The purpose of the International Nutritional Anemia Consultative Group (INACG) is to guide international activities aimed at reducing nutritional anemia in the world. The group offers consultation and guidance to various operating and donor agencies who are seeking to reduce iron deficiency and other nutritionally preventable anemias. As part of this service, INACG has prepared guidelines and recommendations for:

—assessing the regional distribution and magnitude of nutritional anemia;

—developing intervention strategies and methodologies to combat iron deficiency anemia;

—evaluating effectiveness of intervention programs on a continuing basis so that evaluation is a continuing and dynamic procedure;

—research needed to support the assessment, intervention and evaluation of programs.

Monographs published by the International Nutritional Anemia Consultative Group are:

—Guidelines for the Eradication of Iron Deficiency Anemia
—Iron Deficiency in Infancy and Childhood
—Iron Deficiency in Women

These reports are available without charge from:
The Nutrition Foundation
888 Seventeenth Street, Northwest
Washington, D.C. 20006
U.S.A.

The publication of this report was made possible by a grant to The Nutrition Foundation from the United States Army Medical Research and Development Command. Gratefully acknowledged is the support given by the United States Army Natick Research and Development Laboratory; Food and Drug Administration, Bureau of Foods; Department of Agriculture, Science and Education, Human Nutrition, and Department of Agriculture, Food Safety and Inspection Services

The Nutrition Foundation, serving as the Secretariat for the International Nutritional Anemia Consultative Group, receives grant support from The Office of Nutrition of the Agency for International Development.

Library of Congress Catalog Card Number: 82-60413
ISBN 0-935368-28-0
June 1982

THE EFFECTS OF CEREALS AND LEGUMES ON IRON AVAILABILITY

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

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June, 1982

Preface

The International Nutritional Anemia Consultative Group (INACG) is dedicated to reducing the prevalence of nutritional anemia worldwide. In fulfilling this mandate, INACG sponsors scientific reviews and investigations by expert advisory task force groups on issues related to the etiology, treatment, and prevention of nutritional anemias.

Several studies on the bioavailability of iron in different dietary settings with special focus on the influence of cereals and legumes have been initiated in recent years. The need to examine pertinent data from these investigations and to assess their impact on iron nutriture are acknowledged as being important to the establishment of public policy.

At the invitation of the INACG Secretariat, a task force of specialists from various fields met with representatives of the federal government, academia and industry to review recent research findings. This monograph is the result of their collaborative efforts. INACG is grateful for their generous contributions. The members of the task force and the INACG Secretariat are solely responsible for the contents of this monograph.

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I.
Introduction

Cereal products and legumes make up the bulk of staple diets in large parts of the world. In contrast, the take of meat in these areas is usually very small. ..ieat consumption is much greater in Western countries but there has recently been a trend in some parts of the world to reduce the intake of red meat and to increase that of unrefined carbohydrates. These dietary patterns have important implications for iron nutrition because iron is, in general, poorly absorbed from cereals and legumes. Consequently, nutritional iron deficiency reaches its greatest prevalence and severity in populations subsisting predominantly on cereal and legume diets (Bothwell *et al.*, 1979; Hallberg, 1981 a). Meat, on the other hand, has a two-fold beneficial effect on iron nutrition: firstly, the heme iron in meat is highly bioavailable; secondly, meat also potentiates the absorption of the non-heme iron that is the predominant form of iron in other foodstuffs which would otherwise be poor sources of iron (Bothwell *et al.*, 1979). A similar stimulation of iron absorption from a meal can be produced by foods that include enhancers of iron absorption, such as ascorbic acid.

Three meetings of a subcommitte of INACG were convened (September 24 to 25 and November 21 to 23, 1981, in Washington, D.C. and March 15 to 17, 1982, in New York City) to analyze the information that is available on the bioavailability of iron in different dietary settings, with special emphasis on the influence of cereals and legumes. While data concerning cereals are of great relevance in this regard, a more immediate impetus for these meetings was the recent finding that the iron in a major legume, the soybean, is poorly absorbed and that soy products may inhibit the absorption of iron from other foods. These observations are potentially important since soy products are being used increasingly as dietary substitutes for animal protein. In particular, they are used as meat extenders and as a source of protein in weaning foods distributed to developing countries. A major aim of this subcommittee was, therefore, to review present knowledge in this area, and to examine the pertinent experimental data. Many of the studies that are included in this report are unpublished, but our discussion of these data is based on a detailed review of the manuscripts. This report deals almost exclusively with human studies because it has proven difficult to make quantitative or qualitative extrapolations from iron absorption experiments in animals to man.

Since any analysis of this type is predicated on the validity of the available data, it is helpful first to review the basic technique which has been used over the last several years to assess the bioavailability of dietary iron in man. Against such a background it should then be possible to assess the significance of data relating to cereals and legumes, and to develop guidelines for policy actions if this be deemed necessary.

II.
The Measurement of Dietary Iron Absorption

Single Foods

The first attempts to understand food iron absorption were made by Moore and Dubach (1951), who used single foodstuffs which had been biosynthetically labelled with radioiron—vegetables by growing them in hydroponic media containing ^{55}Fe, and meat by injecting animals with radioiron intravenously several months prior to slaughter. The principal finding in this and subsequent studies of the same type was that food iron of animal origin was generally better absorbed than vegetable iron (Layrisse and Martinez-Torres, 1971; Martinez-Torres and Layrisse, 1973). While the information obtained with this approach was useful, its limitations became apparent when two of the foods labelled with different isotopes of iron were administered simultaneously. For example, Layrisse and his colleagues (1968) showed that when veal was eaten with maize (corn), the absorption of the veal non-heme iron was reduced by about half, while that of the maize iron was doubled. When it is remembered how varied typical diets are in different cultures, this strong interaction makes it clear that only limited information can be expected from studies using single, biosynthetically labelled food items, and that data on food iron absorption can really only be useful if there is some way of measuring absorption from the total diet.

Absorption from Meals by the Two-Pool Method

The recent development of a technique for estimating iron absorption from complete meals has yielded much deeper insights into the utilization of dietary iron. This technique was based on the finding that when a small quantity of iron in a soluble inorganic iron salt was mixed with a food item of vegetable origin just before it was eaten, the added iron (extrinsic iron) was absorbed to an extent which was virtually identical to that of the intrinsic food iron. This was established by labelling the extrinsic iron and the intrinsic food iron with two different isotopes (Bothwell *et al.*, 1979; Hallberg, 1981a). While the percentage of iron absorbed varied over a wide range with different foods and in different subjects, the ratio of absorption from the intrinsic and extrinsic labels remained the same.

An additional necessary step was the demonstration that the absorption of radioiron added to a meal containing several vegetable food items was an accurate measure of the absorption of all the non-heme iron in the meal (Cook *et al.*, 1972; Hallberg and Bjorn-Rasmussen, 1972; Bjorn-Rasmussen *et al.*, 1973). It indeed became apparent that when several foodstuffs are consumed in the same meal, the aggregate non-heme iron forms a common pool in the lumen of the gastrointestinal tract and that it is this pool which is labelled by the extrinsic radioisotope.

While current evidence supports the concept of a common intraluminal pool of non-heme food iron, certain exceptions have been noted in which isotope exchange is incomplete. The absorption of the animal iron storage compounds, ferritin and hemosiderin, is significantly lower than that of the vegetable iron in a meal (Layrisse *et al.*, 1975; Martinez-Torres *et al.*, 1976). The same is true of non-heme iron added to unmilled, unpolished rice, possibly due to impaired diffusion of iron across the dense outer aleurone layer (Bjorn-Rasmussen *et al.*, 1973). In addition, insoluble iron compounds, such as ferric pyrophosphate, ferric orthophosphate and ferric hydroxide are poorly absorbed (Cook *et al.*, 1973; Derman *et al.*, 1977), while ferric oxide is not absorbed at all (Derman *et al.*, 1977).

The concept of a common intraluminal pool has been found to be equally valid for the heme iron in food. If a small amount of ^{59}Fe-hemoglobin is added to a meal in which there are other heme-containing items such as meat, the absorption of ^{59}Fe reflects the absorption of the heme iron from the whole meal (Martinez-Torres and Layrisse, 1971). By tagging both the non-heme and the heme fractions it therefore became possible for the first time to measure the total absorption of iron from a whole diet (Layrisse and Martinez-Torres, 1972, Hallberg and Bjorn-Rasmussen, 1972, Bjorn-Rasmussen *et al.*, 1974, Layrisse *et al.*, 1974). The finding that the estimates of iron assimilation agreed closely with estimates of total body iron loss provided additional validation of these results by reflecting the expected balance between absorption and loss. In human studies, most attention has been paid to the absorption of non-heme iron since it forms the major portion of the dietary iron intake, and because its absorption is influenced much more than is heme iron by the composition of the diet and by the iron status of the individual.

Technical Considerations

Method of Administering the Tracer. In early studies on food iron absorption from whole meals, great efforts were made to distribute the extrinsic tracer through all the food items. However, it has been subsequently found that equally valid results can be obtained by labelling only one bulky component of the diet (Bjorn-Rasmussen *et al.*, 1976). Furthermore, it has been shown that the absorption of non-heme iron from freely chosen meals can be reliably measured, provided that the exact intake of different foods is determined and that the radioiron is administered in some bulky component (Hallberg *et al.*, 1979).

Measurement of Radioactivity in Blood vs Whole Body Counting. A unique feature of iron metabolism in relation to isotopic measurements is the fact that most of the absorbed radioactivity is normally incorporated into circulating red cells within 10 to 14 days of oral administration. It is therefore possible to estimate the percentage of radioiron that was absorbed from the radioactivity present in red cells after 14 days, assuming an incorporation of absorbed radioactivity of 80% in normal subjects and of 100% in iron-deficient subjects. For calculation purposes, an assumed blood volume based on the height and weight of the subject is used. The validity of these assumptions has been confirmed by the close agreement between measurements obtained by red cell radioiron and by whole body counting (Larsen and Milman, 1975).

Two Isotopes of Iron. The availability of two isotopes of iron (^{55}Fe and ^{59}Fe) has added further to the flexibility of radioiron absorption measurements since it has meant that more than one measurement can be carried out on each subject. For example, the effect of changing a single item in a diet can be assessed by carrying out absorption measurements with each of the isotopes on succeeding days. Indeed, much of current knowledge concerning the effects of enhancers and inhibitors of non-heme iron absorption has been obtained using this approach (Bothwell *et al.*, 1979, Hallberg, 1981a).

Absorption from a Reference Dose. The degree to which non-heme iron in a diet is absorbed by an individual depends not only on the bioavailability of the iron but also on the iron status of the individual: more iron is absorbed by iron-deficient subjects and less by those who are iron-replete (Bothwell *et al.*, 1979). Without making allowances for variations in iron status, it is therefore difficult to assess the significance of differences between the absorption of iron from test meals. However, the problem can be largely overcome if allowance is made for variations in iron status by obtaining an independent measure of each individual's absorptive capacity. This has been accomplished by determining the absorption from a standard dose of inorganic radioiron given at physiological levels under standardized fasting conditions. The concept of a reference dose of radioiron was introduced by Layrisse and coworkers (1969). An informal agreement has been reached among workers in different countries to use 3 mg of iron as ferrous sulfate together with 30 mg ascorbic acid for this reference dose. In a group of subjects with varying iron status there is a good correlation between the absorption from the reference doses and from the non-heme iron in the meal. Thus, the relationship between the two absorption measurements (meals reference doses) is an index of the bioavailability of the non-heme iron in a meal (Bjorn-Rasmussen *et al.*, 1976). However in iron-balance calculations, it is important to have a measurement of bioavailability that is more concrete and absolute than the slope of such a regression line, and that can be related to a certain iron status. A meaningful measure of the bioavailability of non-heme iron in a meal would be the absorption in subjects who have borderline iron deficiency, i.e., subjects with absent iron stores who have not yet developed anemia. Such subjects are also of interest because they correspond to populations who are at risk of iron deficiency and who would be targets of intervention programs. In practice, subjects with borderline iron deficiency absorb about 40% of a reference dose. Magnusson and coworkers (1981) have therefore proposed that the bioavailability of non-heme iron in a meal should be expressed as the absorption value that corresponds to a reference dose absorption of 40%. To increase the accuracy of this calculated value, it is desirable to include in each study subjects who range widely in iron status.

Validity of the Extrinsic Tag Method to Measure Non-Heme Iron Absorption The use of the extrinsic tag method to measure the total absorption of non-heme iron from composite meals has been validated in a number of ways (Hallberg, 1981a). As mentioned in a previous section, the absorption of the extrinsic tracer has been found to be the same as the iron present in biosynthetically labelled foods in a variety of dietary settings. The absorption of the extrinsic and intrinsic tracers has been found to be the same if the doubly labelled foods are fed immediately after the addition of the extrinsic label. Furthermore, absorption from the two tracers remains the same in the presence of various amounts of inorganic iron and of ascorbic acid or of a potent iron chelator, desferrioxamine. Identical plasma radioactivity curves are obtained in the hours following the administration of doubly labelled foods. Finally, when two biosynthetically labelled foods, namely eggs and white wheat flour, each labelled with a different isotope of iron have been fed together, absorption of the two tracers has been identical despite the fact that their bioavailability when fed individually differs several-fold.

While there is therefore good reason to accept the concept of a common non-heme iron pool in the diet and of the extrinsic tag method as a way of measuring absorption from this pool, there are certain qualifications that must be mentioned. First, it has not always been possible to estimate the size of the common non-heme iron pool labelled by the extrinsic tracer since the diet sometimes also contains contaminating iron from dirt and dust that is of poor bioavailability and that does not mix with the common non-heme iron pool. However, recently an *in vitro* technique has been described which makes it possible to measure the extent to which contaminating iron is labelled by the extrinsic tracer (Hallberg and Bjorn-Rasmussen, 1981). Another precaution that must be taken relates to the amount of iron administered with the extrinsic tag. This should consist of only a tracer dose of radioiron, since it is important to ensure that the chemical properties of the non-heme iron pool in the meal being studied are not altered. The larger the amount of inorganic iron added to a meal, the more it will influence the nature of the dietary pool of non-heme iron.

It should be noted that several of the iron compounds used as iron fortificants (e.g., most forms of reduced iron and iron phosphates) do not enter the common pool completely and are thus only partially labelled by extrinsic tags. Such fortificants are usually relatively insoluble and their bioavailability is thus limited. The method of food preparation (e.g., baking of bread) and the nature of the diet may also affect the bioavailability of fortification iron. In such circumstances, it is possible to measure the relative bioavailability of the iron fortificant by labelling it with one tracer of radioiron and the non-heme iron pool with another tracer. In this way, both the size of the non-heme iron pool and its bioavailability, including that of the iron fortificant, can be accurately determined.

