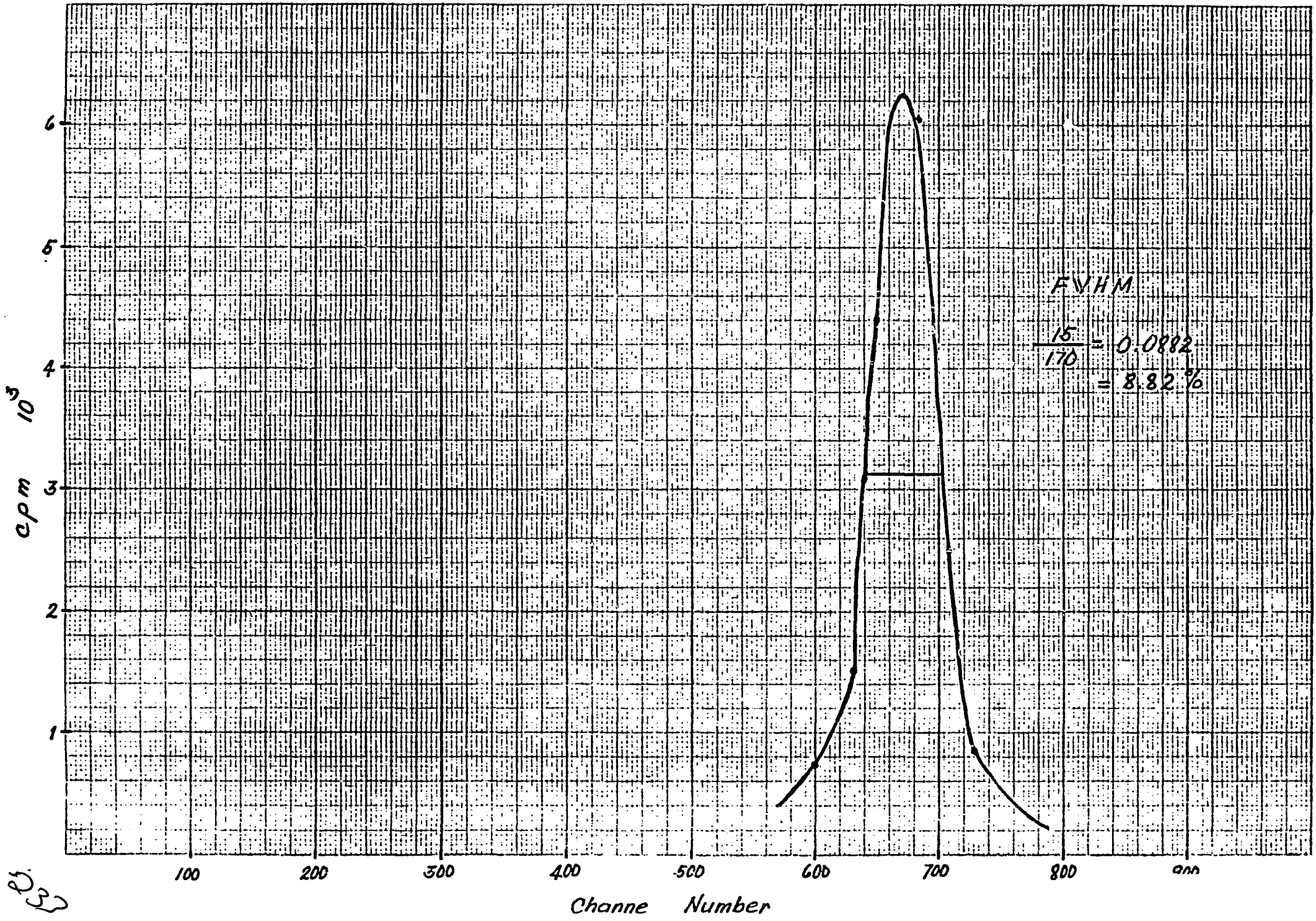

Fig. 3

DRAFT

A SUGGESTED STRATEGY FOR THE CONTROL OF ANAEMIA IN THE ENGLISH-SPEAKING CARIBBEAN

Prepared at the Technical Workshop on the Control of Anaemia in the English-speaking Caribbean

1-5 June 1981
Kingston, Jamaica

Caribbean Food and Nutrition Institute
Kingston 7, Jamaica

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

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PRIORITIES FOR ANAEMIA STRATEGY

Anaemias of public health importance in the English-speaking Caribbean are: nutritional anaemia, anaemias caused from infestations of hookworm and whipworm and sickle cell anaemia. These anaemias mainly affect pregnant and lactating women and pre-school age children. The priorities for the anaemia strategy are as follows:

(1) A Caribbean and individual country strategy should be developed. They should be part of the Regional Food and Nutrition Plan and part of National Health Plans. Programmes for the control of anaemia should not be vertical programmes. Inter-sectoral measures should be used.

(2) The use of appropriate technology through the primary health care system should be stressed.

(3) A mini-model should be developed in one or two countries thereby studying the four recommended approaches by the World Health Organization (WHO) in detail.

(4) Collaborative efforts should be started in each member country of the Caribbean Food and Nutrition Institute (CFNI) to study iron supplementation in ante-natals.

(5) A 'slow release' iron preparation should be tested in the existing ante-natal iron supplementation programme in one country.

(6) Collaborative efforts should be increased in member countries on screening haemoglobins through the primary health care system.

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(7) Collaborative efforts should be increased with the Caribbean Epidemiology Centre (CAREC) and member countries on the control of intestinal parasites.

(8) Consider increasing the quantity of iron in wheat flour from 30 mg/kg to 45-60 mg/kg.

(9) Add 30 mg/kg of iron to cornmeal in one country.

(10) Develop a better anaemia education programme in the Caribbean and stress education in the four recommended approaches by WHO.

(11) Work with member countries on the identification of sickle cell anaemia.

(12) Collaborate with member countries on the individual preparation of ferrous sulphate in syrup for iron supplementation for pre-school age children.

(13) Collaborate with member countries and CAREC on the standardization of haemoglobin determinations.

I. INTRODUCTION

The Problem

Anaemia is a problem in all countries of the English-speaking Caribbean. The prevalence varies from 27.2% to 100% in pregnant and lactating mothers and from 11.1% to 76.0% in pre-school children. The most common cause is a deficiency of iron. This is probably due to an inadequate intake with a low absorption. A deficiency of folate is also probably a responsible factor in the pathogenesis of some of the anaemias. Anaemias associated with parasitic infestations such as hookworm and whipworm and haemoglobinopathies such as sickle cell anaemia are also found.

It is well known that there are many possible causes of anaemia. However, from the public health point of view, there are three main categories:

1. Nutritional Anaemia.
2. Anaemia associated with parasitic infestations such as hookworm and whipworm.
3. Anaemia associated with haemoglobinopathics such as sickle cell anaemia.

There is frequently overlapping among these three categories of anaemia. For example, infestations such as hookworm increase the requirement for haemopoietic nutrients and accelerate the development of nutritional deficiencies.

