Availability of Iron from Infant Foods

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The full-term infant receives a generous and relatively fixed iron supply from the mother. As a result, iron absorption in the early postnatal period is lower than at any later time in childhood; the amount and availability of dietary iron are therefore less important before 2 months of age. The infant then enters a period during which body growth rapidly outstrips the maternal supply of iron, and by 4 to 6 months of age the iron status of the infant becomes almost totally dependent on dietary iron supply.

Iron balance in the infant is characterized not only by this sudden change in iron requirement but by an equally dramatic alteration in the nature of the dietary iron consumed. It is convenient to review dietary iron availability during infancy in relation to three overlapping periods (Fig. 1). Initially, when the infant’s iron needs are lowest, dietary iron is derived largely from milk or milk products. Weaning or transitional foods, mainly processed cereals, are then introduced. In addition, in poor socioeconomic segments of the populations of developing countries there are often programs to enhance caloric and protein intake at this age using so-called infant food supplements. It is during this period in infancy that iron needs are not only the highest but the prospect for meeting these needs by manipulating dietary intake is also greatest. During the latter part of infancy there is an increasing dependence on solid foods, so that by 1 year of age the diet approaches that of other members of the household.

FIG. 1. Dietary iron patterns during infancy.
Studies of food iron availability in infants and children have been conducted for several decades. However, it is only in recent years that the methodology has advanced to the point of furnishing reliable quantitative information. Although a detailed review of this technology will not be attempted here, interpretation of recent studies of food iron absorption does require a thorough understanding of certain basic methodologic concepts.

MEASUREMENTS OF FOOD IRON ABSORPTION

It is possible to make certain inferences about food iron availability by placing infants on differing dietary regimens and comparing their iron status at a later time. However, the body's ability to modulate assimilation according to iron needs is so effective that such therapeutic trials provide only a crude and indirect measure of iron availability. Chemical iron balance studies have also been used to assess iron absorption in infancy, but this approach has proven to be too imprecise to measure the limited iron exchange in infants. Consequently, reliable information on food iron absorption has required the use of radioisotopic methods. The technical aspects of radioiron absorption measurements have been reviewed recently (1). The key features include the techniques for determining retention of administered radioiron, for eliminating the effect of differences in iron status of the participants in the study, and, perhaps most importantly, for isotopic labeling of the test meal.

Radioiron Absorption Measurements

Except for a few early studies in which the retention of administered radioiron was determined by measuring excreted fecal radioactivity, radioiron absorption has usually been determined in infant studies from the radioactivity incorporated into circulating blood 10 to 14 days following administration of the test dose. Although some uncertainty is introduced by the prediction of circulating blood volume from body weight and by the assumption of a fixed red cell incorporation of absorbed radioiron, these errors are negligible in relation to the marked variation in iron absorption between subjects. In adults, an excellent correlation has been obtained between measurements based on red cell incorporation and direct determinations of whole body radioactivity with a specially designed total body counter (2), which indicates that the method for measuring absorbed radioiron is not a critical methodologic consideration.

A major methodologic problem in iron absorption studies is the enormous variability in the percentage absorption. This variation is comprised of day-to-day differences within the same subject and of biological differences between subjects (3). In certain infant studies, day-to-day variability has been reduced to some extent by administering test doses over several days rather than using only a single test meal. However, the largest and most troublesome component of variability is that due to differences in the iron status of the subjects. In studies designed to assess iron bioavailability rather than biological response, the optimal approach is to administer two or more test meals to the same individual to permit comparisons of
absorption from different meals in the same subject. By employing two radioisotopes ($^{55}$Fe and $^{59}$Fe) and performing sequential studies, it is possible to perform as many as four separate iron absorption tests in each subject. This approach eliminates those errors introduced by predicting the size of the blood volume and by assuming a constant red cell incorporation. More importantly, it allows iron assimilation to be examined over a wide spectrum of iron status in subjects with levels of percentage absorption ranging from 1 to over 50%.

Because of the natural concern about administering radioisotopes to infants and children, multiple measurements in this age group have usually been limited to two. In evaluating studies of iron bioavailability it is very important to take into account this particular aspect of study design. If test meals have been administered to separate groups of infants, two- to threefold differences in absorption can easily be obscured by subject-to-subject variability. In contrast, paired measurements in the same subject can usually detect absorption differences of 20 to 30%.