III.
A Summary of Current Knowledge on the Absorption of Dietary Iron

The three major factors influencing the absorption of iron from the diet are the amount of iron ingested, the composition of the meals and the behavior of the mucosa of the upper small bowel. The role of each will be discussed separately.

Dietary Iron Content

The iron content of the diet is determined by its constituent foods. Typical Western diets usually contain about 6 mg iron per 1000 kcal, with surprisingly little variation (U.S. Department of Health, Education and Welfare, 1972; Hallberg and Bjorn-Rasmussen, 1981). In certain circumstances, extrinsic iron, in the form of dirt or solubilized from the surface of containers or cooking vessels, may increase the amount appreciably. In general, iron in the form of dirt is of low bioavailability (Derman *et al.*, 1977; Hallberg *et al.*, 1977) and usually has little effect on iron nutrition, but iron derived from pans or skillets can add significantly to the absorbable iron intake, especially when the pH of the food being prepared in them is low (Moore, 1965).

Bioavailability of Iron

Variations in the bioavailability of dietary iron have been shown to be of greater relevance to iron nutrition than the amount of iron ingested. Radioisotopic absorption studies have established that there is a striking difference between the intrinsic bioavailability of the iron in grain products as opposed to the bioavailability in fish and meat. In one study, the mean absorption of the iron in three major grain staples (wheat, rice, maize) ranged between 1 and 7% (Layrisse *et al.*, 1969; Layrisse and Martinez-Torres, 1971). In contrast, figures for fish and meat varied between 12 and 20%. Much of the iron in the latter foods is in the form of heme, which is highly bioavailable. This can be ascribed to the fact that heme iron is not influenced by the many ligands in the diet which may inhibit the absorption of non-heme iron (Weintraub *et al.*, 1968); furthermore, heme is directly taken up into the mucosal cells by an absorption mechanism different from that of inorganic iron. Heme is also unaffected by the high pH of the upper small bowel, which renders some forms of inorganic iron insoluble. Because of these factors, when heme iron is fed as meat, it contributes substantially to iron nutrition even though it usually forms only a small proportion of the total intake of dietary iron. For example, in one study heme iron made up only 6% of the dietary iron, but accounted for 30% of the total that was absorbed (Bjorn-Rasmussen *et al.*, 1974), while in another it represented 33% of the dietary iron and 74% of the absorbed iron (Layrisse *et al.*, 1974).

The situation with regard to non-heme, food iron is a good deal more complex. When eaten alone, the iron in a number of widely consumed staples, including maize, wheat, rice and black beans, is poorly absorbed, with geometric mean absorptions* varying between 0.8 and 5.7% (Table 1). The only exception so far identified is wheat iron, which is well absorbed once the bran has been removed (see page 14). However, when these various staples form part of a mixed diet, the absorption of the iron contained within them is markedly influenced by other dietary constituents. As mentioned in a previous section, most of the non-

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*Where possible in this report, the results of iron absorption studies have been expressed as geometric rather than arithmetic means. This logarithmic transformation compensates for the highly skewed distribution of percentage absorption when measured in normal individuals. When it has not been possible to calculate geometric means in published reports, arithmetic means have been used, and it should be noted that these values are consistently higher than geometric means except when mean absorption approaches 50%.

TABLE 1.
ABSORPTION OF IRON FROM A NUMBER OF STAPLE FOODSTUFFS WHEN FED ALONE OR TOGETHER

Authors	*Vegetable*	*Number of Subjects*	*Final Iron Content (mg)*	*Geometric Mean Absorption %*		*Total Absorption (mg) Corrected to a 40% Reference Absorption*	
				Intrinsic Label	*Extrinsic Label*	*Intrinsic Label*	*Extrinsic Label*
Björn-Rasmussen *et al.* (1972)	Maize (chapatti)	10	4.6	1.5	1.4	—	—
Cook *et al.*, (1972)	Maize (boiled and baked)	81	2.0	3.3	3.7	0.07	0.08
Layrisse *et al.* (1969)	Maize (baked)	21	4.0	4.2	—	0.21	—
Sayers *et al.* (1974)	Maize (porridge)	5	4.1	0.8	0.8	0.03	0.03
Derman *et al.* (1977)	Maize (porridge)	22	2.0	—	3.8	—	0.08
Björn-Rasmussen *et al.* (1972)	Wheat (white bread rolls)	5	0.3	26.9	25.1	—	—

Björn-Rasmussen *et. al.* (1973)	Wheat (brown bread)	5	1.1	2.7	2.7	—	—
Cook *et al.* (1972)	Wheat (bread)	13	2.0	2.6	3.0	0.1	0.11
Layrisse *et al.*(1969)	Wheat (bread)	18	—	5.7	—	—	—
Björn-Rasmussen *et al.* (1973)	Rice	18	1.0	3.8	4.4	—	—
Sayers *et al.* (1974)	Rice & potato soup	6	5.5	0.8	2.0	0.04	0.09
Sayers *et al.* (1974)	Rice & lentil soup	10	7.8	—	3.2	—	0.28
Cook *et al.* (1972)	Black beans (boiled)	8	3.0	1.5	1.2	0.07	0.06
Layrisse *et al.* (1969)	Black beans (boiled)	15	3.4	2.6	—	0.24	—

heme iron in a meal, whatever its origin, enters a common pool during the process of digestion, and is thus uniformly susceptible to the effects of a number of enhancers or inhibitors of iron absorption (Cook *et al.*, 1972; Hallberg and Bjorn-Rasmussen, 1972). It is now clear that the degree to which the iron in a cereal such as maize is absorbed is dependent on the relative proportions and affinities for iron of other constituents of the meal.

Inhibitors of Iron Absorption A number of compounds have been claimed to interfere with iron absorption. These include carbonates, oxalates, phosphates and phytates (Bothwell *et al.*, 1979). Many diets contain large quantities of one or more of these substances, and it has been suggested, on the basis of *in vitro* studies, that they form large, insoluble complexes with iron which are very poorly absorbed. However, the degree to which such complexes inhibit iron absorption is variable. For example, oxalates have been shown *not* to inhibit iron absorption when tested *in vivo* (Hallberg, unpublished data; Gillooly, Torrance and Bothwell, unpublished data) and inorganic phosphate does not affect dietary iron absorption when given alone, but does so when given as the calcium salt (Monsen and Cook, 1976). Presumably, this is due to the adsorption of iron onto the compound. Data concerning the role of phytate on iron absorption are confusing and are discussed in the section entitled, Effects of Cereals on Iron Absorption.

It has been suggested that some factor or factors present in vegetable fiber may have an inhibiting effect on non-heme iron absorption, and it has been shown that the fiber of wheat and maize binds iron (Reinhold *et al.*, 1981). However, absorption studies with specific constituents of dietary fiber, including pectin and cellulose, have yielded negative results (Gillooly, Torrance and Bothwell, unpublished data; Cook, unpublished data; Hallberg and Rossander, unpublished data).

One of the most potent inhibitors of non-heme iron absorption is Indian tea (Disler *et al.*, 1975a). When Indian tea is consumed with a variety of meals there is a marked reduction in iron absorption, which has been shown to be due to the formation of insoluble iron tannates (Disler *et al.*, 1975b). This observation is likely to have broad relevance to iron nutrition since tea is drunk with meals in many parts of the world. Similarly, polyphenols, of which tannates are but one example, are widely distributed in foodstuffs, and the poor absorption of the iron present in sorghum, horse beans, finger millet, cow peas, spinach and red wines appears to be attributable to their presence (Rao and Prabhavathi, 1982; Gillooly, Torrance and Bothwell, unpublished data).

Egg yolk contains a phosphoprotein which strongly binds iron (Halkett *et al.*, 1958). In addition, when iron is fed with egg albumen, its absorption is low but is significantly increased when the albumen is heated, presumably due to the inactivation of conalbumin (Morck *et al.*, in press). However, some perspective on these observations is provided by the results of a recent study in which the effects of various components of breakfasts were compared. Eggs were found to cause a decrease in the percentage absorption of non-heme iron, but the actual amount of iron absorbed increased slightly due to the higher iron content of the breakfasts that contained eggs (Rossander *et al.*, 1979). In contrast, milk and cheese add very little iron to a meal, and since they do not enhance the absorption of iron from a mixed meal, iron absorption from meals containing milk or cheese as the major protein source is much less than that from meals including equivalent portions of meat, fish or poultry (Cook and Monsen, 1976).

Enhancers of Iron Absorption Two major enhancers of iron absorption are meat and ascorbic acid. The significance of meat for iron nutrition is actually twofold, the effect on the non-heme iron in the meal being over and above the well-absorbed heme iron which it contains (Layrisse *et al.*, 1973). How meat enhances the absorption of non-heme iron is not known, but it is possible that the cysteine present in meat protein may play some role (Martinez-Torres *et al.*, 1981).

A second major enhancer of iron absorption is ascorbic acid (Sayers *et al.*, 1973; Bothwell *et al.*, 1979; Hallberg, 1981b). The effect of ascorbic acid on non-heme iron absorption has been tested in a number of dietary settings and in every case has been shown to be profound. It plays a particularly critical role in diets in which little or no meat is present, such as are consumed by the vast majority of the world's population. In considering the iron nutritive value of such diets it is essential to have a knowledge of their ascorbic acid contents, since the overall absorption of iron may be significantly increased if fruit or vegetables containing ascorbic acid are present in the meal. In this context, oranges, lemons, grapefruit, guavas and papayas are important fruit sources of ascorbic acid, as are broccoli, cauliflower, cabbage, potatoes, beetroot and pumpkin, among the vegetables.

While current evidence underlines the importance of ascorbic acid as a promoter of iron absorption, it is not the only organic acid in food that fulfills such a role. Numerous vegetables and other foodstuffs and beverages contain appreciable quantities of organic acids, including citric, malic, lactic, succinic, and tartaric acids, each of which has been shown to promote the absorption of non-heme iron under certain conditions (Derman *et al.*, 1980. Hallberg, unpublished data. Gillooly, Torrance and Bothwell, unpublished data). However, the relative roles of these various acids on iron nutrition remains to be defined.

Chemical Determinants of Food Iron Bioavailability. Explanations for the marked differences in bioavailability of non-heme iron from various meals must lie in the chemical nature of iron in foods and in the chemical transformations that occur with processing, storage, cooking, interaction of foods in a meal, and digestion. Among the known chemical factors that play important interrelated roles in iron bioavailability are thermodynamic and kinetic stability constants (Clydesdale, unpublished data), reduction potential and pH (Nojeim *et al.*, 1981. Nojeim and Clydesdale, 1981), and the formation of low molecular weight ligands (Saltman, 1965). Solubility, based on pH and particle size, has also been suggested as a means of predicting iron bioavailability (Shah *et al.*, 1977) and as a reason for the greater bioavailability of ferrous iron as compared to ferric iron.

The chemical state of iron in food undergoes changes through reactions that involve a decrease in free energy. For instance, $Fe(OH)_3$ may be produced spontaneously if a food is rendered alkaline, and with time and or processing, it may become insoluble, even when the pH is subsequently decreased to the acidity of the stomach. Thus, the solubility, as well as the free energy of iron interactions, are controlled to some extent by pH and cannot always be considered reversible in the stomach or by acidification of the food. This provides an explanation for the observation that $Fe(OH)_3$ was not found to be interchangeable in the non-heme iron pool of maize porridge since it was absorbed only half as well as the intrinsic iron present (Derman *et al.*, 1977). It may also be one of the factors explaining why other compounds, such as metallic iron, ferric orthophosphate, and ferric pyrophosphate, do not fully exchange with the non-heme iron pool and are thus less available for absorption.

Although some differences in iron bioavailability can be explained in terms of pH or reduction potential, the facilitation or inhibition of the bioavailability of non-heme iron seen in other studies cannot be predicted from these factors. This is due in part to the chemical ligands which form complexes with iron. However, once formed, the characteristics of these compounds will in turn depend on other factors, such as pH. For example, ferric iron will combine with ascorbic acid to produce ferric ascorbate at a low or neutral pH, a complex that will remain soluble even if the pH is later increased (Conrad and Schade, 1968; Gorman and Clydesdale, unpublished data). However, if the pH is raised prior to the addition of ascorbate, the complex will not form. This means that if an alkaline food is fortified with iron it may not be able to form a soluble ascorbate complex and may instead precipitate as $Fe(OH)_3$, becoming unavailable for absorption. Kojima *et al.*, (1981) confirmed the fact that ascorbic acid is most effective at solubilizing iron in pinto beans at a low pH and that citric acid is much more effective at a higher pH. The degree to which iron is bound to various fibers, including cellulose and lignin, was also found to be pH-dependent (Camire and Clydesdale, 1981). The effectiveness of a given ligand will probably depend upon these inherent constants, and should eventually provide an explanation for some of the results which still remain puzzling, such as the apparent dissimilarity between the effects of endogenous and exogenous phytic acid, as well as the differences in various mineral oxalates (Van Campen and Welch, 1980). Ascorbic acid, the best known enhancer of bioavailability, probably owes its action to appropriate thermodynamic and kinetic stability constants as well as its favorable reduction potential and its action as an acid.

In addition to enhancers of iron absorption in foods, certain gastrointestinal secretions, notably hydrochloric acid, also promote the absorption of non-heme iron by rendering it ionizable (Bothwell *et al.*, 1979). Other gastrointestinal secretions are likely to influence iron absorption by promoting digestion with release of iron from the food, but there is little evidence that they contain any component that acts as a specific carrier for iron.

Mucosal Behavior

Iron absorption is markedly influenced by the amount of storage iron in the body. If the storage iron is diminished, a high proportion of the available iron is absorbed, and as the storage iron increases, less iron is absorbed (Kuhn *et al.*, 1968). Thus, body iron content is regulated by the mucosal handling of iron in a manner that favors the maintenance of body iron homeostasis.

Quantitative Aspects of Iron Absorption

The daily intake of iron in industrialized countries normally varies between 10 and 20 mg. In one report, the male intake averaged about 17 mg and the female intake 11 mg (Finch and Monsen, 1972). However, the amounts actually absorbed constitute a relatively small percentage of the total. In the adult iron-replete male consuming a mixed Western-type diet, the average amount of iron absorbed each day matches the obligatory basal losses of about 1.0 mg. In the female during her reproductive years, menstruation increases physiologic losses to an average of about 1.4 mg daily (Hallberg *et al.*, 1966), and the percentage of iron absorbed is substantially greater than in men. When iron requirements rise, whether for physiologic or pathologic reasons, iron absorption from the intestinal mucosa increases in a corresponding fashion, but the amount of dietary iron and its bioavailability ultimately place a ceiling on the amount that can be absorbed. With customary Western-type diets, the maximum is between 3 and 4 mg daily.