The World Health Organization and the International Nutritional Anemia Consultative Group (INACG) recommend four approaches for the eradication of nutritional anaemia. They are as follows:

1. Fortification of one or more food vehicles.
2. Direct oral or intramuscular supplementation with the missing nutrient(s).
3. Educational approaches to alter traditional diets.
4. Public health measures against environmental factors that depress absorption or increase metabolic needs.

All of the countries in the English-speaking Caribbean employ these four approaches to some extent. However, the problem of nutritional anaemia still exists. Is this because of the ineffective implementation of these approaches, or are the approaches themselves at fault?

The Technical Workshop

The Caribbean Food and Nutrition Institute/Pan American Health Organization (CFNI/PAHO) convened a Technical Workshop on "The Control of Anaemia in the English-speaking Caribbean" in Kingston, Jamaica, 1-5 June 1981 to develop a suggested strategy for the control of anaemia in the English-speaking Caribbean.

Participants were drawn from eight of the countries served by CFNI/PAHO and also included representatives from other divisions of PAHO/WHO. Representatives and consultants from the University of the West Indies (UWI) and INACG also attended the Technical Workshop. The financial contribution of INACG and the United States Agency for International Development (USAID) is appreciated. (See List of Participants, page 37).

Available data on the prevalence and cause and current methods of controlling anaemia in each country was discussed.

This included haemoglobin levels in pre-school age children and pregnant women from clinic records, data available on parasites, clinic coverage, iron and folic acid distribution. food fortified with iron. food patterns, sanitation and nutrition education programmes. Suggestions were made how slight modifications could be made to current anaemia control programmes to better control the problem. The use of the primary health care system was emphasised.

The following suggested strategy was developed by the participants on the basis of these various inputs and the ensuing group discussions. It is hoped that the implementation of the suggested strategy will lead to activities which will result in an improvement of the anaemia and nutritional status of people in the Caribbean and, through lessons learnt, elsewhere.

The Deleterious Effects of Anaemia

Severe anaemia limits the oxygen-carrying capacity of the blood and may limit maximum work output. More recent studies indicate that mild anaemia, and possibly even iron deficiency in the absence of anaemia, reduces the maximum level of work. Most daily tasks do not involve sustained physical effort, nevertheless, studies among plantation workers in Indonesia and tea pickers in Sri Lanka, demonstrated that, in ordinary occupation, anaemic individuals had a decreased work output compared with that of non-anaemic colleagues.

In pregnancy, severe anaemia is associated with an increased risk of premature delivery and increased foetal and maternal morbidity and mortality. Milder degrees of anaemia may also be detrimental and have been shown to be

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associated with premature delivery, lower birth weight, placental hypertrophy and increased maternal estriol excretions.

It is generally considered that anaemic individuals are more likely to develop infections than non-anaemic ones, but epidemiological studies relating the incidence of infections to the prevalence of anaemia are difficult to control and interpret. Also, some studies have indicated that anaemia may have an effect on mental development.

II. STRATEGY

Broad Considerations

After detailed discussions it was agreed that the four approaches recommended by WHO and INACG were applicable to the English-speaking Caribbean. However, a Caribbean strategy and National programmes should take into account the following considerations:

(1) All four approaches should be employed in an integrated manner. Failure to control anaemia in the Caribbean is probably due to an unbalanced approach. There is a need to devise the most efficient and effective programme through controlled trials in one or two Caribbean countries. Such a trial should not delay improvements in other countries. It will facilitate adjustments later.

(2) Approach 3 (education) should read: Modification of diets where necessary to encourage a sufficient intake of haematinic elements and their adequate absorption.

(3) Any programme for the control of anaemia should emphasise the development of appropriate technologies and delivery through the primary health care system.

(4) Any programme for the control of anaemia should not be a vertical programme but should be part of an overall health and nutrition programme.

(5) The suggested strategy as set forth in this document should be an integral part of the Caribbean Food and Nutrition Plan.

(6) Monitoring at the individual level and surveillance at the national level are essential components of the anaemia strategy. Consequently, the use of simple haemoglobin screening techniques in the primary health care system and a system of standardization of the measurement of haemoglobins throughout the region should be linked to a system of data flow and analysis. CFNI should continue to develop innovative approaches here.

Fortification

Background

Fortification is the addition of iron and/or folate to some foodstuff. It can be directed at the public at large using a widely consumed product such as wheat flour or sugar. Alternatively, it can be directed at a particular risk group, for example infants who are not breastfed using artificial formulas as the vehicle.

There are three options for increasing iron absorption by fortification. The first and most obvious is to add an absorbable iron salt to some vehicle in the diet; the second is to add some facilitator of iron absorption such as ascorbic acid; and the thrid is to do both. All three approaches have been shown by radioisotopic studies to be feasible.

In considering *iron compounds* it is essential to review not only the chemical and physical properties of each compound but also those of the vehicle to be fortified. Unfortunately, many iron compounds, especially those most readily absorbed, are highly reactive and produce a variety of

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undesirable changes in foods to which they are added. The following examples of the chemical properties of iron compounds must be kept in mind:

- ferrous salts may be oxidized to form yellow, green, or black ferric oxide;
- iron salts may react with phenolic compounds such as tannins and propyl gallate to form blue-black colours;
- iron compounds may react with sulphur compounds to produce a black colour;
- iron compounds may increase the activity of oxidation enzymes that cause off-colours, -odours and -flavours;
- iron is a known pro-oxidant and an iron compound may catalyze the development of oxidative reactions;
- high density products such as reduced iron powders may require special measures to achieve a stable and uniform distribution;
- the colour and flavour of the iron compounds themselves may be undesirable and carry through into the food. These problems may be especially noticeable in iron-fortified beverages; and
- the solubility characteristics of the iron may not be compatible with the food to be fortified.

All of the pre-ground wheat flour imported into the Caribbean is fortified with vitamins and iron. All wheat flour imported into the Caribbean and ground locally is fortified with vitamins and iron except in Suriname. Wheat flour is fortified at the following levels:

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

Fortification with calcium is optimal and is usually not done.

The iron added in both pre-ground as well as locally ground wheat is in the form of either ferrous sulphate or reduced iron. Ferrous sulphate has a higher bioavailability than does reduced iron of a large particle size. The reduced iron used is 30-40 mμ in size. Studies have shown that this large particle size reduced iron is absorbed to a limited extent.

To monitor the level of fortification and type of iron compound in pre-ground wheat flour would be difficult since most of the imported wheat flour comes from Canada, the United States of America, Puerto Rico and West Germany as is shown in Figure 1. Some wheat flour is ground and fortified with iron in St. Vincent and re-exported to other Caribbean countries. Specifications such as the type of iron compound used and the level of fortification would require legislation and governmental control. At the present time the imported wheat flour is not checked in the countries of import as to its type or level of fortification. Wheat flour is fortified by law in Jamaica, Trinidad and Tobago and Guyana.

The factors involved in a good fortification programme are many and complex. Many important facts such as iron overload, effects on haemochromatosis

and thalassemia, the best types of iron, and possible induction of a deficiency of other trace minerals and its effects on infections are still unknown.