**Corrections for Iron Status (Reference Dose)**

Although using multiple tests in the same subject is an effective way of comparing iron bioavailability from any pair of test meals, it is very desirable to be able to compare data obtained in different studies and in different subjects. Since the percentage absorption is profoundly influenced by iron status, and iron status invariably differs from study to study, it is necessary to adjust absorption data for such differences. The use of a reference dose for this purpose has been a major technical advance in iron absorption studies. The method involves administering a standard dose of inorganic radioiron to all subjects as one of the test meals. By general agreement among investigators, a dose of 3 mg iron as FeSO$_4$ containing a 2:1 molar ratio of ascorbic acid to iron is now used. By expressing absorption from a given test meal as a ratio of the reference dose, a measurement is obtained which is largely independent of the iron status of the subject (4).

The use of a reference dose makes it possible not only to compare the results in different studies but also to estimate the relative absorption of iron from different foodstuffs in subjects with widely differing iron stores. Current data suggest that an iron-replete male absorbs 15 to 20% of the reference dose as compared with 50 to 60% in a subject with advanced iron deficiency. It has been suggested that all food iron absorption data be adjusted to a reference value of 40%, which is taken to represent the absorption figure for individuals with depleted iron stores but without frank iron deficiency anemia. Reference dose measurements in iron-deficient infants have indicated that 60% is more appropriate in this age group (5).

One important question relating to reference dose corrections is whether absorption measurements of iron-replete individuals can be used with confidence to estimate absorption in individuals with severe iron deficiency. This is especially relevant to infants and children because of the concern about administering isotopes to younger age groups. Therefore, studies of iron availability from infant foods are often performed in adult subjects and the results are extrapolated to iron-deficient
infants who have a different caloric intake. Although additional validation of reference dose corrections would be desirable, at present there is no concrete evidence that the approach is not methodologically sound (6).

Isotopic Labeling

Biosynthetic Labeling

The most critical aspect of food iron availability in relation to isotopic studies is the method by which the label is introduced into the test meal. The technique of biosynthetically labeling was first introduced in the early 1950s by Moore and Dubach (7), who tagged vegetable foods with a so-called intrinsic label by growing them in hydroponic media containing radioiron. Animal food (including fish, poultry, and dairy products) was prepared in a similar manner by injecting radioiron into the animals several weeks or months prior to obtaining the food. Because of the marked food interactions which occur when several foods are included in the same meal, an intrinsic tag provides useful information only if the test meal consists of a single food item. Probably the one dietary situation in which synthetic labeling is still relevant is in the newborn infant whose dietary intake consists solely of milk. Nevertheless, although biosynthetic labeling is cumbersome and time-consuming, it remains the standard by which the accuracy of other methods for measuring food iron absorption is assessed (1,6).

Extrinsic Tag Labeling

Since the introduction in the early 1970s of extrinsic radioiron tagging, numerous disadvantages of biosynthetic labeling have been circumvented (8-10). Extrinsic tagging is based on the observation that when one adds a small quantity of inorganic radioiron (0.1 mg was used in early studies) to a food that has been biosynthetically tagged with an alternate radioiron form, absorption of the two labels is virtually identical (9,11,12). This has held true when tested with a large variety of foods (wheat, maize, sorghum, soybean, and black bean), when the dose of the extrinsic tag varies from between 1 to 2 μg and 5 to 10 mg, and when iron availability of the test meal is either markedly decreased by adding desferrioxamine or sharply enhanced by adding ascorbic acid (6,9,12–15). Although extrinsic radioiron tagging has been explained on the basis of complete isotopic exchange of the intrinsic and extrinsic labels within the gastrointestinal lumen, absorption of the radioiron tags is also identical in patients with complete achlorhydria (1). Thus, the low pH of gastric contents, which would favor iron solubility and isotopic exchange, is not a prerequisite.

Extensive experience with extrinsic tagging has identified some important technical considerations. The extrinsic tag must be thoroughly mixed with the food before administration because inappropriately high absorption occurs if the tag is simply consumed in a drink after the bulk of the meal has been eaten (1). When the test meal contains several food items, the extrinsic tag should be mixed with
the bulkiest item or distributed on the food items in rough proportion to iron content (6). It is not necessary to introduce the label before cooking the food; FeCl$_3$ and FeSO$_4$ (dose of iron 1–100 μg) are equally suitable as extrinsic labels.