Analysis of diets in developing countries indicates that the iron content is not unusually low and may even be high, with a significant proportion derived from contaminating sources, such as soil, dust and water. As mentioned in a previous section, iron is poorly absorbed from diets in which the major staples are cereals and legumes, unless moderate amounts of meat or ascorbic acid are also present. For example, it has been calculated that when the daily dietary iron content is 15 mg and there is less than 30 g of meat or less than 25 mg ascorbic acid present, then the total amount absorbed by a subject with no iron stores would be expected to be less than 1 mg (Monsen *et al.*, 1978). Since meat is expensive and virtually absent from the usual diet in most developing countries, the bioavailability of the dietary iron is largely dependent on the amount of ascorbic acid and other organic acids in the fruits and vegetables consumed, and on the extent to which they are retained after cooking.

Technical Intervention to Increase Bioavailability

Food processing affects the chemical forms of iron in food and thereby influences iron bioavailability (Lee and Clydesdale, 1978; Lee and Clydesdale, 1981). In order to monitor such chemical changes in iron, a method for determining "iron profiles" in food was developed (Lee and Clydesdale, 1979) which included elemental, total nonelemental, soluble, insoluble ionic, soluble complexed, ferrous, and ferric iron. Application of this method showed dramatic changes in iron profiles for various foods under different processing conditions. Iron fortification sources (including ferrous sulfate, ferric orthophosphate, ferric sodium EDTA, and elemental iron) became solubilized and were found in the ferrous form to varying degrees depending on addition of ascorbate, duration of ambient storage, and spray or freeze drying (Lee and Clydesdale, 1980a). The same iron sources became insoluble as a result of baking in a non-yeast leavened product (Lee and Clydesdale, 1980b). Thermal processing of spinach caused 93% of the endogenous iron to be rendered insoluble, whereas iron added to spinach varied in solubility depending upon the source and the presence of ascorbate. Ferric sodium EDTA was found to be minimally affected by thermal processing (Lee and Clydesdale, 1981).

A technique to bind iron to several proteins (e.g., wheat gluten, soy isolate, and casein complexes) was developed in an attempt to enhance the bioavailability of iron (Nelson and Potter, 1979). When the protein-iron complexes were subjected to digestion in an HCl-pepsin or an HCl-pepsin-pancreatin system most of the iron was released, suggesting that the protein-bound iron might be freed for absorption in the gastrointestinal tract.

There are several stages in the processing of cereal grains where pH alterations might increase bioavailability. Fiber binding of iron in wheat bran is affected by pH (Camire and Clydesdale, 1981; Reinhold *et al.*, 1981), and neutralization of soy products has been shown to influence the availability of zinc. This is an area where technological intervention might be effective. As well as a simple decrease in pH, a pH adjustment with later neutralization might be considered at various points in the processing of soy products (see discussion on pp. 23*ff.*). Such alterations at strategic points in heating might decrease iron binding with fibers. These *in vitro* findings underline the potential importance of processing in influencing the chemical characteristics of food iron. The major need now is to ascertain the relevance of the *in vitro* findings to human nutrition by iron absorption studies in man.

Presence and/or Addition of Ligands. The most familiar example of enhanced bioavailability due to complex formation with a ligand is that formed by iron with ascorbic acid. The excellent facilitation of iron absorption by ascorbic acid is probably due to a combination of factors, including pH and favorable reduction potential in its half reaction, in addition to its activity as a ligand (Clydesdale, unpublished data). However, ascorbic acid is heat labile, and its irreversible oxidation will eliminate the properties that allow it to facilitate iron absorption. For this reason and others, it would also be advantageous to investigate other ligands, such as the carboxylic acids involved in the tricarboxylic acid cycle which might have the advantage of being heat stable. The key to choosing appropriate ligands is to have greater knowledge about both the thermodynamic and kinetic stability constants of the complexes formed (Forth and Rummel, 1973; Gorman and Clydesdale, unpublished data) and the range of stability which provides the greatest facilitation of iron absorption. The formation of such ligands within foods could be potentiated by modifications in processing, or ligands could be added at critical points in the manufacturing process.

IV. Cereals

Level of Utilization

Cereal grains are the world's most important source of calories. Major cereals are rice, maize (corn) and wheat which are consumed as such, processed into flours, starch, oil and bran, or fed to livestock for conversion into animal protein. World production figures for 1979 for the three major cereals were as follows:

Cereal	*Million Metric Tons*
Rice	369
Wheat	403
Maize	406

Maize and wheat are produced in nearly equal amounts followed by rice, but the latter is the most important because it supplies the major food for more than half the world's population.

Maize and wheat are primary ingredients of blended foods, such as corn-soy-milk (CSM) and wheat-soy blend (WSB), estimated to have an annual value of $68 million in fiscal year 1979 and distributed world-wide under United States Public Law 83-480 (PL480), Food for Peace Program.* CSM, for example, contains 59% maize meal, 17.5% soy flour, 15% nonfat dry milk, 5.5% soybean oil, plus vitamin and mineral premixes. The mineral premix includes ferrous fumarate as an iron supplement, while the vitamin premix contains ascorbic acid. These blended cereal foods are intended to be used as supplements at levels ranging from 65-125 g/day. CSM contains 40 mg of ascorbic acid and 18 mg of iron/100 g, a molar ratio of ascorbic acid to iron of about 0.7. At the suggested levels of daily intake it supplies 12-23 mg of iron in the diet.

Other cereals include sorghum, which is grown in semiarid regions of Asia and Africa where it is a major food crop. Although the United States produces more than one-third of the sorghum in the world, most of this production is used as animal feeds. In Asia and Africa, food uses of sorghum include flat, unleavened breads, rice substitutes, and porridges which may be fermented or unfermented. Additionally, sorghum is used for beers which are generally high in solids and relatively nutritious (Derman *et al.*, 1980).

Processing

Some salient features of cereal processing are summarized in this section because they influence the concentration of iron and other nutrients, the bioavailability of iron, and the opportunity for fortification.

Rice. As it comes from the field, the grain still contains the hulls or husks and is referred to as rough or paddy rice. The major objectives of milling are to remove the hull and the bran layers (including the germ) and to recover the maximum amount of whole rice kernels. Rice processing consists of cleaning, hulling (shelling), milling, polishing and sizing (separation of whole and broken rice). Figure 1 outlines the various steps involved and shows the approximate distribution of the various fractions obtained during standard milling of rice as conducted in the United States (Luh, 1980). The first step consists of

*The Food for Peace Program was authorized in 1954 by the United States Congress under Title II of Public Law 83-480. The Agricultural Trade Development and Assistance Act of 1954, with the objective of combatting hunger and malnutrition overseas by distributing surplus food commodities. In subsequent years emphasis has been placed on using the commodities in formulated foods as nutritional supplements for feeding weanling infants, young children, and pregnant and lactating mothers. The Food for Peace Program is administered by the Agency for International Development with the United States Department of Agriculture.

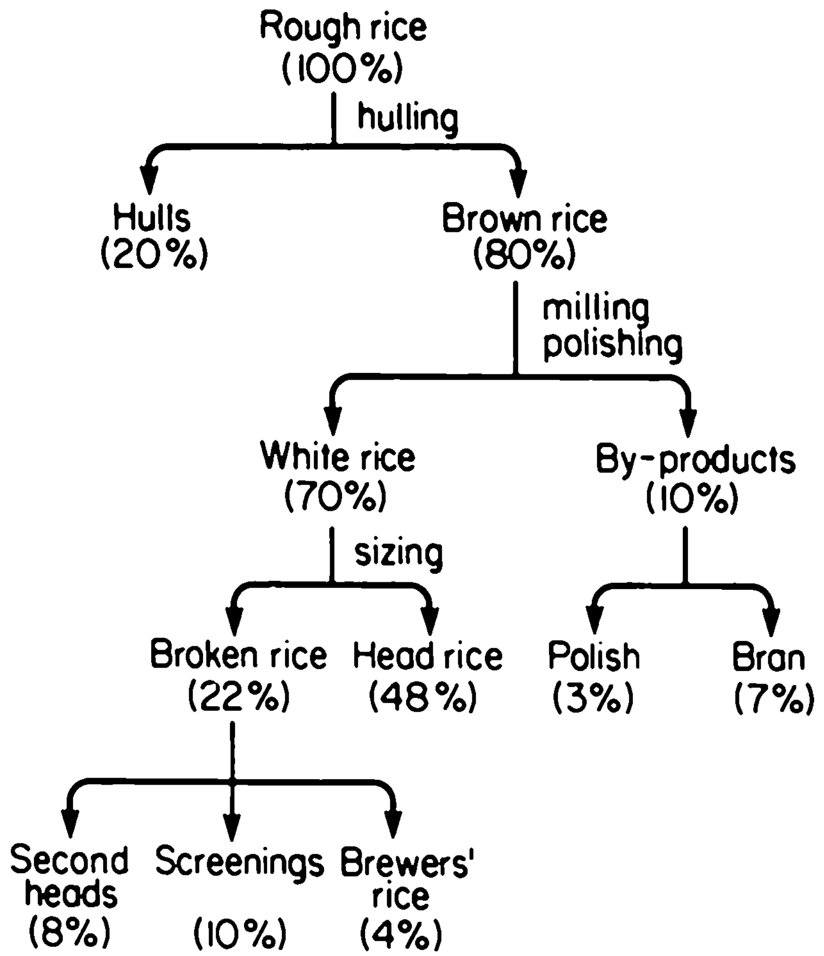


FIGURE 1

Outline of rice processing and the distribution of the fractions obtained.

TABLE 2.
COMPOSITION OF BROWN AND WHITE RICE AND RICE BRAN[a]

Component	*Brown Rice*	*White Rice*	*Bran*
Protein (%)	7.1-13.1	5.6-13.3	12.1-17.2
Crude fat (%)	1.8- 4.0	0.2- 1.1	14.6-21.7
Crude fiber (%)	0.2- 2.6	0.1- 0.6	8.7-13.1
Ash (%)	1.0- 2.4	0.3- 0.7	9.0-12.2
Carbohydrates (%)	74.5-90.2	84.0-93.5	40.9-49.1
Iron (mg/kg)	7-54	2-27	130-530

[a]Dry basis
Source: Luh (1980)

passing the rough rice between rubber rollers to remove the hulls by shearing forces and then screening. The brown rice that results is milled by abrasion (either by rubbing the kernels against a rough surface or against each other) to loosen the bran, which is then separated by screening. The next step is polishing, which removes particles of loose bran. The milled rice is then sized to separate whole kernels (head rice) from broken ones. Whole kernels constitute less than one-half of the original rough rice. The broken kernels are further fractionated according to size: second heads are the largest and brewers rice consists of the smallest pieces. Table 2 shows the changes in proximate composition and iron content that occur when brown rice is milled. Protein remains nearly constant, but fat, fiber, ash and iron tend to decrease when brown rice is processed into white rice because these constituents are concentrated in the outer layers of the kernel. White rice has only about one-half the iron content of brown rice whereas bran is very high in iron.

Wheat. Processing of wheat consists of removing the bran (14.5%) and the germ (2.5%) from the endosperm (83%) of the kernel (Anonymous, 1965). The endosperm is recovered in finely divided form as white flour, which is widely used in bread and other baked goods. Modern milling of wheat consists of a complex series of operations including cleaning, tempering, grinding and sifting to yield flour and associated by-products (Figure 2). The by-products—bran, shorts, (fine particles of bran, germ, flour and offal), red dog (milling residue plus fine particles of bran, germ and flour), and germ—are referred to collectively as mill feeds and used primarily for animal feeds. An average of 72% of the wheat is recovered as flour. Table 3 shows the changes in chemical composition that occur when wheat is milled into flour. Flour is lower in protein, ash and fat, but higher in starch than wheat. Of special significance is the low iron content of flour as compared to the starting wheat. In contrast, the bran, one of the by-products of milling, is several-fold higher in iron content than the original wheat.

Because of the low iron content of white flour and its widespread use in bread and other foods in the United States, it has been common practice since 1941 to enrich flour with iron. Present iron levels in enriched flour are 28.6-36.3 mg/kg (13.0-16.5 mg/pound) and they are scheduled to be increased to 44 mg/kg (20 mg/pound) on July 1, 1983 (Anonymous, 1982).

Maize. In the United States, processing of maize is conducted either by wet milling or dry milling (Inglett, 1970). The wet milling industry, however, produces only starch and derived products for food use. The by-products, hull (bran), gluten and maize germ meal, go into animal feeds.

The dry milling industry in the United States uses two general systems—nondegerming or degerming. The

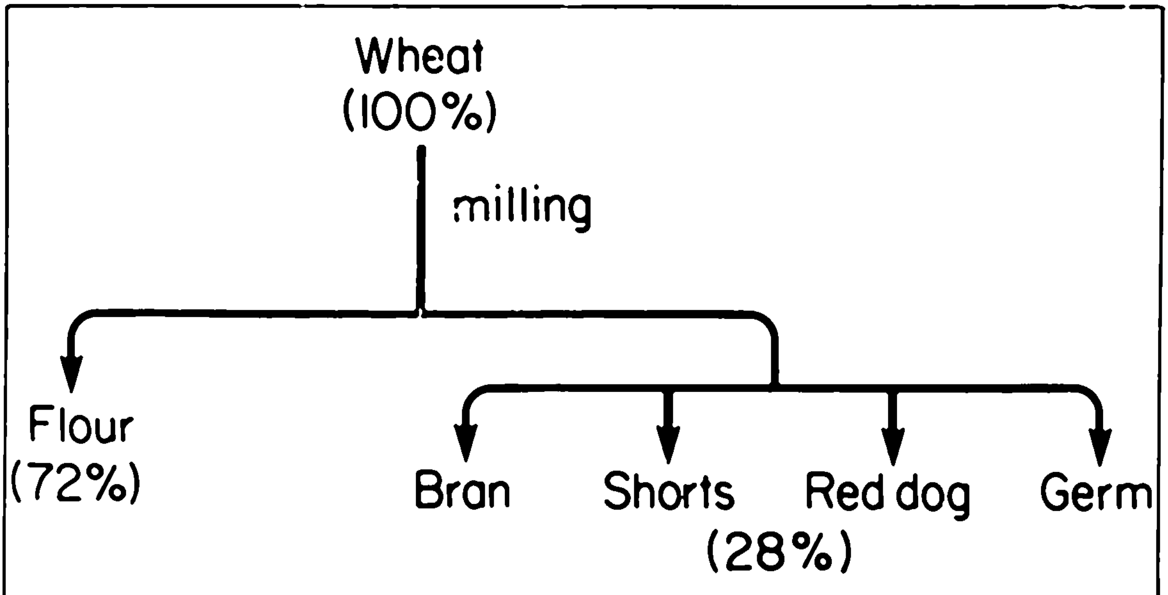


FIGURE 2

Distribution of the fractions obtained by the milling of wheat.