If the present fortification programme was changed, factors such as price, cost-effectiveness, rancidity, uniform distribution in the vehicle and a careful monitoring of persons with haemochromatosis and thalassemia should be considered.

As can be seen in Figures 2 and 3, most of the infant formulas and foods are imported into the Caribbean from Canada, the United States of America, England, Belgium and Holland. The infant food produced in Guyana is already fortified with iron. One possibility would be to add iron to cornmeal in Jamaica since it is used as the principal weaning food.

Suggested Strategy for an Iron Fortification Programme

1. Objective

The objective of a change in the fortification programme would be to increase the average dietary intake of iron by 25%.

2. Technical Aspects

2.1. Vehicles for fortification

2.1.1. Wheat flour

It is recommended that studies start as to the efficacy of increasing the amount of iron added in wheat flour from 30 mg/kg of wheat flour to 45-60 mg/kg of wheat flour. The iron should be either ferrous sulphate or reduced iron of a small particle size (80% should be 10-20 mμ in size).

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2.1.2. Cornmeal

Cornmeal is the principal weaning food in Jamaica and at present no additional iron is added. All of the cornmeal is processed at a central plant. It is recommended that studies start immediately to determine the feasibility of adding iron to the cornmeal. The iron should be either ferrous sulphate or reduced iron of a small particle size and should probably be added at the level of 30 mg/kg of cornmeal.

2.1.3. Brown sugar

Molasses used for making rum and not otherwise widely used contains approximately 34.0 mg/100 g of iron.

Brown sugar contains approximately 3.4 mg/100 g of iron. In the Caribbean the average per caput consumption of sugar is 60 g/day. Therefore, a person could conceivably consume 2.04 mg of iron per day from sugar. However, the amount of iron in sugar that is absorbed is questionable.

It is therefore suggested that the fortification of brown sugar with iron sodium EDTA be considered. White sugar is presently being fortified with iron sodium EDTA in Guatemala and Venezuela. However, one study done in Guatemala has shown that if moisture is present, there is settling of the iron and this causes uneven distribution of the iron and produces colour changes. This would require a careful study before

deciding on brown sugar as a vehicle. It would have to be determined if iron sodium EDTA forms a black precipitate in tea.

2.1.4. Rice

The technology of adding iron to rice has not been fully explored. At the present time it is not recommended to fortify rice in the Caribbean with iron.

2.1.5. Sweetened condensed milk

This product is widely used by a large cross-section of the population particularly in one Caribbean country. It is produced locally by two plants in that country.

It is recommended that this product be considered for the addition of iron. Studies should be done to determine:

(a) type of compound suitable for fortification;

(b) cost;

(c) shelf life;

(d) palatability and consumer acceptance.

2.1.6. Salt

Salt is imported into Jamaica and processed at a central factory. In other Caribbean countries all salt is imported and there are no central processing plants. In India the fortification of salt with iron is still at an experimental stage. It was recommended not to consider salt as a possible vehicle for the addition of iron.

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2.1.7. Macaroni and spaghetti

Macaroni and spaghetti that are imported into the Caribbean are fortified with iron. However, the macaroni and spaghetti that are produced in Barbados, Jamaica and Trinidad and Tobago are not fortified with iron.

It is recommended that a study be undertaken to ascertain the consumption of these products by the lower socio-economic group with the possibility of recommending fortification with iron.

2.1.8. Non-traditional vehicles

It was suggested that each country examine some of the other popular food products that are widely used, e.g. the chocolate beverage powder, Milo. Most of the Milo sold in the Caribbean is produced in Jamaica and has 6 mg/oz of ferric oxide added. This type of iron is not absorbable. It is recommended that a different and more available type of iron be added if it is technically feasible. If it is not, then the product should not be promoted by the distributors as an iron-containing food.

3. Institutional Framework

The overall responsibility for any project to fortify foods with iron would rest with the Ministry of Health with assistance from CFNI. Initial research should be done at a research institute in the Caribbean into (a) the feasibility of fortifying wheat flour at twice the present level of fortification and (b) the fortification of cornmeal.

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Absorption studies from typical Caribbean meals as well as absorption from meals with the higher fortified cereals could be conducted at the Tropical Metabolism Research Unit (TMRU), University of the West Indies, Jamaica.

The monitoring of the fortified product could be done by the Bureau of Standards or a scientific agency which should also check the level of fortification of imported wheat flour. The responsible importation agency could discuss with suppliers the possibility of increasing the level of fortification in imported wheat flour.

Measurable improvements in anaemia status are likely to occur with increased fortification but they will take some years to become manifest.

4. Training Needs

An individual from the Caribbean should be sent for two months to the University of Kansas, Kansas City, Kansas to learn iron absorption techniques should absorption studies be needed. A person from Jamaica and Trinidad and Tobago should also be sent to the University of Kansas to learn the technique of serum ferritin.

5. Educational Methods, Messages, Materials

Planners and administrators must be sensitised to the need for fortification. The population should also be alerted with regard to fortified products. Importance of labelling for identical products with different levels of fortification should be made known to the public.

6. Data Gaps

6.1. Need for research into effects of doubling fortification of flour.

6.2. Consumption patterns of certain foods (e.g. flour and cornmeal) by area and socio-economic groups.

6.3. Strategy for reaching persons who will not be reached by fortification programme.

6.4. Application of serum ferritin methodology.

6.5. Absorption of iron from traditional diets.

6.6. Technology of fortifying foodstuffs with folate.

7. Monitoring and Surveillance

7.1. Any fortification programme should be carefully monitored measuring changes of body iron stores in the recipients. This can be done through a newly developed technique of estimating serum ferritin levels (the level of serum ferritin is proportionate to the amount of stored iron present).

Supplementation

Background

As there is a high prevalence of iron deficiency anaemia in the population, supplementation is needed to treat the most severely affected or at-risk individuals. This is the only way to improve iron nutrition status within a period of weeks. Supplementation is particularly important in pregnancy where the objective is to achieve satisfactory haemoglobin levels prior to delivery. From considerations of iron balance it is clear

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that the requirements of pregnant women in the Caribbean cannot be met during pregnancy by diet alone.

Supplementation programmes may be influenced by a variety of factors such as the nature of the diet, the properties of the iron supplement (tablets) used, whether or not folate is used, the prevalence of parasitic infestations (particularly hookworm), local customs and beliefs, acceptability and the organization of the health services. For this reason, a programme successful in one area may not succeed in another; each programme must be carefully tailored to fit the local situation.

The Caribbean Food and Nutrition Institute/Pan American Health Organization (CFNI/PAHO) have collaborated with the Government of Jamaica in evaluating the copper sulphate method for screening haemoglobins in the primary health care system. CFNI/PAHO have also developed another simple instrument for screening haemoglobins in the field. There is need to further evaluate these simple techniques for screening haemoglobins in the primary health care system.

The target population for supplementation must first be identified. In most areas the groups with the highest prevalence of iron deficiency anaemia are likely to be infants (particularly those born with a low birth weight), children and women of child-bearing age, especially pregnant women.