Although the use of an extrinsic tag for measuring absorption of nonheme iron has been adequately validated, certain exceptions have been noted. In one such study, absorption of the extrinsic tag remained higher than that of the intrinsic tag when rice was fed as a whole grain but not when it was first thoroughly ground (14). Apparently the extrinsic tag did not completely permeate the polished rice grain (16). Layrisse et al. (17) demonstrated that iron absorption from the storage iron compounds ferritin and hemosiderin cannot be measured by an extrinsic label. There is also evidence that insoluble iron salts such as sodium iron pyrophosphate and ferric orthophosphate are also less well absorbed than an extrinsic tag (18). Thus it is clear that absorption of all forms of iron added to or contained in a meal are not measured by this technique and some forms of extraneous food iron, such as that resulting from soil contamination or food processing, undergo little if any exchange with an extrinsic radioiron tag (16,18).

While the discussion of extrinsic tagging has focused thus far on nonheme iron, it is noteworthy that the absorption of heme iron, the second major pool of dietary iron, can by the same principle be similarly measured (8,19). Radioactive iron is injected into rabbits and the animals are subsequently phlebotomized to obtain labeled hemoglobin. A small quantity of this hemoglobin is then extracted and added to the test meal in order to obtain a measure of the total absorption of the heme iron contained in the meal. However, during infancy the absorption of heme iron is of lesser importance because of the small amounts that are ingested.

**Fortification Iron**

One of the pitfalls in using an extrinsic tag is to assume that any form or amount of radioiron added to a meal will adequately measure absorption from the nonheme pool. As noted previously, both FeCl$_3$ and FeSO$_4$ can be used for extrinsic tagging in doses ranging from one to several hundred micrograms of iron. Even at much higher quantities of the extrinsic tag, absorption of the intrinsic and extrinsic labels remains equivalent if the latter is in a relatively soluble form. This is not true, however, for all the forms of fortification iron that are currently used and it is therefore essential that the bioavailability of such preparations be assessed prior to their introduction. In such studies it is important that the labeled iron compound be prepared under the same conditions as those for large scale commercial preparation and they should then be added to a meal that has been extrinsically labeled in the standard way using another radioisotope of iron. If absorption of the tags is identical, availability of the fortification iron can be measured in subsequent studies by extrinsic tagging. Studies of this type have shown that some forms of fortification iron, such as FeSO$_4$ or reduced iron of small particle size, undergo complete exchange with the dietary pool, whereas other forms, such as orthophosphate and sodium iron pyrophosphate, do not (20).
Another assumption to be avoided when using an extrinsic tag is that the amount of iron added as the label is unimportant. Even if complete isotopic exchange can be established between the fortification iron and the dietary nonheme iron, larger amounts of added iron can be expected to alter the percentage absorption from the dietary nonheme pool. Although the relative effects of different dosages of added iron have not been determined, it should not be assumed that this iron is a true extrinsic tag when it exceeds 10% of the native iron content of the meal.

SALIENT FEATURES OF FOOD IRON ABSORPTION

Heme and Nonheme Pools of Dietary Iron

The importance of extrinsic tagging extends far beyond the technical convenience of avoiding the need to prepare biosynthetically labeled foods. A critically important outcome of studies using the extrinsic tag is the realization that all forms of nonheme iron in a meal, regardless of the source, form a single common pool within the gastrointestinal lumen. This was initially established by adding an extrinsic tag to a homogenized meal containing several food items, one of which was a small quantity of biosynthetically tagged maize. Absorption of the two radioiron forms was virtually identical (9). It has been shown that when two biosynthetically tagged foods (which have markedly different absorptions when fed separately) are given in the same meal, percentage absorption becomes identical. For example, in one study absorption of labeled eggs and bread averaged 1 to 2% and 30%, respectively, when fed separately, but when fed together nearly identical mean absorptions of 5.0% were observed (14). The ability to measure total absorption of nonheme iron from a complex meal has become the cornerstone of our approach to studying food iron availability.