TABLE 3.
COMPOSITION OF WHEAT, WHITE FLOUR AND BRAN

Component	*Wheat*[a]	*White Flour*	*Bran*
Moisture (%)	14.0	13.5	10.7
Protein (%)	11.1	10.4	12.1
Ash (%)	1.5	0.4	6.2
Fat (%)	1.8	0.9	4.2
Crude fiber (%)	2.1	—	10.9
Starch (%)	56.8	66.1	5.2
Iron (mg/kg)	22	4	81

[a]Data for hard red spring wheat. Farrel, *et al.* (1967); Waggle, *et al.* (1967).

TABLE 4.
COMPOSITION OF MAIZE MEAL, GRITS AND FLOUR

Component	*Whole*	*Bolted*	*Degermed*	*Grits*	*Flour*
Moisture (%)	12.0	12.0	12.0	12.0	12.0
Protein (%)	9.2	9.0	7.9	8.7	7.8
Fat (%)	3.9	3.4	1.2	0.8	2.6
Carbohydrate (%)	73.7	74.5	78.4	78.1	76.8
Fiber (%)	1.6	1.0	0.6	0.4	0.7
Ash (%)	1.2	1.1	0.5	0.4	0.8
Iron (mg/kg)	24	18	11	10	—

Source: Inglett (1970)

nondegerming type of milling consists essentially of grinding the maize with little, if any, removal of germ to yield whole maize meal, which has the composition of whole maize (Table 4). Because whole meal contains all of the original fat, it has a relatively short shelf life. Whole meal is sometimes bolted (sifted) to remove coarse particles of hull and some of the germ which constitute 4-6% of the maize. Fat, fiber and iron contents are lowered as a result of bolting (Table 4).

Most of the maize processed by the dry milling industry is degermed by the tempering-degerming system. Objectives of the tempering-degerming process are: (a) to remove all germ and hull thereby leaving the endosperm low in fiber and fat; (b) to recover a maximum yield of endosperm, and (c) to recover much of the germ clean and of large particle size. The multi-step process consists of cleaning the maize; tempering by adding moisture; freeing the hull, germ and tip cap in a degermer, which is a cone shaped attrition mill; drying and cooking; fractionation by multi-step milling, sifting, separating and purifying; further drying if necessary; and recovery of crude oil from germ fraction.

A wide variety of primary products can be produced in a tempering-degerming mill. Expressed percentages of the original maize are as follows: hominy feed (35%); regular grits (23%), coarse grits (15%), cereal flaking grits (12%), flour (4%); coarse meal (3%); dusted meal (3%); and oil (1%). Four percent is lost due to shrinkage. Other products are made by blending. Brewer's grits, for example, are made by mixing coarse and regular grits. Hominy feed is a by-product including the hulls, maize germ meal remaining after removal of the oil, fines from the degerming, and other streams of feed quality. Compositions of degermed meal, grit and flour are given in Table 4. Dry milled maize products, such as grits and meal, are frequently enriched by adding vitamins and iron; enriched maize grits and meals contain 29 mg iron/kg (13 mg/pound).

Sorghum. Traditional milling in Africa and India is accomplished by pounding with a mortar and pestle or by grinding with a hand-operated stone mill. After pounding or grinding, the outer bran is removed by winnowing. Mechanical milling is practiced in villages and cities. Dry milling as practiced in the United States yields four fractions: grits (67%), bran (12%); germ (11%), and fines (10%). The grits are low in oil and fiber and about the same in protein content (10%) as the whole grain. The oil is concentrated in the germ and the fiber is found in high concentrations in the bran (Hoseney *et al*., 1981).

Effects of Cereals on Iron Absorption

The bioavailability of dietary iron is determined by several factors that enhance or inhibit non-heme iron absorption, as previously outlined. Cereals are the staple food in most diets. Besides containing different forms of starch, cereals contain phytates and various fiber materials. Certain cereals contain other components such as polyphenols, which may influence the absorption of non heme iron. Consequently cereals can be expected to have varying effects on non-heme iron absorption. An appropriate way of comparing the effects of cereals on the absorption of non-heme iron would be to study composite meals of identical composition but containing different cereals. Although no such studies are published, an attempt has been made to compare data from meals in which (1) one cereal made up the bulk of the meal; (2) no factor known to enhance or inhibit non-heme iron absorption was present in an appreciable amount; and (3) the iron status of the subjects was fairly well defined by the use of a reference dose of iron or by investigating normal men exclusively.

Rice. Meals in which milled rice was the major constituent were investigated in five studies comprising 221 subjects (Table 5). All results are adjusted to correspond to a reference dose absorption of 40%. Because of marked variations in the iron content of the meals, the results are only given in terms of percentage of iron absorbed. The meals contained no meat or ascorbic acid-rich food. In addition to rice, the meals also contained some vegetables and spices. The mean absorption in this group of studies was 6.5% (weighted according to the number of subjects in each study).

Wheat. Results of iron absorption from white wheat flour with an extraction between 60 and 80% are summarized in Table 6. Wheat was given as bread, and the iron intake in these studies was usually much lower than in the absorption studies of rice and maize. In some of the studies no reference doses were given. Nevertheless, these studies are included as all the subjects were normal men. The 40% reference absorption values were estimated by doubling the observed mean values in these groups (see Maize below). The mean absorption in the 86 subjects reported in seven studies was 30.9%.

Maize. Results from seven studies comprising 167 subjects are shown in Table 7. Most of the meals studied contained only maize. In two studies, no reference dose was given. These investigations involved a group of normal young men who were found to have a reference dose absorption of about 20% in other studies. Estimated 40% absorption values were thus

TABLE 5.
NON-HEME IRON ABSORPTION FROM RICE MEALS[a]

Number of Subjects	*Iron Intake (mg)*	*Mean Iron Absorption (%)*[b]	*Authors*
6	7.6	1.2	Aung-Than Batu *et al* (1976)
89	1.9	8.1	Hallberg *et al* (1978)
60	5.1-7.6	3.7	Sayers *et al* (1974)
60	14.6	6.9	Rao (unpublished data)
6	14.6	11.6	Rao (unpublished data)

[a]All meals are mixed meals composed of rice, vegetables and spices.
[b]Corrected to a 40% reference absorption.

TABLE 6.
NON-HEME IRON ABSORPTION FROM WHEAT MEALS[a]

Number of Subjects	*Iron Intake (mg)*	*Mean Iron Absorption (%)*[b]	*Authors*
8	3.0	12	Disler *et al.* (1975)
5	0.3	56	Björn-Rasmussen *et al* (1972)
5	1.0	25	Björn-Rasmussen *et al* (1973)
28	0.7	32	Björn-Rasmussen *et al.* (1974)
10	0.6	28	Hallberg *et al* (1977)
10	0.6	28	Hallberg *et al* (1977)
20	4.0	35	Rossander (unpublished observations)

[a]60-80% extraction
[b]Corrected to a 40% reference absorption.

TABLE 7.
NON-HEME IRON ABSORPTION FROM MAIZE MEALS

Number of Subjects	*Iron Intake (mg)*	*Mean Iron Absorption (%)*[a]	*Authors*
17	4.0	6.0	Layrisse *et al* (1969)
12	2.9	2.4	Layrisse *et al* (1974)
15	1.6	4.2	Layrisse *et al* (1974)
48	2.0	4.4	Cook *et al* (1972)
22	2.0	3.0	Derman *et al* (1977)
12	2.5	3.1	Layrisse *et al* (1973)
11	4.6	3.5	Hallberg and Bjorn-Rasmussen (1972)
30	5.0	2.6	Bjorn-Rasmussen and Hallberg (1974)

[a]Corrected to a 40% reference absorption.

obtained by doubling the observed mean absorption figures. The weighted mean absorption of all results was 3.7%.

Comparison Among Rice, Maize and Wheat. The results summarized above suggest that iron absorption is highest from wheat, intermediate from rice, and lowest from maize meals. However, any comparison of the effects of these three cereals on non-heme iron absorption based on the studies summarized in Tables 5-7 must be tentative since the cereal contribution, the iron content, and the composition of the meals varied over a wide range.

There is a firmer basis for comparisons between wheat and rice flour, and between starch derived from wheat and rice or from wheat and maize in recent unpublished observations of Hallberg and Rossander. Non-heme iron absorption from rolls made with white wheat flour was compared with that from rolls made of rice flour. Each of the two kinds of rolls was labelled with a different radioisotope of iron and served to normal male subjects in random order. As shown in Table 8, there was a significantly higher absorption from the wheat rolls in spite of the fact that the content of iron was nearly the same as in the rice rolls. In a similarly designed study, rolls made of

TABLE 8.
NON-HEME IRON ABSORPTION FROM ROLLS MADE FROM DIFFERENT KINDS OF FLOUR OR STARCH

	Number of Subjects	*Iron Content (mg)*	*Phytate-Phosphorus Content (mg)*	*Mean Iron Absorption (%)*[a]	*Absorption Ratio in Relation to Wheat*[b]
Wheat rolls	6	1.8	0	11.8	0.24
Rice rolls		1.4	34	3.0	
Wheat starch rolls	8	2.1	0	34.9	0.37
Rice starch rolls		1.6	0	12.8	
Wheat starch rolls	8	1.5	0	16.9	0.59
Maize starch rolls		1.9	0	10.0	

[a]Not corrected for reference absorption. Comparison of absorption values among the three groups can therefore not be made.
[b]See Figure 3
Source: Hallberg and Rossander (unpublished data)

wheat starch and rice starch were compared. As shown in Table 8, a much higher iron absorption was also obtained from the rolls made of wheat starch.

The difference in iron absorption between wheat and rice was greater when rolls were made of flour than when they were made of starch derived from wheat or rice. A possible basis for the more profound difference in iron absorption from the two kinds of flour is the greater content of phytate in the rice flour.

Iron in maize meals is less well absorbed than iron in meals having wheat or rice as their main source of energy. The average absorption was only 13% of the absorption from wheat when pooled results were compared (Figure 3). A comparison of rolls made of wheat starch and maize starch showed a statistically significant lower absorption from the maize rolls. However, the ratio of maize starch to wheat starch absorption was 0.59 compared with the ratio of 0.13 for maize compared to wheat meals. A possible explanation for the relatively good absorption from rolls made of maize starch is that they do not have the high content of phytates present in the maize porridge meals (Table 7). These results also indicate that maize starch has a less inhibitory effect on non-heme iron absorption than rice starch.

Sorghum. Iron absorption from meals consisting largely or solely of sorghum has been studied by several workers (Radhakrishnan and Sivaprasad, 1980; Derman *et al.*, 1980; Morck, Lynch and Cook, unpublished data; Gillooly, Torrance and Bothwell, unpublished data). The results of these studies indicate that the iron in sorghum (sorghum vulgare) is of low bioavailability. For example, in one investigation the absorption of iron from three porridge meals was compared, one contained red sorghum, the other white sorghum and the third maize meal. Geometric mean absorptions, corrected to a reference iron absorption of 40%, were 3.6%, 2.8% and 4.4%, respectively (Morck, Lynch and Cook, unpublished data). Some improvement in iron absorption from sorghum was noted in another series of experiments in which the sorghum was fermented; this was shown to be due to several factors, including the low pH, the lower solids content, and the formation of lactic acid and alcohol (Derman *et al.*, 1980). The outer coat of red sorghum contains both tannins and phytates, while that of white sorghum only contains phytates. In one study, removal of the outer coat of red sorghum led to an increase in iron absorption (6.0% as compared with 2.4%), while the bioavailability of iron in white sorghum was modestly greater than in red sorghum (Gillooly, Torrance and Bothwell, unpublished data).

Bran. An inhibitory effect of bran on iron absorption was first recognized by Widdowson and McCance (1942), using classical iron balance methods. Recently, the effect of bran has been more quantitatively evaluated by the double radioisotope method (Bjorn-Rasmussen, 1974). The amount of bran added to bread was found to inhibit non-heme iron absorption in a dose-dependent fashion. Recently, Simpson *et al.* (1981) investigated the mechanisms for the inhibiting effect of wheat bran. Bran was found to maintain its inhibition of iron absorption after its phytate had been destroyed by endogenous phytase. This finding and other results in the same study indicated that the inhibition was not caused by the phytate present in bran but rather by a water soluble, phosphate-rich fraction. In another series of studies, the effects of wheat bran and oat bran on non-heme iron absorption were compared in subjects who were served a breakfast meal (Hallberg and Rossander, unpublished data). These two kinds of bran were compared because their content of phytate differs markedly (Table 9). In spite of this difference, their inhibitory effect on non-heme iron absorption was the same. The results of the above two studies are in accord with the finding by Lipschitz and coworkers (1979) that monoferric phytate, the major form of iron in bran, was as well absorbed by dogs from a mixed meal as was ferrous sulfate. However, results in man that are apparently conflicting were obtained in another laboratory (Hallberg and Rossander, unpublished data). Washing of bran with water did not reduce its inhibiting effect. Washing of bran with dilute hydrochloric acid, however, removed the phytates and reversed the inhibition of iron absorption. A replacement of phytate in the hydrochloric acid-washed bran led to a reappearance of the inhibiting effect of bran on non-heme iron absorption (Figure 4). Although these results indicated an inhibition of iron absorption by sodium phytate, it remains uncertain whether phytate was the factor responsible for the inhibiting effect of bran on iron absorption. It is evident that further studies are needed to fully understand the mechanisms of the inhibitory effects of bran and phytate on non-heme iron absorption in man.