The numerous pharmaceutical preparations of iron on the market differ widely in their elemental iron content, the iron compounds employed, their pharmaceutical properties (such as their disintegration and dissolution rate in the intestine) and their cost.

All readily soluble ferrous iron compounds suitable for oral administration are, in general, equally well absorbed and produce the same type of side effects with similar frequency. Ferrous sulphate, one of the

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cheapest available compounds, is recommended for use in public health programmes.

The way in which tablets are manufactured (e.g. the amount of pressure used to form the tablet, the nature of the excipient and the type and thickness of coating) considerably influence the biological availability of the iron. As there seems to be no fully reliable *in vitro* method, the bioavailability of the iron in the tablets must be studied by performing an *in vivo* trial.

Iron tablets or iron and folate tablets are distributed at ante-natal clinics in all countries in the English-speaking Caribbean. These tablets vary in dosage and are usually ferrous sulphate, ferrous fumerate, or ferrous gluconate, folate or ferrous sulphate/folate. The reasons for such a high prevalence of anaemia in pregnancy are probably a low clinic coverage in some countries, side effects in some people, low compliance, poor iron reserves and an inadequate monitoring of the haemoglobin level in some countries at the ante-natal clinics.

Liquid iron in the form of ferrous sulphate or ferrous ammonium citrate with or without folate is used in child health clinics in most of the English-speaking Caribbean countries. The haemoglobin level of preschool age children attending child health clinics is checked in only some of the Caribbean countries.

The side effects of therapeutic doses of oral iron fall into two main categories: (a) those related to the upper gastrointestinal tract - nausea, epigastric pain and vomiting; and (b) those related to the lower gastrointestinal tract - namely diarrhoea and constipation. The prevalence of these side effects, especially those arising from the upper gastrointestinal tract, is dose-related. All iron compounds appear to produce the same prevalence of side effects for the same amount of available iron.

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The prevalence of upper gastrointestinal side effects may be reduced if iron is slowly released in the gastrointestinal tract. There are some slow release preparations on the market in which the bioavailability of the iron appears to be reduced. A slowly dissolving preparation with a high bioavailability of the iron, if associated with a reduction in side effects and if available at a competitive price, might represent a significant advance in therapy enabling appropriate supplementation to be given with a single daily dose when otherwise several doses of a readily soluble iron tablet might have to be given to achieve the same results. Trials of any such potential products should be undertaken if absolutely safe and if there are likely benefits to the participants.

Suggested Strategy for a Supplementation Programme

1. Objective

   1.1. To reduce the prevalence of severe anaemia (Hb <8.0 g/dl) in ante-natals by 75% in five years.

   1.2. To reduce the prevalence of mild to moderate anaemia (Hb <10.0 g/dl) in ante-natals by 75% in five years.

   1.3. To reduce the prevalence of anaemia in pre-school age children by 50% in five years (Hb <10.0 g/dl).

   1.4. To reduce the prevalence of anaemia in school age children by 50% in five years (Hb <11.0 g/dl).

2. Technical Aspects

   2.1. Ante-natals - supplementation for all ante-natals is recommended and should continue throughout pregnancy.

   2.1.1. Type of supplements

   Supplementation with iron and folic acid with each tablet containing 60 mg ferrous elemental iron,

100 µg folate and an unspecified quantity of ascorbic acid as an enhancer.

2.1.2. Dosage

Supplements should be one to two tablets per day and the specified tablet set out in 2.1.1. should be administered independent of haemoglobin status or stage of gestation, except that none should be given in the first trimester.

2.1.3. Reducing side effects

2.1.3.1. It is inadvisable to increase dosage as this would increase the possibility of side effects.

2.1.3.2. It is advisable to avoid the administrations of any drugs including iron supplements, during the first trimester of pregnancy because of (a) the remote possible teratogenic effects (b) the risk of non-compliance linked with hyperemesis gravidarum (c) the low risks associated with anaemia at this time.

2.1.3.3. The iron tablet should be taken, at bedtime and between meals. If any side effects threatening non-compliance are noted, the tablets should be taken with meals or ferrous fumerate should be used.

2.1.3.4. A locally made acid fruit drink should be taken with the tablets.

2.1.4. Haemoglobin tests should be done on pregnant women at least at the first visit and at 36 weeks with further investigations being done every four to six weeks where feasible. Haemoglobin tests using a technology appropriate at the primary health care level should be done on pregnant women at least at the first visit. One suggested schedule is shown in Annex 1.

2.1.5. An algorithm be developed for intervention at different levels of haemoglobin and different gestational periods.

2.1.6. One iron/folate tablet should be given for six weeks post-partum.

2.2. Pre-school age children - 0-5 years of age

2.2.1. Type and dosage

Ferrous sulphate liquid is the best iron preparation for supplementation in children. It should be available at a strength of 25 mg/ml. The maintenance dose of supplemental iron for a child is 2 mg elemental ferrous iron per kilogram per day (6 mg ferrous sulphate per kilogramme per day). The therapeutic dose is three times as much for six weeks. Inexpensive ferrous sulphate solution can be made in the Caribbean. The therapeutic dose of folate is 2-5 mg per day. Folate therapy should continue for three to four weeks.

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2.2.2. Low birth weight babies (2.5 kg)

All babies of low birth weight - and those not weighed at birth - should receive maintenance doses of iron and folate from six weeks to six months of age.

2.2.3. Haemoglobin tests should be performed routinely at child health clinics starting at the first visit after the age of six months. There is no need to routinely check haemoglobin before this age because iron stores should be adequate until about four months of age.

2.2.4. Pre-school children with low haemoglobin levels (<10 g/dl) should be given iron therapy.

2.2.5. Pre-school children with haemoglobin levels below 8 g/dl should be referred for a complete examination and probably given therapeutic doses of iron and folate for six weeks and haemoglobin determinations repeated to check the therapeutic response.

2.3. School age children

2.3.1. It is suggested that there should be a full medical examination (including Hb) of all children on entering school (Hb <11.0 g/dl).

2.3.2. Where a high incidence of anaemia (30% of children under 10 g/dl) is detected there should be iron supplementation for all children at the school over a specified period. The iron tablet should contain 30.0 mg of elemental iron.

2.3.3. About 30% of the children from the school in 2.3.2. should be monitored annually throughout school life. Where a fall in haemoglobin levels is detected blanket supplementation should again be done for a set period.

2.3.4. Calculations for iron and folate supplements are shown in Annex II.

3. Institutional Framework

The responsibility for any applied nutrition programme to change the present system of iron supplementation should rest with the Ministry of Health with assistance from the Caribbean Food and Nutrition Institute. Supplementation programmes should be conducted within the framework of primary health care services, the coordinating body being the Maternal and Child Health Committee or the Ministry of Health.

4. Training Needs

Personnel should be trained in principles of primary health care in the preparation and use of algorithms for monitoring the effects of supplementation.

5. Educational Methods, Messages, Materials

A programme should be started to encourage ante-natals to visit the ante-natal clinics by the 16th week of pregnancy. At the sessions of the ante-natal clinics pamphlets and nutrition education should be given on the use of iron supplementation and better nutrition.