In recent studies emphasis has been placed on the availability of nonheme iron because this constitutes the major fraction of dietary iron. However, there is a second compartment consisting of heme iron which enters the mucosal cells as an intact porphyrin complex and is therefore affected little if any by the nature of the meal (1). There is a striking difference in the availability of iron from the heme and nonheme iron pools. In a study by Bjorn-Rasmussen et al. (8), meals containing proportional amounts of all foods consumed in a typical 6-week diet were labeled with double extrinsic tags of heme and nonheme iron and given to 32 young men. The daily intake of iron from this diet was 17.4 mg, of which 1 mg was heme. The absorption of heme and nonheme iron averaged 37 and 5.3%, respectively, corresponding to daily absorptions of 0.37 and 0.88 mg (Fig. 2). Thus, in this meat-eating population, heme iron provided nearly one-third of the daily iron requirement even though it represented less than one-tenth of the dietary iron.

Biochemical Determinants of Food Iron Availability

The objective of current studies on iron absorption is not simply to determine iron availability from different foods but to catalog and quantitate the potency of
IRON AVAILABILITY FROM INFANT FOODS

FIG. 2. Absorption in 10 subjects of nonheme and heme from a diet in which almost all the iron was present as nonheme iron (8).

various biochemical determinants in the diet which either facilitate or impair absorption from the common pool. The assumption by earlier workers that iron assimilation can be explained simply on the basis of valency and solubility is now recognized as an oversimplification and there is increasing awareness of the complex nature of the iron-binding reactions that occur in the gastrointestinal tract. The effect of a given ligand depends on its concentration, its chelating efficiency which is often sharply pH dependent, and the types and concentrations of competing ligands in the meal. Although it may eventually be possible to design in vitro models which can predict iron assimilation from a given meal, at present it is necessary to measure iron absorption in human subjects under different dietary conditions to obtain meaningful data.

Enhancers of Iron Absorption

The quantity of animal tissue in a given meal is probably the single most important determinant of iron availability. Except for milk intake, it is the only dietary factor that has been found to correlate with iron status in population studies (1,21). The beneficial effect of meat is partly explained by the high content of heme iron. For example, the percentage absorption of nonheme iron from a meal containing only meat is 20 to 30% as compared to 2 to 3% in vegetable foods or dairy products (8,10). Meat fulfills a second role: its addition to a meal enhances the absorption of nonheme iron by a factor of 2 to 3 (22). Despite continued study, the biochemical factor responsible for the facilitating effect of animal tissue is still unknown. Martinez-Torres et al. (23) have suggested that cysteine is the factor but this has not yet been confirmed. Others have assumed that the enhancing effect of iron absorption is related to the protein fraction of the meat; if so, however, it must be the type of protein rather than protein per se. In studies of the effect of macronutrients, protein but neither fat nor carbohydrate was found to inhibit iron absorption...
It is possible that the stimulating effect of meat relates to the rate or products of protein digestion.

The enhancing effect of ascorbic acid on nonheme iron absorption is profound whether it is contained naturally in the food or is added as the synthetic vitamin (1,15,18,24-26). The facilitating effect of vitamin C relates not so much to the valency state of iron but more to its ability to form a soluble complex with iron at a low gastric pH. This complex then promotes iron absorption by preventing the formation of the insoluble hydroxides at the higher pH of the small intestine. However, it is not easy to predict the importance of naturally occurring ascorbic acid on food iron absorption because much of the vitamin may be rendered inactive during preparation of the food. For example, ascorbic acid is destroyed by baking (15). The relative roles of other organic acids in potentiating iron absorption have not been defined. There is, however, preliminary evidence that lactic acid (27) and citric and malic acids do exert some effect (28). It is noteworthy that all the vegetables associated with higher iron bioavailability contain citric, malic, and ascorbic acids in various combinations (28). The vegetables include potato, beetroot, pumpkin, tomato, broccoli, cauliflower, cabbage, and turnip.

**Inhibitors of Iron Absorption**

The importance of dietary inhibitors first became apparent when a dramatic inhibiting effect of tea on iron absorption was demonstrated (29). It was shown that a single cup of tea reduced the absorption of FeCl$_3$ from 22 to 6% and of nonheme dietary iron from 11 to 2.5% (Fig. 3). This inhibition, which also occurs to a lesser extent with coffee, results from the formation of insoluble iron tannates. Tannins are widely distributed in vegetable foods and may be partly responsible for the overall low bioavailability of iron in many such foods (28).