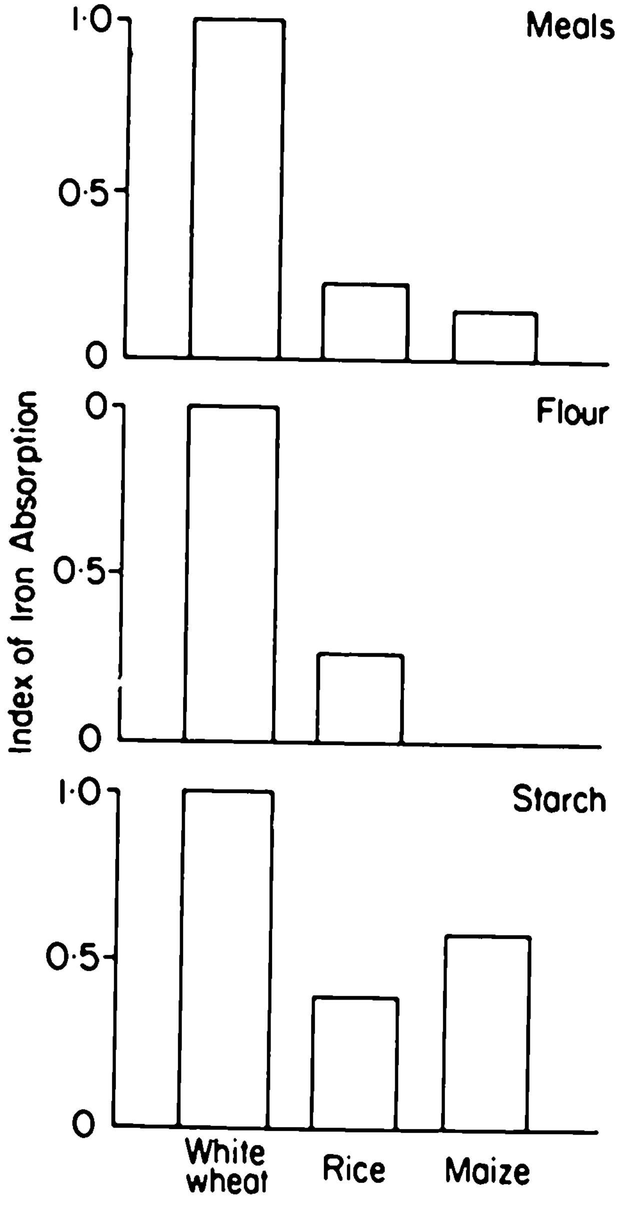


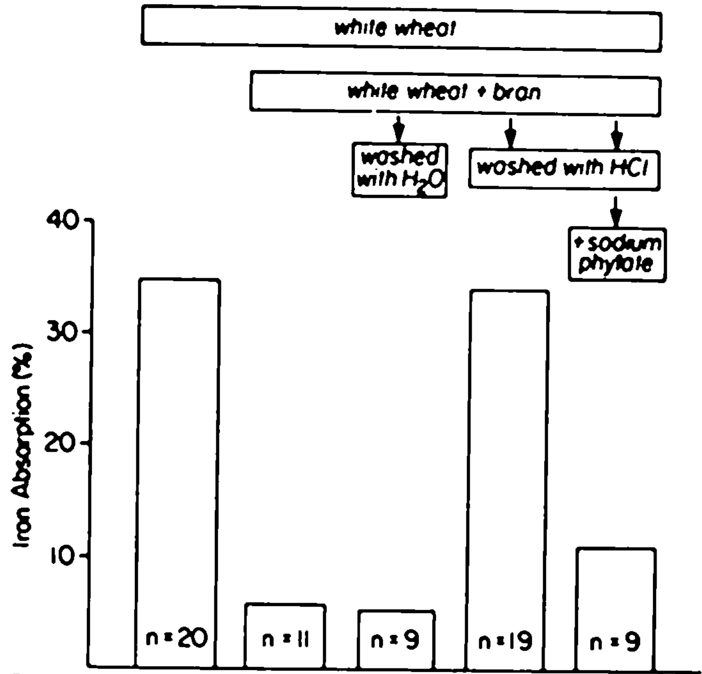
FIGURE 3

Relative absorption of non-heme iron from meals composed mainly of white wheat, rice or maize (data from Tables 5-7), from rolls prepared from wheat flour and rice flour, and (bottom part of figure) from rolls made of starch prepared from wheat, rice or maize (data in Table 8). (Hallberg and Rossander, unpublished data)

TABLE 9.
EFFECTS OF WHEAT BRAN AND OAT BRAN ON NON-HEME IRON ABSORPTION FROM A BREAKFAST MEAL[a]

	Number of Subjects	*Iron Intake (mg)*	*Phytate-Phosphorus Intake (mg)*	*Mean Iron Absorption (%)[b]*
Breakfast with wheat bran	10	4.1	74.0	3.2
Breakfast with oat bran		3.9	3.0	3.2
Breakfast without bran	9	3.9	0	4.8[c]
Breakfast with wheat bran		3.9	74.0	2.8[c]

[a]Meal consisted of coffee, rolls, butter, marmalade, and sour-milk.
[b]Corrected to 40% reference absorption.
[c]Statistically significant differences ($p < 0.05$)
Source: Hallberg and Rossander (unpublished data)



Non-heme Iron (mg)	native	0·65	3·5	4·5	0·75	0·8
	added	3·15	-	-	3·15	2·9
	Total	3·8	3·5	4·5	3·9	3·7
Phytate - P (mg)		0	235	230	0	230

FIGURE 4

Iron absorption from wheat bread when given alone or with bran which had been treated in various ways. (Hallberg and Rossander, unpublished data)

V.
Soy Products and Other Legumes

Level of Utilization

The average consumption of soy food in the United States is thought to approximate five pounds per year. The largest growth in consumption is occurring in the traditional, low technology consumer soy foods, especially tofu (or bean curd) and tempeh (fermented soybean patty), but among the other more highly processed soy products, there is a trend toward greater use of those that are most highly refined.

Apart from Asian populations, those that consume soy products in greatest quantity are vegetarians, the military, consumers of school lunches, infants taking soy-based formula (an estimated 20% of infant formula sales) and recipients of foods under PL 480. Annual use of defatted soy flour in the PL 480 programs alone is 300 million lbs. In infants who consume soy-based infant formulas, soy protein may be virtually the only source of protein.

Processing

Soybeans are different in composition from cereal grains in that more than half of the bean consists of protein and fat (40 and 20%, respectively). The remainder is composed of carbohydrates, ash, and minor ingredients (Wolf and Cowan, 1971). In contrast, carbohydrate is the major constituent of cereal grains. As a result of the high economic value of the major soybean constituents, the processing procedures that have been developed for soybeans are quite different from the classical milling procedures that have been used for cereal grains for centuries. Modification of the processing of soybeans and grains should be considered as one of the possible means of increasing iron bioavailability, and one that warrants research in the future.

The protein products that are currently derived from soybeans are classified in the three following groups on the basis of their protein content (Wolf and Cowan, 1971):

Product	*Protein Content %*
Flours and grits	40—50
Concentrates	70
Isolates	90—95

The refining procedures utilized in obtaining these products are shown in Figure 5. Both flours and concentrates may be further modified by an extrusion process, which involves the application of pressure and heat to produce textured vegetable protein. It should be remembered that the processing of all vegetable proteins, including soy, is not simply an isolation and purification procedure. The steps involved must produce a product with physical, chemical, and functional properties that are compatible with its intended food usage.

All soy protein products listed above have a common history up to the point of hexane extraction and subsequent removal of the solvent (desolventizing) (Figure 5). Soybeans are broken and the hulls loosened by cracking rolls, after which the hulls are removed by screening and aspiration. The cracked beans next move into a conditioner to adjust their moisture content to 10-11 percent and temperature to about 160°F. These tempered beans then go through smooth rollers to produce soy flakes which are extracted with hexane to remove the oil, followed by removal of solvents and cooking in the presence of moisture (toasting) in a desolventizer-toaster. The toasting step is necessary to inactivate a trypsin inhibitor and perhaps other factors present in raw soybeans that inhibit animal growth. Products intended for human consumption undergo special processes for hexane removal (Wolf and Cowan, 1971).

Protein concentrates may be prepared by one of three methods as follows:

(1) Aqueous alcohol leach: defatted flakes are leached with 60-80% aqueous alcohol that dissolves the sugars, leaving the proteins and polysaccharides, which are insoluble. After drying, the concentrate has a pH near neutrality.

(2) Acid leach: the defatted flakes are subjected to an acid leach (pH ≈ 4.5) where the proteins are at their isoelectric point and are therefore insoluble, as are the polysaccharides. The wet concentrate is neutralized before drying to make the proteins soluble when added to aqueous food systems.

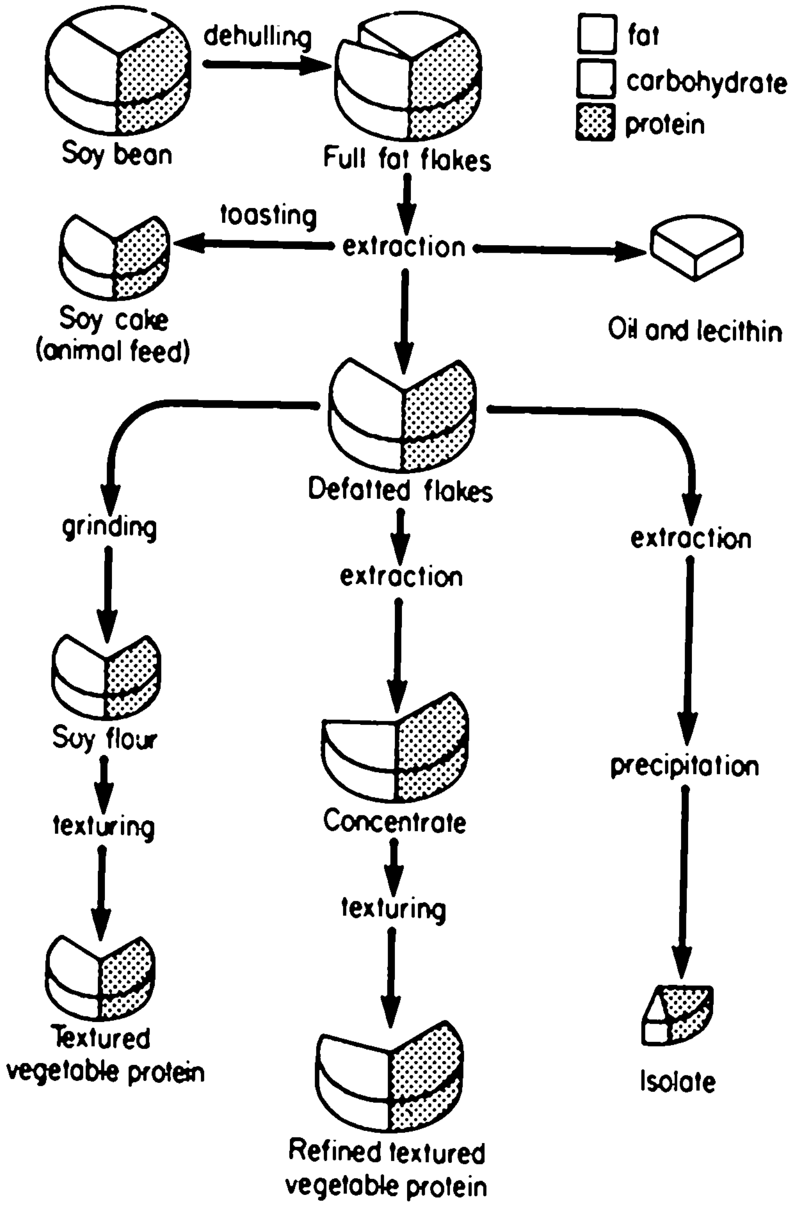


FIGURE 5

Schematic diagram of the changes in the composition of soy beans as a result of processing. (Courtesy of L. Schutte)

(3) Moist heat, water leach: flakes or flours are steamed to heat-denature and insolubilize the proteins. A water wash then removes the soluble sugars and on drying a concentrate results.

The protein content of the three end-products is similar, but the acid-leached products show greater protein solubility. It might be valuable to evaluate the effects of each of these processes on mineral binding and on the chemical forms of the endogenous iron, as has been done with wheat bran, fractions of dietary fiber, and maize (Camire and Clydesdale, 1981; Reinhold *et al.*, 1981)

Isolates are prepared by extracting undenatured, defatted flakes with dilute alkali at about pH 8.5 and centrifuging to remove the spent flakes (polysaccharides plus residual protein). The clarified extract is then acidified to pH 4.5 to adjust the proteins to their isoelectric point where they precipitate. This protein curd is then centrifuged to remove the whey (soluble sugars, ash and minor proteins). After washing, the curd may be spray dried to yield the isoelectric form of the proteins, but more commonly, the proteins are resolubilized by neutralizing with alkali, after which they are spray dried to produce a more food-dispersible product.

Soy products may take different forms in food. Meat extenders are generally made with textured soy flour after hydration to 18% protein (a little over 2 parts water: 1 part flour). When hydrated soy products are used as meat extenders they generally replace from 15-30% of the meat in the United States, and up to 50% in some parts of the world. Meat analogs (e.g., products resembling bacon) are generally prepared from soy concentrates which are combined with water, binders (wheat gluten, egg white solids) and flavors, and then cooked by extrusion, a high pressure-moderate temperature process. The extrudate is fried in oil to yield the analog which may be fortified.

Dairy analogs used as infant formulas are generally made from soy isolates and are heated to modify their physical properties and to destroy anti-nutritional factors. Nutrients, sweeteners, vegetable fats, emulsifiers, and stabilizers are added to the formula which is then heat sterilized and packaged.

The iron content of soy preparations is generally high. Analyses of 37 different samples from five different suppliers of soy products (Schriker, Miller, and Van Campen, unpublished data) showed a mean value of 8.6 mg iron/100 g (range 7.4 to 10.9) in 18 preparations of soy flour. The values were 11.9 mg/100g (10.5 to 15.2) and 15.0 mg/100g (11.6 to 19.8) for four soy concentrates and 18 soy isolates, respectively.

The mean bioavailability of iron from intrinsically labelled soybeans differs over a more than eight-fold range in the studies outlined in Table 10, and this variability has never been satisfactorily explained. Factors such as differences between batches and in the stage of maturity at harvesting, differences in methods of preparation and in the iron content of the test meals, and differences in the iron status of the test subjects may have played a part. One valuable point that did emerge and that has relevance for later studies was established in two of the early studies. When an extrinsic tracer was fed together with the soybeans, its absorption was the same as the intrinsic iron present in the beans (Bjorn-Rasmussen *et al.*, 1973; Sayers *et al.*, 1973). This finding helps to validate the results of subsequent studies in which only extrinsic labels were used.

Effects of Soy Products and Other Legumes on Iron Absorption

Of the legumes which have been tested for iron bioavailability, most attention has been directed at soy, since it has been extensively used as a substitute for animal protein. Because of this, virtually all the discussion that follows will be concerned with the effects of soy products on iron absorption. By comparison, there is only limited information on the bioavailability of the iron present in the many other legumes which are widely consumed throughout the world. However, the information that is available is of a uniform pattern - iron in legumes is of low bioavailability. The first legume to be investigated was the black bean. When intrinsically labelled black beans were fed as a single foodstuff, geometric mean absorptions of 1.5% and 2.6% were obtained in two separate studies (Table 1). Similar findings were noted with lentils—there was a geometric mean absorption of only 1.2% in a group of subjects who absorbed 16.3% of a reference dose of iron. While the reason for these low absorption figures has not been systematically studied, it may well relate to the high polyphenol content of most legumes (Rao and Prabhavathi, 1982). It is, however, almost certainly not the only factor, since iron in soybeans is also poorly absorbed (see following section), despite the fact that it has a low polyphenol content.