6. Data Gaps

6.1. Supplementation research including evaluation should be conducted to test the compliance and efficacy of the slow release floating tablet in a small population.

6.2. Supplementation research to evaluate the correct level of iron supplementation.

6.3. A field evaluation on a country basis of the CFNI haemoglobin screening device.

7. Monitoring and Surveillance

7.1. Any supplementation programme should have a built-in surveillance component by using the haemoglobin measurement.

Behavioural and Dietary Modifications

Background

The design of an educational campaign to control anaemia is dependent on several factors including the collection of baseline information on current intakes of dietary iron, eating patterns and beliefs, availability and cost of iron-fortified foods, etc. This information when collated and analysed can give the policy-makers and programme designers a better idea of how nutritional messages should be developed so as to bring about desired changes in dietary and health behaviours.

Dietary change depends on (a) the availability of the needed foods at prices that consumers can afford and (b) the willingness of those buying and preparing the family food for consumers to choose and eat such foods.

Nutrition education efforts for anaemia prevention and control must be designed for these target audiences:

Policy-makers and Opinion Leaders

Before a national educational effort can be launched, influential members of a society must be sensitised to the importance of the problem and the need for programmes of control and prevention to be planned and implemented. Their commitment to educational programmes is essential if community based activities are to have adequate support.

Community at Large

Educational programmes also require the understanding, support and involvement of community members in order to be successful. Participation from the community should begin in the early planning stages so that any programmes developed reflect the needs of the community as they identify them along with the nutrition needs identified by professionals in the field.

Schools

In any country where the educational system reaches large numbers of citizens at an early age, the schools, particularly at the primary levels, are valuable channels for educational messages in nutrition, especially those geared to bringing about changed attitudes and behaviours. During these formative years, attitudes and values are established. Carefully planned nutrition education activities should be integrated into those subjects taught at pre-school, primary, secondary and tertiary levels which lend themselves to such integration, e.g. health and family life education, science, home economics, agriculture, etc.

School feeding supervisors and staff must be reached with nutrition information so that their work supports and influences the nutrition education in the classroom.

Vulnerable Groups

Special educational efforts in nutrition must be directed through and outside the primary health care services to all population groups which are especially vulnerable to anaemia. These include pregnant and breast-feeding women, infants under one year, children from one to five years of age, children from five to twelve years of age, adolescents and others identified as vulnerable through surveys and surveillance.

Enhancers and Inhibitors

The concept of enhancers and inhibitors is used in nutrition education and is an extremely important concept.

The availability of food iron is best discussed under the headings of haeme and nonhaeme iron, since considerable experimental data indicates that each of these form a different pool of iron. Haeme iron, which comes from haemoglobin and myoglobin, is absorbed directly as the intact iron porphyrin complex and its iron is freed in the intestinal mucosal cell. The proportion of haeme iron which humans usually absorb from their diet is about 35% in an individual who has no iron stores.

The nonhaeme iron pool consists of iron from other foods such as vegetables, grains, fruits, eggs and dairy products as well as from the nonhaeme iron of meats, poultry and fish and from soluble iron supplements.

Unique to nonhaeme iron is the fact that the amount absorbed can be modified markedly by components of foods ingested concomitantly. Dietary factors which dramatically increase the absorption of nonhaeme iron, as much as four fold, are ascorbic acid and a 'meat factor' present in meat, poultry

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and fish. As the quantities of these substances in a complex or composite meal increase, iron absorption increases. These enhancing factors play a very important role, for if they are not present in a meal, the absorption of nonhaeme iron is very low. It is also apparent that a group of other less well defined substances act to decrease nonhaeme iron availability. Tannic acid in tea, phosvitin of egg yolk, phytates, calcium and phosphate salts and antacids clearly suppress absorption when present in sufficient amounts.

The absorption of nonhaeme iron depends not only on the general composition of the meal but along with haeme iron is further affected by the iron status of the individual. Absorption of nonhaeme iron in the iron deficient individual may be as much as 20% when enhancers are abundant (to be contrasted with about 35% haeme iron absorption). A much greater difference is observed in the iron replete subject with a meal lacking enhancers and/or with high levels of blocking substances, where nonhaeme absorption is about 2% as contrasted to about 15% haeme iron absorption. Even though the percent absorption of nonhaeme iron is considerably below that of haeme iron, the quantity of nonhaeme iron in the diet is many folds above that of haeme iron; thus, in most meals, the major contribution of available iron is made by nonhaeme iron.

Suggested Strategy to Achieve Behavioural and Dietary Modifications

1. Objectives

1.1. To ensure a regular consumption each day of suitable amounts of iron-containing foods. This implies a good mixed diet according to the multimix principle.

1.2. The consumption of enhancers of the absorption of dietary iron along with and immediately after each meal. The main enhancers are meats and ascorbic acid.

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1.3. The avoidance of inhibitors of absorption of dietary iron during or soon after meals. The most important inhibitors in the Region appear to be in tea or coffee.

2. Technical Aspects

2.1. Target groups - these should include at-risk groups, school population, private sector, persons in industry and commerce. Ongoing nutritional surveillance will enable planners to identify continuously specific target groups not previously identified and monitor progress toward achievement of behavioural change.

3. Institutional Framework

The Food and Nutrition Council and the Health Education Unit should be responsible for coordinating programmes in dietary modification and other educational programmes.

Nutrition messages should be known, understood, supported and transmitted by change agents at all levels and by people living in and working with communities.

4. Training Needs

There should be an updating of agricultural extension officers, home economics teachers, 4-H groups, science teachers and community groups in new information about iron absorption, use of enhancers and effect of inhibitors.

Training in motivational and educational techniques will be required by all those involved in educational programmes aimed at the achievement of objectives in all four aspects of anaemia control and prevention.

5. Educational Methods, Messages, Materials

Educational messages should be identified from the objectives, for example:

(a) increase use of foods high in ascorbic acid with meals;

(b) plant more fruit trees, particularly West Indian cherry and citrus;

(c) shop for iron-fortified foods; and

(d) include some animal foods whenever possible.

Misconceptions should be corrected in education programmes.

6. Data Gaps

6.1. To determine dietary patterns using ongoing surveys, e.g. teenage pregnancies.

6.2. Sociological surveys to determine response to nutrition education messages.

6.3. Determine the effect of bush teas on iron absorption.

The Control of Intestinal Parasites

Background

It had long been appreciated that hookworm infestation was frequently associated with anaemia and that there was some relationship between the severity of the infestation and the degree of anaemia. WHO, recognising the probable importance of hookworm infestation in the pathogenesis of tropical iron deficiency anaemia, recommended that detailed studies, including radio-isotopic measurements of intestinal blood loss, should be undertaken. In a community based study, Layrisse and Roche were able to demonstrate that with egg counts of over 2000/g faeces, there was a significant correlation between the severity of the infestation and the degree of anaemia. The blood loss

produced by the worms was found to range from about 2 ml/day in lightly infested subjects, to about 100 ml/day in subjects with heavy infestation. It was further demonstrated that blood loss caused by *Necator americanus* is about 0.03 ml/worm per day and, with *Ancylostoma duodenale*, it is about 0.02 ml/worm per day. A proportion of the iron in the blood, shed by the worm into the intestine, is reabsorbed lower down, so that the net daily iron loss with a worm load represented by an egg count of 1000 eggs/g faeces, is about 0.8 mg for *N. americanus* and about 1.2 mg for *A. duodenale*.