Another important inhibitor of iron absorption is bran, which impairs iron absorption in a dose-dependent fashion (30,31). It is widely assumed that the effect is due to phytate. This has been inferred from studies in which the effect of adding large amounts of sodium phytate to a meal has been measured but it is not certain whether naturally occurring phytate also impairs iron absorption. For example, wheat and oat bran appear to be equally inhibitory despite widely differing phytate contents (32). In addition, destruction of the phytate content of whole bran by

![FIG. 3. The inhibitory effect of tea on the absorption of the nonheme iron in a meal containing rice, potato, and onion soup, and 100 mg ascorbic acid (29).]
enzymatic hydrolysis did not diminish its inhibiting effect in a recently reported study (33). Moreover, monoferric phytate, the major form of iron in bran, has relatively high bioavailability as determined by studies in both animals and humans (34). The inhibitory effect of bran may also be due to its fiber content or possibly to a component contained in a soluble extract of bran.

The propensity of iron to form highly insoluble complexes with phosphates has led to the assumption that they are important inhibitors of iron absorption (32). Certainly, egg yolk contains a phosphoprotein that seems to explain its very low bioavailability. However, in a more recent study the addition of either a soluble calcium salt or soluble phosphate salt had little effect on iron absorption, whereas marked inhibition was observed when these were added simultaneously. Apparently, absorption was inhibited by the adsorption of iron onto an insoluble complex of calcium phosphate (35).

Another potent inhibitor of food iron absorption is EDTA, which is widely used as a food preservative. The effect of EDTA on iron absorption depends on its molar ratio to iron. When only small amounts are added to a meal, nonheme iron absorption is actually enhanced (36,37). This observation has led to the suggestion that sodium iron EDTA may be useful for iron fortification. However, when EDTA is added in a molar ratio greater than 2:1, absorption of nonheme iron is progressively impaired (38). There is reason to believe that such quantities of EDTA may exist in a broad range of prepared foods.

Many workers have suggested that fiber is an important inhibitory substance but this is based largely on indirect studies of in vitro iron binding (39). There is little direct evidence that fiber inhibits the absorption of nonheme iron in humans and, in fact, we have observed no effect whatsoever of certain purified fibers such as pectin and cellulose. Moreover, in a recent study, isocaloric meals were matched in terms of their major biochemical determinants but differed markedly in their content of naturally occurring fiber. Iron absorption from the low fiber meal was significantly higher but the relatively modest twofold difference between the extremes in fiber content suggests that fiber does not represent a major inhibitory factor in the diet.

**Fortification Iron**

There is evidence that a number of compounds that were used in the past as iron fortificants are poorly absorbed. These include large particle reduced iron, ferric orthophosphate, and ferric pyrophosphate (18,20,40). The relative bioavailability of such compounds in relation to a well-absorbed form of iron such as ferrous sulfate has been assessed in a number of ways. These include in vivo animal models using rats or chicks (41) and several in vitro studies aimed at testing the relative solubility of different iron compounds and of dietary iron in conditions similar to those operating in the upper gastrointestinal tract (42,43). However, before any fortificant is added to a diet, its bioavailability should be directly measured in humans by administering test meals that are representative of the diets consumed by the population concerned (44,45).
Currently the most commonly used fortification compounds are ferrous sulfate and various iron powders, including reduced iron, electrolytic iron, and carbonyl iron. Former problems relating to the absorbability of iron powders have been largely overcome by reduction of their particle size and surface area, and it has been shown that the reactive surface area and the dissolution rate in hydrochloric acid are good predictors of absorbability (46). The particular compound chosen depends not only on its bioavailability but also on its compatibility with the chosen vehicle and with other constituents in the diet. Thus high-density iron powders may be difficult to distribute evenly in powdered foods, whereas metallic iron may be removed by the magnets employed in food processing to detect metallic contaminants. On the other hand, more reactive compounds such as ferrous sulfate may be associated with undesirable changes in the color, odor, and flavor of the food (45).

IRON ABSORPTION FROM INFANT FORMULAS

Infant Formulas

Iron absorption from milk is of critical importance during infancy since this food often represents the only source of dietary iron during the first few months of life (47). In discussing this subject it is important to distinguish between human and cow’s milk—the iron in breast milk is much more bioavailable than that in cow’s milk (48–50). Since iron absorption from breast milk is the subject of another report, the major emphasis here will be on cow’s milk. In this connection, it is essential to distinguish between the fortified and unfortified products, as the percentage absorption is affected significantly by the iron content. There is no clear evidence that commercial processing has a significant effect on iron availability. Therefore, no distinction will be made between fresh cow’s milk, pasteurized milk, condensed milk, evaporated milk, or reconstituted powder. Moreover, since carbohydrate and fat have relatively little effect on iron absorption, milk and milk-based formulas will be considered equivalent. Attention must be paid, however, to the presence of nutritional additives and supplements such as ascorbic acid since they can be expected to affect the amount of iron absorbed.