Soy Products. In one group of studies (Cook *et al.*, 1981; Morck *et al.*, in press) iron absorption from semi-synthetic diets with protein equivalent quantities of egg albumen, casein or isolated soy protein

TABLE 10.
IRON ABSORPTION FROM *IN VIVO* LABELLED SOYA BEANS PREPARED AT THE UNIVERSITY OF WASHINGTON

Authors	*No. of Subjects*	*Preparation*	*Fe Content (mg)*	*Geometric Mean Absorption %*
Layrisse *et al.* (1969)	17	Ground whole beans boiled and baked for 1 hour at 300° F	4.0	11.0
Sayers *et al.* (1973)	10	Ground whole beans baked at 400° F for 45 minutes	4.6	12.3
Björn-Rasmussen *et al.* (1973)	15	Ground whole beans boiled and baked for 25 minutes at 410° F	2.5	1.5
Ashworth and March (1973)	10 (children)	Ground whole beans boiled for 15 minutes	0.5	2.6
	16 (children)	Ground whole beans boiled and baked for 1 hour at 300° F	0.5	6.7

TABLE 11.
COMPARISON OF IRON ABSORPTION FROM ANIMAL AND VEGETABLE SOURCES[a]

Number of Subjects	*Iron Content (mg) Native*	*Added[b]*	*Source of Protein*	*Geometric Mean Absorption (%)*	*Total Iron Absorption (mg)*
	0.4	3.7	Albumen	2.5	0.10
15	0.3	3.8	Casein	2.7	0.11
	4.0	0.1	Isolated soy protein	0.5	0.02

[a] Each meal contained 68 g carbohydrate derived from dextrimaltose, 35 g fat from corn oil and 29.4 g protein from various sources.
[b] Added as ferric chloride
Source: Cook *et al.* (1981)

was measured (Table 11). The geometric mean absorptions from the three meals were 2.5% (egg albumen), 2.7% (casein) and 0.5% (isolated soy protein). It should be noted that because of the very low iron contents of egg albumen and casein in relation to isolated soy protein, the total iron contents of the diets were equalized with exogenous iron. The cooking of egg albumen was associated with a significant increase in absorption to 6.2%, a finding that may be explained by heat inactivation of conalbumin, the iron-binding protein in egg white.

Three sets of studies were done to determine the effect of cooking or of ascorbic acid addition on the inhibitory effect of soy products on iron absorption (Morck *et al.*, in press). The first set of experiments involved the feeding of various soy preparations as part of an otherwise standard semi-synthetic diet. There was a small though significant difference between iron absorption from uncooked and baked isolated soy protein (0.64% and 1.28%, respectively), and between boiled and baked soybeans (1.06% and 1.60%, respectively). The absorption from isolated soy protein was significantly less than from a similar quantity of egg albumen (0.56% as compared with 5.05%).

In an extension of the previous experiment, the semi-synthetic diet was again fed, but this time albumen was compared with three major forms of soy products, namely full fat soy flour, textured soy protein and isolated soy protien (Table 12). When egg albumen in the semi-synthetic 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.

Cereal-Soy Blends. This group of products is widely used in developing countries and provides additional iron to populations at high risk of iron deficiency, such as pregnant women, infants and children. The major products in this category include corn-soy-milk (CSM) and wheat-soy blend (WSB) which are provided under the PL480 program (see footnote page 12). In iron-replete males, mean percentage absorption from CSM and WSB ranged from 0.6 to 1.4%. By relating these results to 40% absorption from a reference dose of inorganic iron, it was estimated that infants with borderline iron deficiency would absorb between 1.1 and 2.8% of iron contained in the blended foods (Table 13), or the equivalent of 0.2 to 0.5 mg per 100 g of blended food. Both CSM and WSB are fortified with 15 mg of iron as ferrous fumarate per 100 g of product, yielding a total content of 18 and 21 mg of iron per 100 g, respectively (Table 14). The iron in a 100 g serving is equivalent to the U.S. Recommended Daily Allowance (RDA) for pregnant and lactating women, and a 75 g serving would correspond to the RDA for infants after six months of age and for young children. However, iron in ferrous fumarate which is highly bioavailable when used therapeutically between meals becomes poorly available when added to CSM or WSB. Studies of iron bioavailability from these products indicate that they are

TABLE 12.
COMPARISON OF THE AVAILABILITY OF IRON FROM ALBUMEN-CONTAINING MEALS AND MEALS CONTAINING VARIOUS SOY PROTEIN FORMS[a]

Number of Subjects	*Iron Content (mg)*		*Sources of Protein*	*Geometric Mean Iron Absorption (%)*	*Total Iron Absorption (mg)*
	Native	*Added*			
10	0.1	3.9	Albumen	5.5	0.22
	3.4	0.6	Full fat soy flour	1.0	0.04
	3.3	0.7	Textured soy protein	1.9	0.08
	2.0	2.0	Isolated soy protein	0.4	0.02

[a] Each meal contained 68 g carbohydrate derived from dextrimaltose, 35 g fat from corn oil and 14.7 g protein from various sources.
Source: cook, *et al.* (1981)

TABLE 13.
IRON AVAILABILITY FROM INFANT FOOD SUPPLEMENTS[a]

Number of Subjects	*Preparation*	*Geometric Mean Absorption*	
		% Iron Absorption Corrected to a 40% Reference Absorption	*Total Absorption of Non-Heme Iron (mg) Corrected to a 40% Reference Absorption*
13	Corn-soy-milk	2.1	0.19
	Wheat-soy blend	1.1	0.10
	Wheat protein concentrate-soy	1.1	0.10
14	Corn-soy-milk	1.7	0.15
	Corn-soy blend	2.8	0.25
	Wheat-soy drink	2.0	0.18

[a] All meals contained 50-59 g dry product, 20 g sucrose, 1 g salt, 170-225 ml water, 9 mg iron as ferrous fumarate and 20 mg ascorbic acid.
Source: Morck *et al.* (1981)

TABLE 14.
IRON AND ASCORBIC ACID CONTENT OF CEREAL AND SOY BLENDS AND SOY-FORTIFIED PRODUCTS

Product	Added Iron (mg/100 g)	Total Iron* (mg/100 g)	Ascorbic Acid (mg/100 g)
Wheat-soy blend	15	21	40
Corn-soy-milk (CSM)	15	18	40
Instant CSM	15	18	40
Whey-soy drink mix (WSDM)	15	18	47
Soy-fortified bulgur	0	5	0
Soy-fortified flour 12%	2.9-3.6	5	0
Soy-fortified cornmeal	2.9-5.7	5	0
Soy-fortified sorghum grits	0	2	0
Soy-fortified rolled oats	0	5	0
Bulgur	0	4	0
All purpose flour	2.9-3.6	3	0

*Estimates taken from technical bulletins and information provided by manufacturers.

unlikely to meet the total iron needs as was originally intended. These findings point to a need for improved fortification strategies.

The independent effect of each of the three components of CSM on iron availability was studied by removing one component at a time from the mixture (Cook *et al.*, unpublished data). Geometric mean absorption from the complete supplement was 0.6%. There was no significant increase when either corn was withdrawn (0.6%) or when milk was withdrawn (0.5%). On the other hand, absorption rose to 1.2% when soy was withdrawn. These data show that soy decreases the percentage of iron absorbed from CSM and WSB. Since the amount of iron added as ferrous fumarate is much greater than the amount that is contained in the soy, one can infer that soy decreases the absolute amount as well as the percentage of iron absorbed.

Ferrous fumarate, which is used to fortify cereal-soy blends, is less soluble than dessicated ferrous sulphate, especially in an acid environment. As pH increases toward neutrality, the solubility of ferrous fumarate approaches that of ferrous sulphate. Since there is no information on the efficacy of ferrous fumarate relative to other iron fortificants in cereal-soy blends, it would be advisable to directly measure bioavailability of fortificants under conditions that simulate the storage and use of cereal-soy blends.

Ascorbic acid enhances the absorption of iron even in the presence of soy (Morck, in press; Bothwell, Derman and Torrance, unpublished data). CSM and WSB contain 40 mg of ascorbic acid per 100 g of product yielding a molar ratio of ascorbic acid:iron of 0.7 (40 mg ascorbate: 18 mg iron). Figure 6 indicates that ascorbic acid would be more effective in enhancing iron absorption if the molar ratio were increased from 0.7 to between 1.0 and 1.5. This is equivalent to increasing ascorbic acid to between 60 and 85 mg per 100 g of cereal-soy blend (or from about 2 mg ascorbic acid/mg iron to between 3 and 5 mg ascorbic acid/mg iron).

An important consideration is the oxidation of ascorbic acid under many conditions of processing and storage. Until recently, cereal-soy blended foods under Title II, PL 480, contained uncoated ascorbic acid, which was vulnerable to inactivation. However, as of September 1981 (USDA announcement CSSM-1), CSM and WSB products are to be formulated using a more stable ethyl cellulose-coated ascorbic acid (Bookwalter *et al.*, 1980). Use of coated ascorbic acid should enhance the absorption of iron from cereal-soy blended foods with very little increase in cost. Cost figures for iron and ascorbic acid fortification are shown in Table 15.

Soy-Fortified Products. Some food commodities distributed under PL 480 (e.g., corn meal, wheat flour,

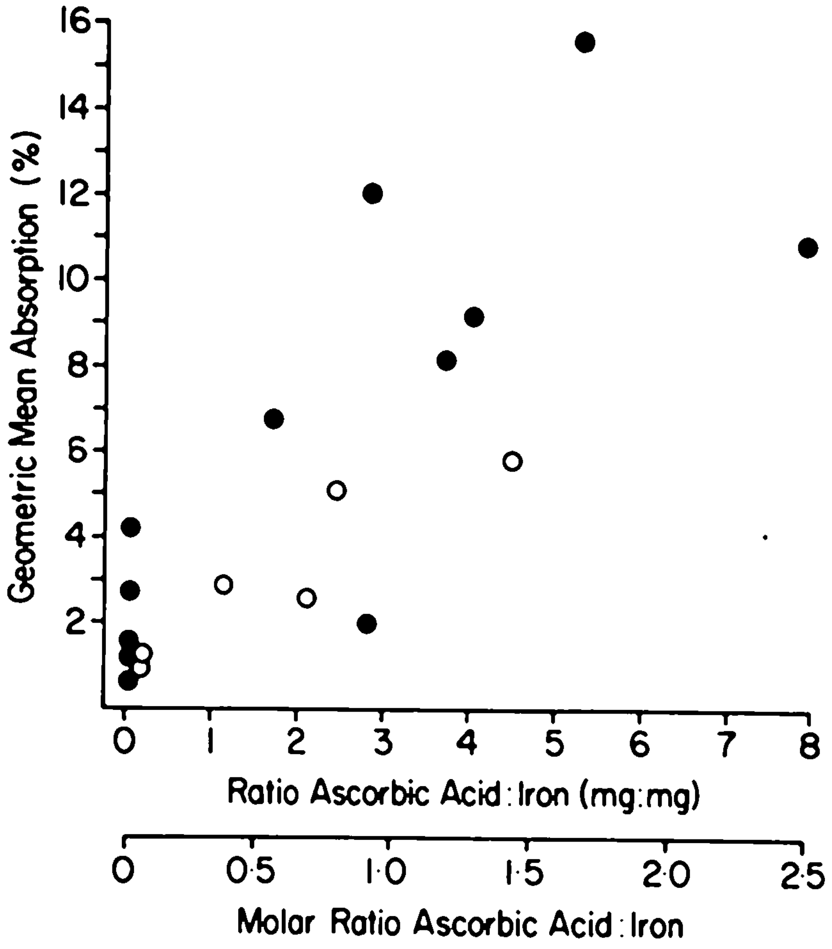


FIGURE 6

Effects of various quantities of ascorbic acid on the absorption of iron from a number of infant foods (supplements and weaning foods). Soy protein (14 to 30%) was present in all foods represented by the closed circles, but was absent from those shown as open circles (Derman *et al.*, 1980. Bothwell *et al.*, unpublished data)

bulgur, sorghum grits, rolled oats) are fortified with 12 or 15% defatted soy flour or grits. Estimates for the iron content of these products are listed in Table 14. Only three of the products are fortified with iron. No ascorbic acid is added to these soy-fortified foods.

Soy-Based Infant Formulas In contrast to the evidence of poor availability of iron from cereal-soy biends, the limited data on iron absorption from soy-based infant formulas are relatively reassuring. Rios *et al.* (1975) studied the absorption of iron from soy-based and cow milk-based formulas and from an infant cereal. The subjects were non-anemic, healthy infants between four and seven months of age. Within this age range, neonatal iron stores are normally diminishing, but iron deficiency anemia is rare until later infancy. This population is also of particular interest because it is one in which soy-based formulas are sometimes used as the sole or major source of calories and protein. In a group of 13 infants, the mean percent iron absorption from a soy-based infant formula was 5.4%, a value that was at least equivalent to or actually higher than the values of 3.9% (N = 14) and 3.4% (N = 15) obtained with two iron-fortified (12 mg ferrous sulfate/liter) cow milk-based formulas. Cow milk formulas fortified with iron at this level have been shown to be effective in preventing iron deficiency during the first year of life. The intrinsic iron content of the soy formul. was 5 mg/liter and contained an additional 12 mg o' iron as ferrous sul-

TABLE 15.
COST OF FORTIFYING CSM WITH ASCORBIC ACID IN DOLLARS[a]

	Ascorbic Acid Cost/Ton of CSM	*Total Cost/Ton of CSM*	*Increased Cost/Ton %*
Present level of fortification at 0.7 moles of ascorbic acid/mole iron			
Unstabilized ascorbic acid	$ 3.79	$211.60	—
Stabilized ascorbic acid	3.90	211.71	<0.1
Proposed levels of fortification with stabilized ascorbic acid			
1.0 moles ascorbic acid/mole iron	5.85	213.66	0.9
1.5 moles ascorbic acid/mole iron	8.27	216.08	2.0

[a]Based on cost of $10.40/kg unstabilized ascorbic acid and $10.70/kg stabilized ascorbic acid.

fate to yield a total of 17 mg of iron, substantially more than the cow milk-based formulas. These values for iron content and percent absorption would lead one to infer that the soy formula would be adequate as the major source of iron for infants under one year of age

The apparent discrepancy between adequate bioavailability of iron from the soy-based infant formula as compared to the poor iron bioavailability from the cereal-soy blends is intriguing, and suggests that the method of food processing might play an important role The infant formula is processed in liquid form, has a low pH, is fortified with ascorbic acid, and is marketed in an air-impermeable container that preserves its ascorbic acid content

Soy Products as Meat Extenders Recently, the use of soy products as meat extenders* has increased. For example, all ground beef (bulk and patties) purchased by the U.S. military since 1979 is extended 20%, on a weight basis, by blending 80 parts of ground beef with 20 parts of hydrated granular soy protein concentrate. There are no regulations that require the fortification of the soy concentrate to achieve nutrient equivalency with the portion of meat that is replaced. Military personnel who eat most of their meals in the dining hall consume an average of approximately 100 g/day of the soy extended beef with maximal values approaching 225 g/day. Currently, approximately 15% of the United States military is composed of women of childbearing age. Federally-sponsored school lunch programs in the United States allow local authorities the option of substituting up to 30% of ground beef with hydrated soy products.