In some populations, there is a high prevalence of *Trichuris trichiura* infestation, particularly in children. Measurement of the blood loss caused by these parasites indicates that each worm is responsible for a loss of about 0.005 ml/day. Since the parasites live in the large intestine, there is no reabsorption of haemoglobin iron, so with heavy infestation, a significant degree of iron loss may result.

In schistosomiasis, also, blood loss may be sufficiently great to cause iron deficiency anaemia.

In a recent study Tikasingh of CAREC studied the prevalence of intestinal parasites in eight countries of the Caribbean. The Countries surveyed were: Antigua, Bahamas (New Providence), Belize (three villages), Dominica, Guyana, Montserrat, St. Vincent and Trinidad (two counties). A total of 2266 samples was examined from 5 and 9 year old school children. Some 67 percent of the children were positive for one or more parasites. Trichuris (49%). Ascaris (28%) and the hookworms (8%) were the commonest helminths found. Rates of infestations however, varied from country to country for each species of parasite. For example, rates for Trichuris ranged from 17 percent (Guyana) to 88 percent (Dominica). Intensity of the infestations was generally mild to moderate.

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A Strategy for the Control of Intestinal Parasites such as Hookworm and Whipworm

1. Objective

As a matter of priority determine the extent to which parasites (particularly hookworm and whipworm) infestation is a problem in respective countries and if found to be, incorporate suitable relevant programmes aimed at reducing the prevalence.

2. Technical Aspects

2.1. Identify areas where hookworm and whipworm are endemic.

2.2. Implement an anti-parasite programme for mass treatment leading to eradication of parasites (hookworm, trichuris).

2.3. Improvement of faecal and solid waste disposal in collaboration with Environmental Sanitation Department.

2.4. Any study on the etiology of anaemia should include parasitology.

3. Institutional Framework

The overall responsibility of any project to improve the public health aspects would be a collaborative effort among the Ministries of Health, CAREC and CFNJ.

The Environmental Sanitation Department is the best department for promoting public health measures within the framework of primary health care services.

4. Training Needs

4.1. Public Health - already existing technical staff and family nurse practitioners should be trained in quantitative analysis of stool examination for helminths particularly hookworm and whipworm

5. Educational Methods, Messages, Materials

5.1. Education Programmes should be geared toward improving hygiene practices thus decreasing the opportunities for contamination. Where possible all such programmes should be mounted in collaboration with CAREC.

6. Data Gaps

6.1. Data collection and analysis to determine the extent of parasitic infestations. The assistance of CAREC should be requested.

6.2. The effect of parasites on the anaemia status in the Region.

6.3. Conduct research to determine whether dosage presently used in the treatment of trichuriasis is effective for large numbers. The cost of treatment should be determined.

6.4. Research should be conducted to determine the best drug to control hookworm in a community where it is endemic.

FIGURE 1: MOVEMENT OF WHEAT AND WHEAT FLOUR IN THE COMMONWEALTH CARIBBEAN AND SURINAME

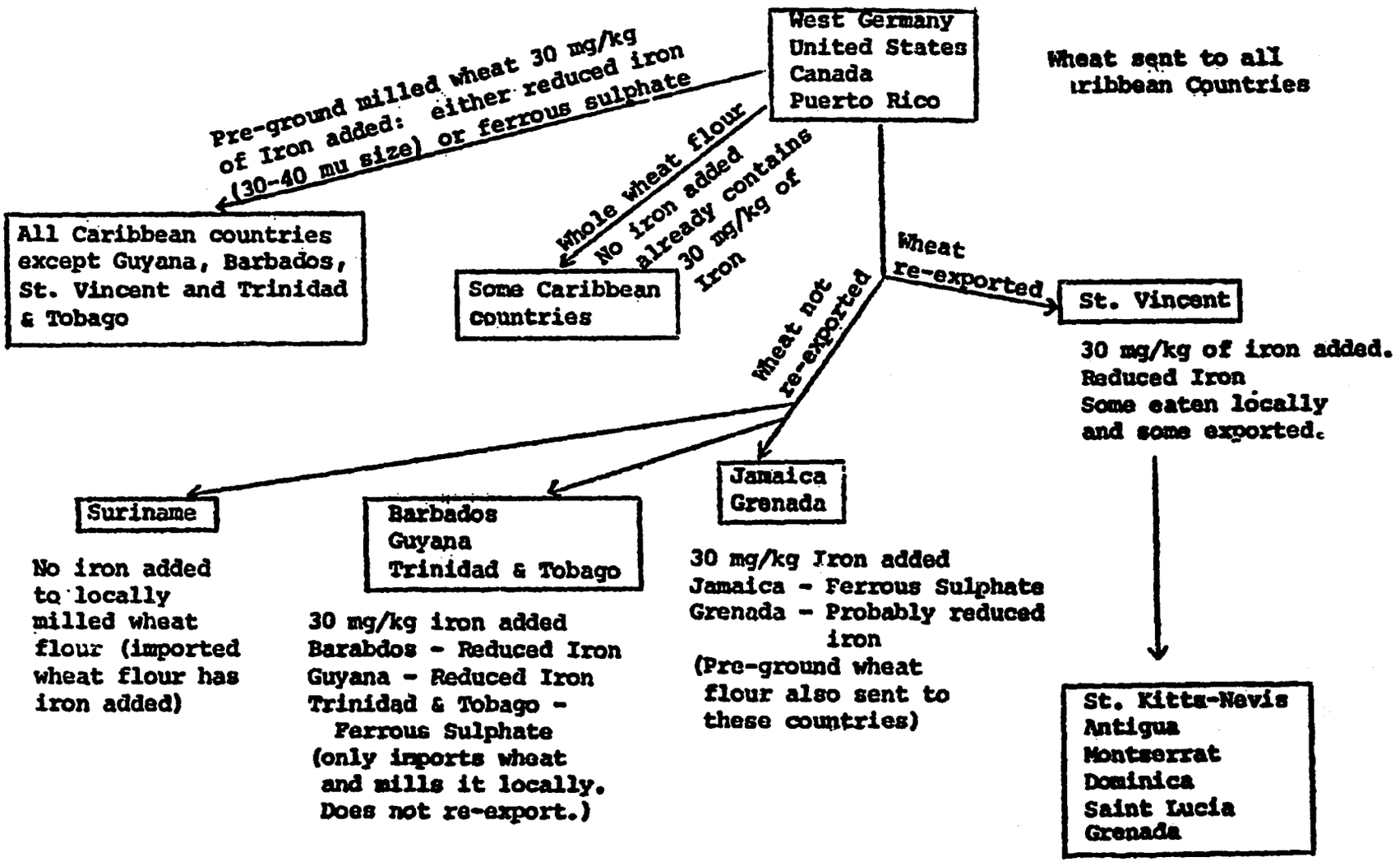


FIGURE 2: AVAILABILITY AND MOVEMENT OF MILK AND INFANT FORMULAS INTO AND THROUGH THE ENGLISH-SPEAKING CARIBBEAN: TOTAL NUMBER AVAILABLE AND THOSE FORTIFIED WITH IRON