In one study, the absorption of non-heme iron was measured in three meals (Cook *et al.*, in press). The basic meal consisted of a 100 g broiled beef patty on a bun, French fries, and a milk shake. Textured soy protein (30 g) was either added to the meal or substituted for 30 g of the 100 g broiled beef. The percentage of non-heme iron absorption dropped from 3.2% with the basic meal to 1.2 and 1.5%, respectively, when soy was present. However, when total iron absorption (i.e., heme and non-heme iron) was calculated, the differences were not as great, being 0.44 mg (basic meal), 0.41 mg (soy addition) and 0.36 mg (soy substitution), respectively (Figure 7). This can be ascribed to the relatively high non-heme iron content of the soy products.

In a series of experiments from another laboratory, Hallberg and Rossander (in press) investigated the effects of decreasing the meat content of hamburgers even further than the 30% substitution described above. One of two soy products (textured soy flour or defatted soy flour) was substituted in a protein equivalent amount for half of the meat protein in a

*The term meat extender can be ambiguous. In common usage and in this report it refers to the partial substitution of certain soy products for meat.

hamburger meal. In the first experiment, the meat content was reduced from 82 to 41 g, but no soy was added. There was a drop in the absorption of the non-heme iron in the meal from 11.2 to 8.4%, which is equivalent to reduction in non-heme iron absorption from 0.34 mg to 0.20 mg (Figure 8). When textured soy flour and defatted soy flour were added, there was further reduction in the percentage of non-heme iron absorption to 7.2 and 5.6%, respectively. However, because of the high iron content of the soy protein, the actual amounts of non-heme iron absorbed were greater than when a 41 g meat hamburger was consumed alone (0.27 mg and 0.22 mg, respectively). Dephytinization of the soy product did not increase the amount of non-heme iron absorption. Total iron absorption was, however, substantially improved by the addition of a small amount of blood (1.05 mg heme iron) to the hamburger, with total iron absorption from the meal rising to 0.51 mg (Figure 9).

Studies from a third laboratory were reported by Stekel (unpublished data). In the first experiments, the basic meal consisted of 100 g beef fed with bread. Fifty grams of hydrated isolated soy protein were

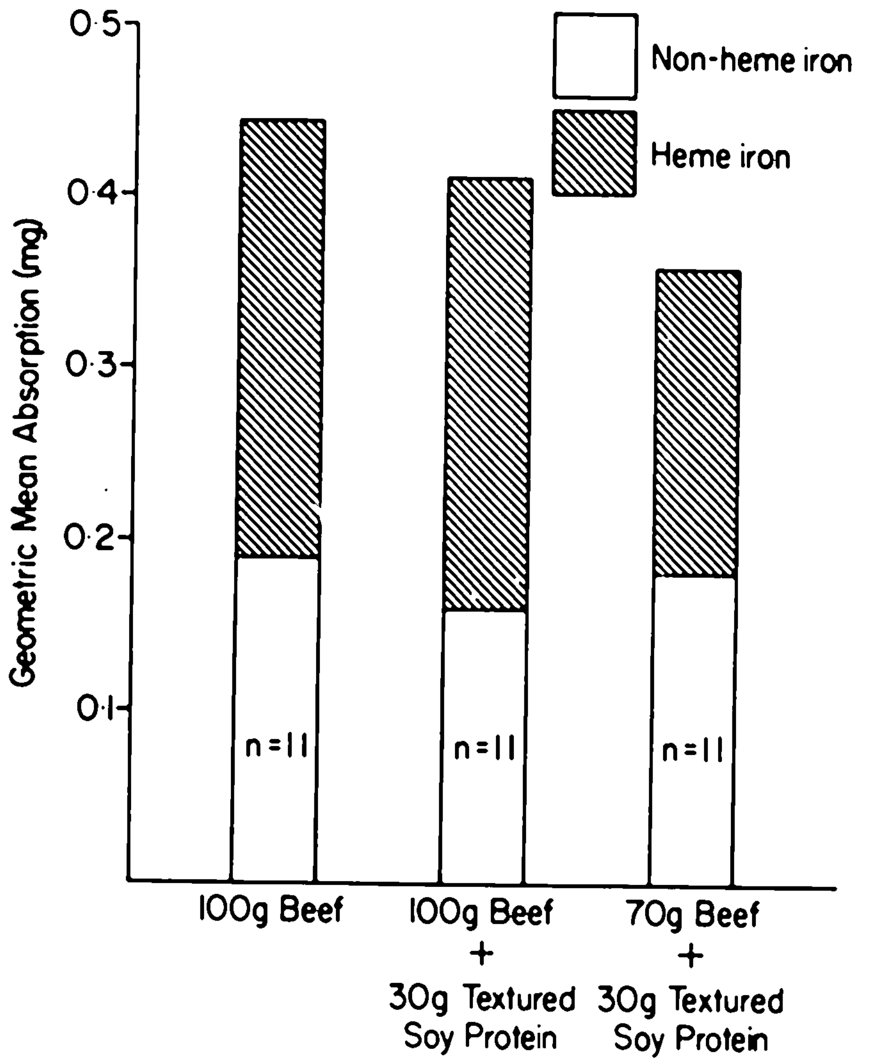


FIGURE 7

Effects of textured soy protein on the absorption of iron from meat-containing meals. Each meal consisted of broiled beef patty on a bun, 55 g French fries and 180 ml vanilla milk shake containing 0.1 mg $^{59}FeCl_3$. Soy was either added to the beef or replaced part of it. It was assumed that 25% of the heme iron in meat was absorbed. (Cook *et al.*, in press)

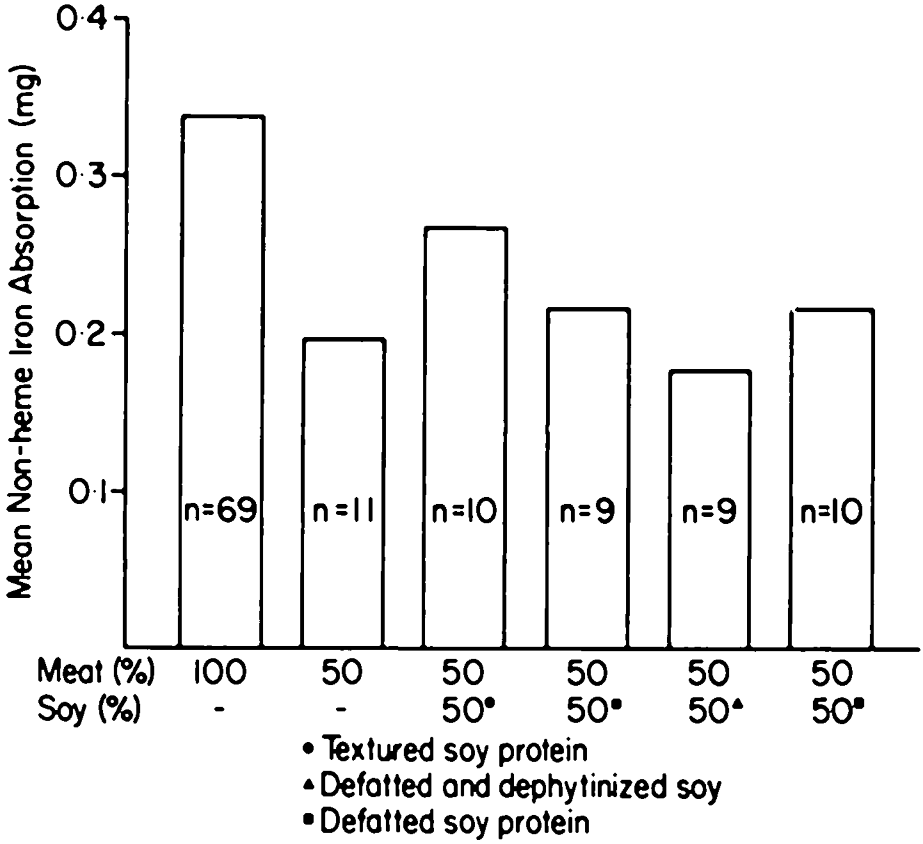


FIGURE 8

Effects of different forms of soy protein on the absorption of non-heme iron from a hamburger meal. The basic meal consisted of 82 g meat, 60 g string beans and 150 g mashed potatoes. (Hallberg and Rossander, American Journal of Clinical Nutrition, in press)

either added or substituted for half of the beef (Figure 10). Percentage absorptions (corrected to a 40% reference dose) were 12.4% (basic meal), 9.2% (soy added) and 9.3% (soy substituted), respectively, while calculated figures for total iron absorption were 0.63 mg, 0.54 mg and 0.42 mg, respectively. Since the basal meal had less iron than those containing soy, the non-heme iron content was adjusted with ferrous sulfate so that it contained the same amount of iron (3.1 mg). If this had not been done, total absorptions from the meals would presumably have been very similar. In a second experiment the absorption of iron from intrinsically labelled meat and from extrinsically labelled isolated soy protein was measured when consumed alone and together (Table 16). There was a striking difference in the percentage absorptions, with 25% for meat and 2.1% for isolated soy protein. When half the meat was replaced by soy protein there was a slight reduction to 19.6% in the absorption from meat and a rise to 6.6% from the non-heme iron in soy and meat. While certain assumptions are required to calculate the total absorption from these three meals, figures of 0.38 mg (meat), 0.05 mg (soy) and 0.22 mg (meat and soy), respectively, seem reasonable approximations.

When these different studies from three laboratories are considered together it is apparent that the use of soy products as meat extenders in mixed meals is associated with an overall reduction in total iron absorption. When 30% of the meat is replaced by a soy product, 18% less iron is absorbed from the meal. When 50% of the meat is replaced by a soy product, the amount of iron absorbed from the meal is decreased by between 30 and 42% (Table 17).

Hallberg and Rossander (unpublished data) performed a group of experiments using a basal meal

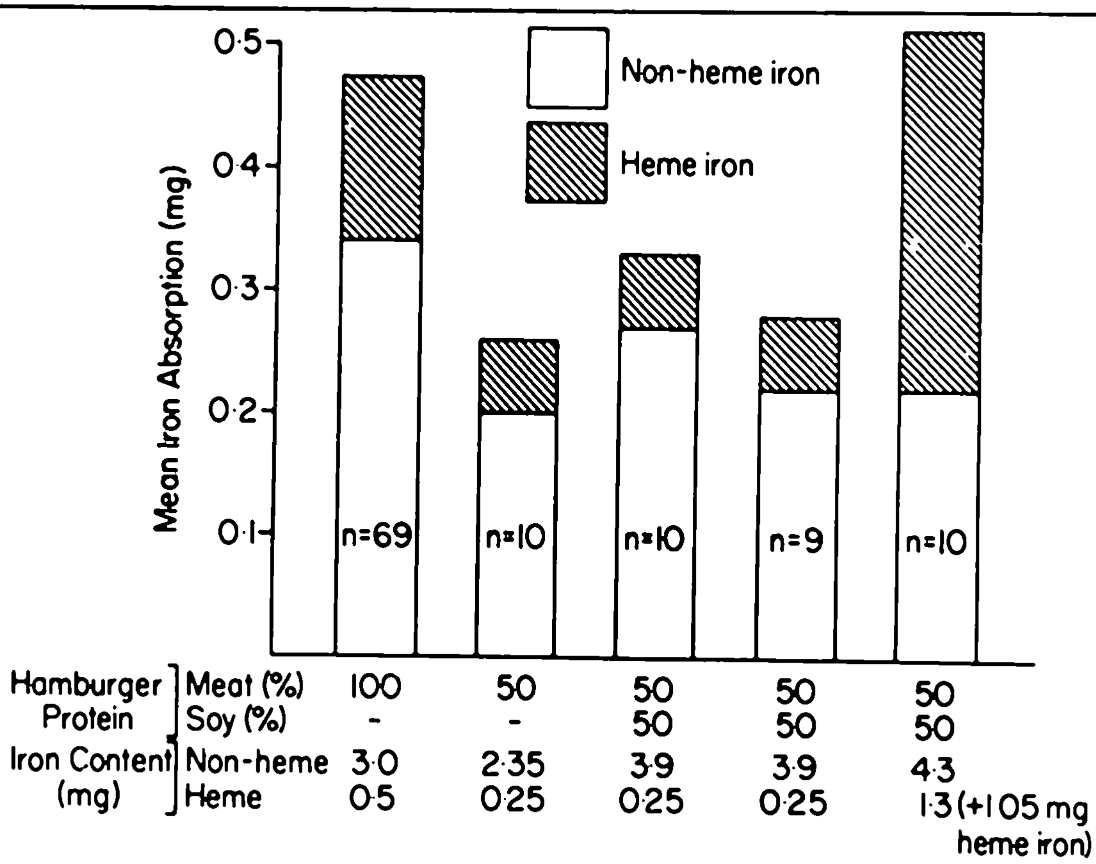


FIGURE 9

The effects of soy protein on total iron absorption from a hamburger meal. The basic meal consisted of 82 g minced meat, 60 g string beans and 150 g mashed potatoes. It was assumed that 25% of the heme iron in meat was absorbed. (Haliberg and Rossander, in preparation)

(maize, rice and black beans) of low iron bioavailability (Figure 11). When 75 g meat (16 g protein) was added, the percentage absorption of non-heme iron, expressed in terms of a 40% absorption from a reference dose, rose from 3.2% to 8.4%, the calculated figures for absorbed iron being 0.18 mg and 0.63 mg, respectively. When 15 g fat-free soy flour was fed instead of meat, the percentage absorption (4.8%) was actually higher than that obtained with the meat-free basal meal (3.2%), and because of the high iron content of the soy, the actual amount of iron absorbed was much greater (0.51 mg as compared with 0.18 mg). Indeed, when ferrous sulfate was added to the basal meal in an amount equivalent to that present in the soy flour, the total absorption of iron was only slightly greater than when soy was present (0.64 mg and 0.51 mg, respectively). In this instance, a soy product substantially augmented the amount of iron absorbed when it was added to a vegetarian meal of low iron bioavailability.

Effects of Ascorbic Acid on the Absorption of Iron from Soy Morck and coworkers (in press) found that the addition of 100 mg ascorbic acid enhanced the absorption of iron from either isolated soy protein or egg albumen, but the absorption from isolated soy protein was still much lower than from albumen (Table 18). In terms of the actual amounts of iron absorbed, ascorbic acid caused an increase from 0.03 mg to 0.18 mg iron from the soy-containing meal, and of 0.28 mg to 0.57 mg from the albumen-containing meal. Related findings of Hallberg and Rossander (Figure 11) showed that the addition of cauliflower, containing 65 mg ascorbic acid, to another meal of low bioavailability (maize, rice and black beans) increased iron absorption from 0.18 to 0.58 mg.