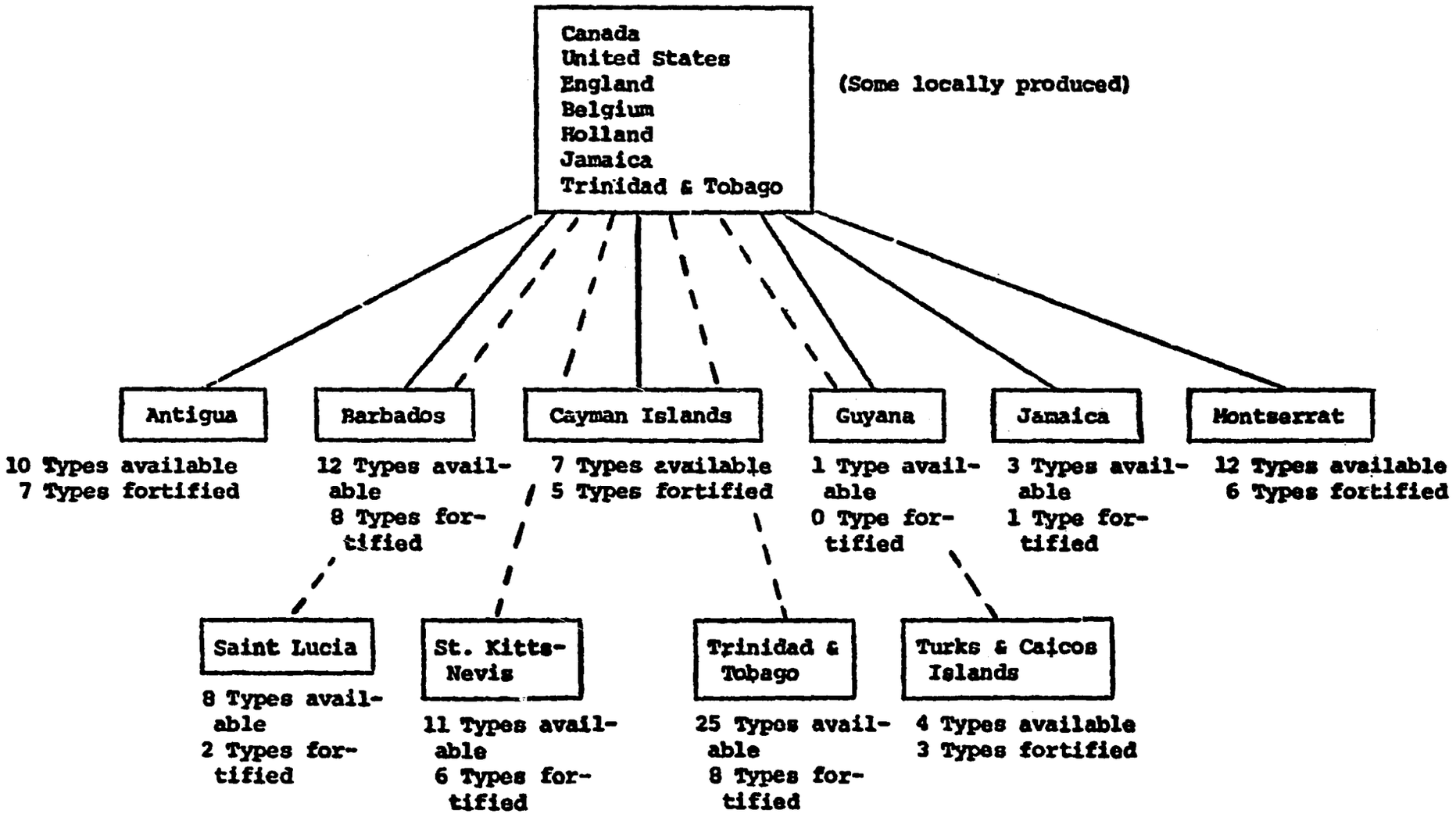
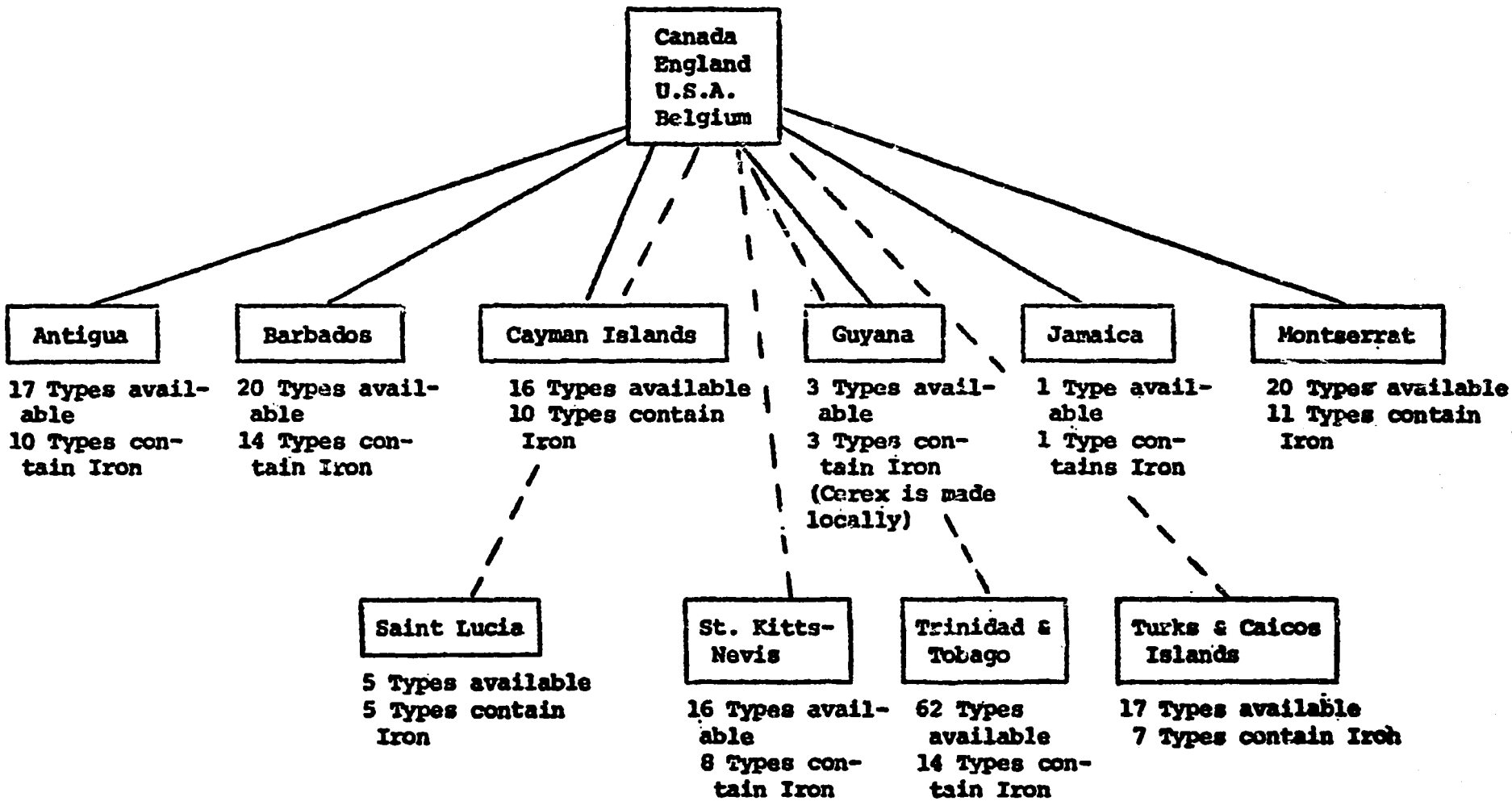