Further evidence for the role of ascorbic acid in potentiating the absorption of non-heme iron was obtained in a series of experiments using six cereal-based infant foods, four of which contained soy protein and two of

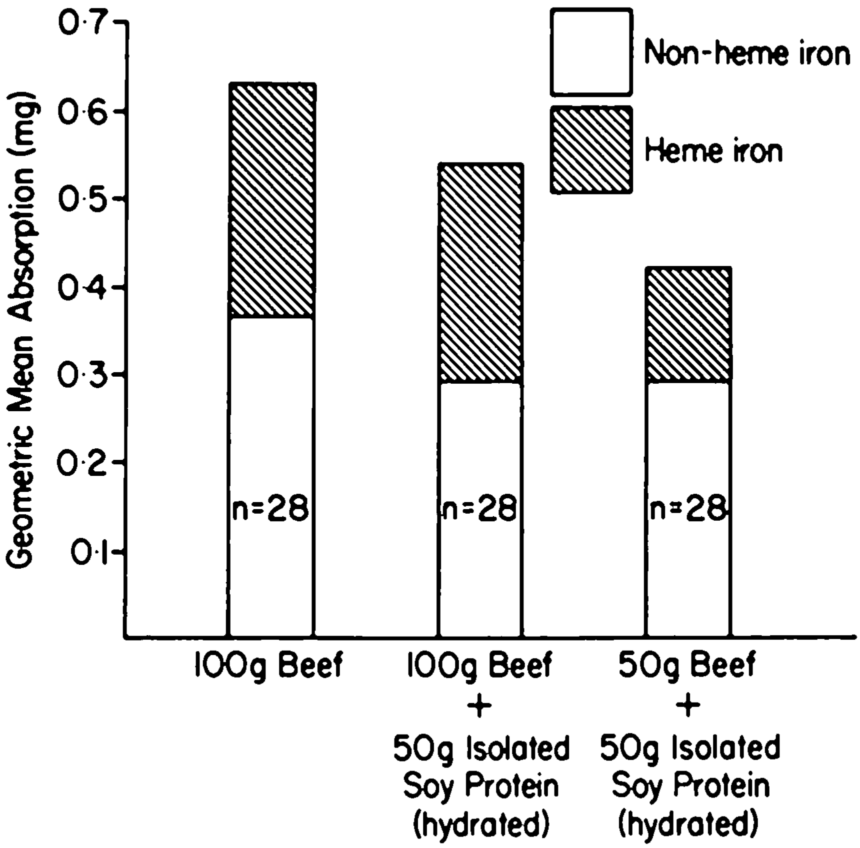


FIGURE 10

Total absorption of iron from meals containing various amounts of beef and soy protein. The first two meals also contained bread (100 g wheat flour), while the third meal had half the amount of bread. Since the basal meal had less iron than those containing soy, the non-heme iron content was adjusted with ferrous sulfate so that it contained the same amount of iron. It was assumed that 25% of the heme iron in the meat was absorbed (Stekel, *et al.*, unpublished data)

which did not (Bothwell *et al.*, unpublished data) (Figure 6). The bioavailability of iron from all of the products was low, with geometric mean absorptions varying between 0.4 and 4.1%. The addition of ascorbic acid was associated with a significant increase in iron absorption from all of the infant foods, the effect being dose-related. The mean increase was more than three-fold with an ascorbic acid: iron molar ratio of 1.5:1 (about 5 mg ascorbic acid per mg of iron) (Derman *et al.*, 1980). There were no apparent differences between the products which contained soy protein and those that did not.

In view of the substantial enhancement of iron absorption from cereal and soy products by ascorbic acid, it is noteworthy that certain encapsulated preparations of ascorbic acid are far more stable than ordinary ascorbic acid during storage. When ethylcellulose-coated ascorbic acid is used in fortifying corn-soy-milk, it is much more resistant than ordinary ascorbic acid to moisture and high temperature during storage (Bookwalter *et al.*, 1980). For example, about 50% of the ascorbate was still present after 56 days of storage at 49°C and 10% moisture, whereas less than 10% of the uncoated ascorbate remained. Coated ascorbate is widely used by the food industry and can be a practical means of enhancing iron absorption from blended foods. At present, the ethyl-cellulose-coated ascorbic acid costs only 3% more than uncoated ascorbic acid.

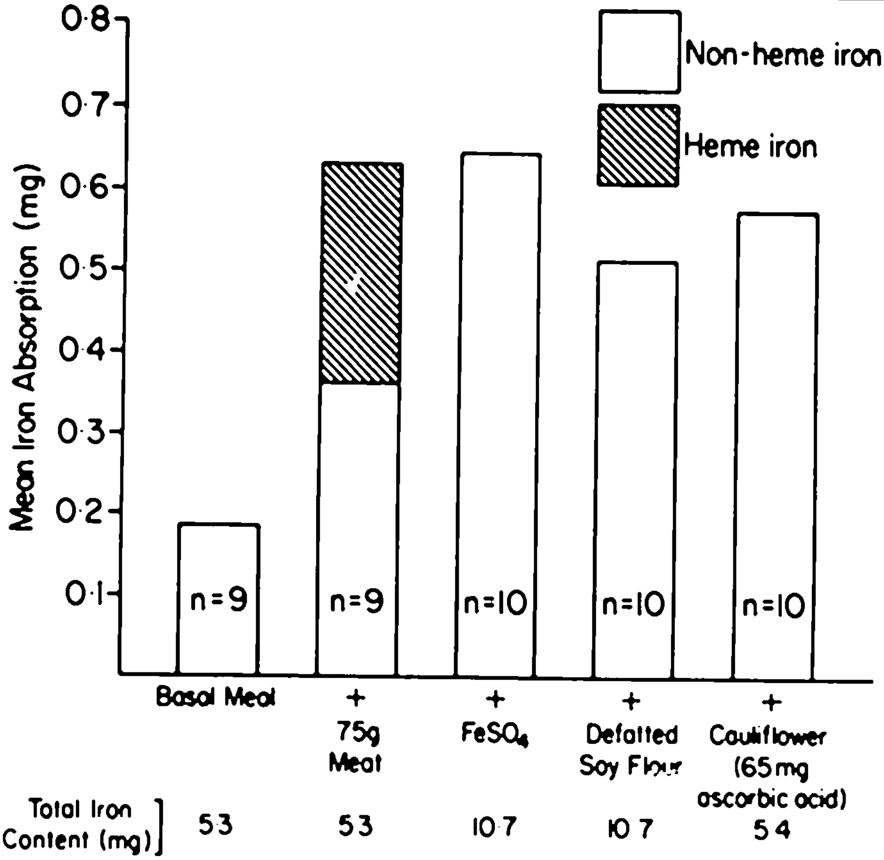


FIGURE 11

Iron absorption from different meals. The basal meal consisted of 81 g of a mixture of maize (chapatties), rice and black beans. It was assumed that 25% of the heme iron in meat was absorbed. (Hallberg and Rossander, unpublished data)

TABLE 16.
IRON ABSORPTION FROM MEAT AND SOY PROTEIN ISOLATE WHEN FED SEPARATELY AND TOGETHER

Number of Subjects	Preparation	Iron Content (mg)	Geometric Mean Absorption: % Absorption	Geometric Mean Absorption: Calculated Total Iron Absorption (mg)
	100 g Meat	± 1.5 (1 mg heme iron)	25.0	0.38
29	22 g Isolated soy protein	± 2.6	2.1	0.05
	50 g ^{55}Fe Meat & 11 g ^{59}Fe isolated soy protein	± 2.05 (0.5 mg heme iron)	19.6 (^{55}Fe) 6.6 (^{59}Fe)	0.22

Source: Stekel *et al.* (unpublished data)

TABLE 17.
EFFECTS OF SOY PROTEIN ON IRON ABSORPTION WHEN USED AS A MEAT EXTENDER

Authors	Replacement %	Total Iron Absorption (mg): − Soy	Total Iron Absorption (mg): + Soy	Reduction in Iron Absorption (%)
Cook *et al.* (in press)	30 (Fig. 7)	0.44	0.36	18
Hallberg and Rossander (in press)	50 (Fig. 9)	0.47	0.28	40
	50 (Fig. 9)	0.47	0.33	30
Stekel *et al.* (unpublished data)	50 (Fig. 10)	0.63	0.42	33
	50 (Fig. 10)	0.38	0.22	42

TABLE 18.

COMPARISON OF THE EFFECTS OF ASCORBIC ACID (AA) ON IRON ABSORPTION FROM ALBUMEN AND SOY PROTEIN WHEN FED AS PART OF A SEMI-SYNTHETIC DIET.

Sources of Protein	*Iron Content (mg)*			*Geometric Mean Iron Absorption (mg)*
	Native	*Added*		
Albumen	0.2	5.4	− AA	0.28
			+ AA	0.57
Isolated soy protein	2.1	3.5	− AA	0.03
			+ AA	0.18

Source: Morck *et al.* (in press)

VI. Conclusions

1. *Bioavailability of Iron in Cereals and Legumes.* In general, iron is poorly absorbed from cereals and legumes. An exception is wheat that has been refined to 60-80% extraction, but even in this instance, the advantage of a high percentage of iron absorption is offset by a low iron content. In the case of soy products, increased refinement and processing have not been effective in significantly augmenting iron bioavailability.

Factors that depress iron absorption from cereals include some phytates and certain components of fiber. Tannates decrease the absorption of iron from a number of legumes, but are not responsible for the low percentage of iron absorbed from soy products. However, there must be additional factors responsible for the poor availability of iron from cereals and legumes that remain undefined and that require further study.

At present, the most reliable means of increasing the percentage of iron absorbed from cereal and legume meals is the inclusion of meat and/or ascorbic acid with those meals. In addition, the amount of iron absorbed is increased by fortifying a dietary staple, such as flour, with iron. Conditions of growth, maturity at harvest, and storage conditions may well be of importance, and there is *in vitro* evidence that there are chemical changes that occur during processing which may influence bioavailability (Lee and Clydesdale, 1980a, 1980b, and 1981).

2. *Partial Substitution of Meat by Soy Products.* When soy is added to a meat-containing meal (e.g., to make a bigger hamburger) the *percentage* of non-heme iron absorbed is usually decreased, but the actual *amount* of iron absorbed is increased as a result of the substantial iron content of the added soy product. In contrast, when soy is used as a meat extender or meat substitute (e.g., to replace a portion of the meat in a hamburger) there is a decrease in the total amount of iron absorbed from the meal, the decrease being in proportion to the degree of substitution. For example, the substitution of 50% of the meat in a mixed meal reduces the total amount of iron absorbed by about 35%, whereas a 30% substitution decreases it by only 20%. Part of the reduction in iron absorption is due to a decrease in the heme iron content of the meal, part to a decrease in the enhancing effect of meat on the absorption of non-heme iron, and part due to an inhibitory effect of soy.

The degree to which soy products might be expected to affect iron nutrition adversely when used as meat substitutes will depend on the iron status of the population, the amount of soy in the diet, and the bioavailability and amounts of other dietary iron. In a population, such as that in the United States, subsisting on diets containing adequate amounts of meat, fish, poultry and ascorbic acid, the substitution of up to 30% of the meat with soy products should pose few problems relative to iron nutrition. This is particularly so in those segments of the population in which nutritional iron deficiency is uncommon (e.g., adult males and postmenopausal women).

Conclusions in respect to infants, children, and women during the reproductive years must be qualified to some extent. Iron nutrition is more marginal in these groups, but iron deficiency anemia in developed countries is becoming relatively rare (except in infants) and is usually very mild when it does occur. These considerations make the use of up to 30% soy substitution for several meals per week justifiable if there are adequate amounts of meat, fish, poultry and ascorbic acid in the diet. In specific terms, the present levels of substitution of soy for meat being used by the United States Armed Services and school lunch programs seem reasonable if the diet contains enhancers of iron bioavailability in adequate amounts.

In other parts of the world, the advisability of soy substitution for meat will depend on the quantities of meat consumed. In areas where the basic meat consumption is relatively low, a significant substitution of this meat with soy protein would be expected to have a deleterious effect on the iron nutrition of the most vulnerable groups, namely infants, growing children, and women of reproductive age.

3. *Cereal-Soy Blended Foods* Data showing low bioavailability of iron from cereal-soy blends, such as corn-soy-milk, is of concern because these commodities are extensively used to help alleviate malnutrition in developing countries and among poorly nourished populations. These products are directed to infants, young children, and pregnant and lactating women, who are likely to be at greatest risk with respect to iron deficiency. The blended foods are intended as dietary supplements, but they sometimes form a major part of the diet. The blended cereal-soy products are fortified with both ferrous fumarate and ascorbic acid. Iron in ferrous fumarate is well absorbed when used therapeutically between meals, but is poorly absorbed from the blended foods. Ascorbic acid is added to enhance the absorption of iron from blended foods, but it is likely that a substantial proportion of the uncoated ascorbic acid is lost with storage, leading to decreased iron bioavailability. Use of stabilized ascorbic acid may, at least in part, overcome this problem and merits further investigation, both in terms of its stability and its effect on iron absorption. However, stabilized ascorbic acid is sufficiently promising to justify its immediate field use.

4. *Addition of Soy Products to Protein-Poor Diets.* When soy products are added to the diet to improve protein nutrition in developing countries where diets are of low iron bioavailability or in vegetarian diets, there is no indication that iron absorption will be impaired. Actually, a modest increase in total iron absorption can be expected, due to the extra iron present in the soy products. However, if soy products are substituted for the small amount of meat in the diet, the risk of iron deficiency will be increased.

VII. Recommendations

1. *Bioavailability of Iron from Diets in which Cereals and Legumes Predominate.* Iron absorption from such diets should be enhanced by inclusion of ascorbic acid-containing foods and/or at least small quantities of meat. Because legumes are rich in iron, their inclusion in the diet can be encouraged because they are likely to increase the total amount of iron absorbed from meals.

2. *Meat Extenders.* Soy protein may substitute for up to 30% of the meat protein without adverse effect on iron nutrition of population groups (adult males, postmenopausal females) at low risk of nutritional iron deficiency. Soy protein may substitute for up to 30% of the meat protein in several meals per week for populations at risk of nutritional iron deficiency (infants, children, adolescents, and females of child-bearing age), provided that the diets contain adequate amounts of meat, fish, poultry and ascorbic acid.

3. *Cereal-Soy Blended Foods.* These foods should be fortified with an adequate amount of stabilized ascorbic acid in order to more effectively enhance the bioavailability of fortification iron. In addition, other means of making these foods better sources of bioavailable iron should be sought.

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