FIGURE 3: AVAILABILITY AND MOVEMENT OF BABY CEREALS AND FOODS INTO THE ENGLISH-SPEAKING CARIBBEAN: TOTAL NUMBER AVAILABLE AND THOSE FORTIFIED OR CONTAINING IRON



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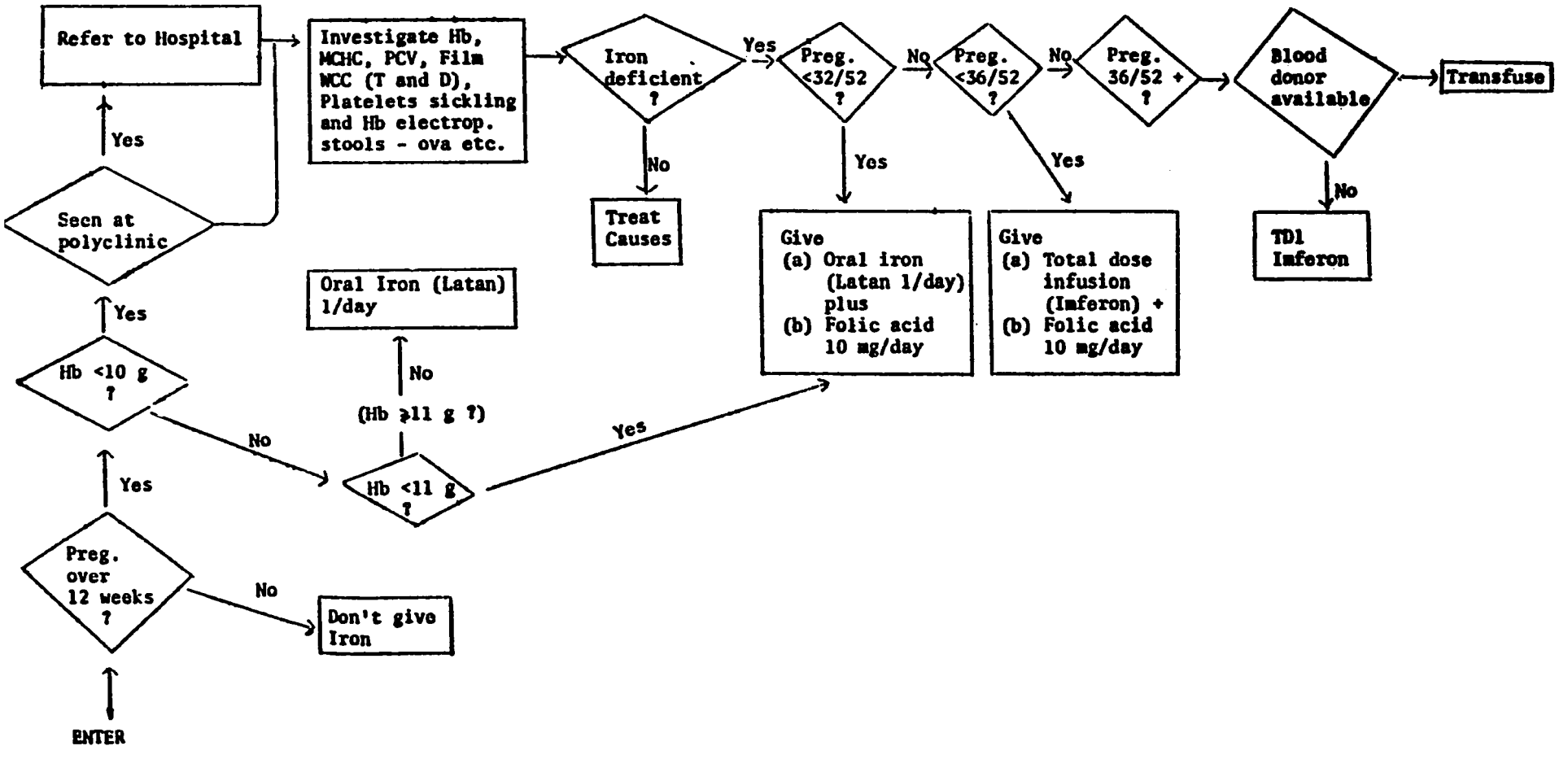
AN ALGORITHM OF THE GUIDELINES FOR ANAEMIA CONTROL THROUGH ANTENATAL CLINICS IN ONE COUNTRY



CALCULATIONS FOR SUPPLY OF IRON SUPPLEMENTS FOR SMALL ISLAND AS RECOMMENDED BY GROUP B

Island: 100,000 population
4,000 births/year
400 low birth weight children/year

FERROUS
SULPHATE (25 mg/ml)
ELIXIR

Each low birth weight child needs iron supplementation for approximately 150 days at average dose of 1 ml/day = 150 ml.

150 x 400 = 60,000 ml = 60 litres
One (1) 48-gallon drum contains about ++++++ 240 litres

Allowing for (1) Wastage
(2) Maldistribution
(3) Other use of the iron, e.g. therapeutic

I suggest 3 48-gallon drums as initial stock
1 48-gallon drum as absolute minimal stock

and re-order 1 48-gallon drum per year.

For pregnant women: 4,000 women/year
200 days of supplementation each
(1 tablet per day)

FERROUS
IRON 60 mg
+
FOLATE 100 µg

= 800,000 tablets per year

I suggest 2 million tablets as (minimal) initial stock*
1 million tablets/year as RE-ORDER.

*Depends on shelf-life

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For FERROUS SULPHATE ELIXIR:

Each health centre needs 2 5-litre containers

Minimal stock = 1 full container

Re-order rate ≃ 1 container per 6 months.

For ANTENATAL Iron Supplementation tablets:

Each health centre needs 25,000 tablets basic stock

ROUGHLY 10,000 tablets/year re-order, but this depends on population served by the health centre (very variable).

Each island also needs 1,000 dark 60 ml containers, each with dropper-stopper graduated in 0.5 ml (Re-order 1,000 per year)
LABEL "Keep out of reach of children".

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