Cow’s Milk

A frequent question that is asked in relation to infant formulas is whether milk in general is a good or poor source of dietary iron. There is no clear-cut answer to this question because it involves comparison with other foods and there is no agreement at present on what constitutes a reference food with respect to iron availability. In addition, it is not clear whether comparisons between test meals should be made on the basis of equal calories, equal bulk, equal protein, or equal iron content. Despite these conceptual problems, there are several observations in the literature that have a bearing on the question.

One of the few studies in infants and children in which iron absorption from milk as a single food item was compared with other foods was that reported a
number of years ago by Smith and Schulz (51). Using biosynthetically tagged foods, they observed that iron absorption from cow's milk averaged 9.1% in 10 children under the age of 5 as compared with 8.3% from eggs in 49 children. These findings imply low availability of iron in milk since the phosphoprotein of egg has been shown in adults to be a strong inhibitor of iron absorption (1). Iron absorption from liver and cereals was found to be similar to eggs and milk. However, there are two defects in this particular study that make interpretation difficult. First, no details of the iron status of the subjects were provided. Second, iron absorption was measured by fecal radioiron balance, which was subsequently shown to be a relatively inaccurate method.

The most stringent way to evaluate iron absorption from milk is to administer it with iron and compare the absorption to a similar iron dose given with water. When Schulz and Smith (52) fed 30 mg iron as ferrous sulfate with 180 cc milk instead of water, the average absorption dropped from 15 to 5%. Surprisingly, the same reduction was observed when the iron was given with 100 cc orange juice containing 42 mg of added ascorbic acid. It therefore seemed as if milk was no more inhibitory than orange juice in terms of fortification iron. Similar findings were reported by Davis and Bolin (53) who gave 5 mg iron as ferric ammonium citrate with either 300 ml full-cream powdered milk or with water and then measured iron absorption by whole body counting. The use of milk as a vehicle for the iron resulted in a moderate decrease in absorption from 23 to 10%. In a more recent study carried out by Heinrich et al. (54) in infants, the absorption of 5 mg iron as FeSO₄ in water fell from 18 to 4% when given with 50 ml cow's milk.

While these studies suggest that milk does not have a major inhibitory effect on iron absorption, the iron it contains is distinctly less available than some other types of animal protein. This point was underlined by the findings in one study in which the effects of various sources of animal protein were assessed by substituting them in protein equivalent amounts in complete meals (22). When milk was substituted for beef in a typical American meal, absorption fell from 5.1 to 2.6%, but no difference was observed when milk replaced egg albumen as the protein source in a meal containing semipurified ingredients. In addition, it was found that iron absorption from several dairy products, including milk, cheese, egg, and ovalbumen, was similar and distinctly lower than from animal tissue such as beef, pork, lamb, liver, chicken, and fish.

From a pediatric standpoint, it is pertinent to ask whether the iron in milk products is less or more bioavailable than the iron in foods which are used as substitutes for milk during infancy. The evidence suggests that iron absorption from milk is relatively high. For example, Ashworth and March (5) noted that when maize was added to a formula consisting of dried skim milk and sugar, absorption of the fortification iron which was present as FeSO₄ fell significantly from 9.5 to 6.3% in 16 iron-deficient children (reference absorption 59.6%). More recently, Oski and Landaw (55) studied the effect of strained pears on the absorption of iron from milk. In 5 iron-replete adult males, the mean percentage absorption from
100 ml human milk fell significantly from 24 to 5.7% when it was given with one jar (128 g) of this common baby food. This result might be partly explained by an increase in the amount of iron, neutral detergent fiber, volume of the meal, and perhaps EDTA. Nevertheless, these various studies suggest that while milk does not promote iron absorption, it has a significantly less inhibitory effect than infant cereals or solid food.

**Human Milk**

One of the most intriguing observations in relation to food iron absorption that has been made in recent years is the exceptionally high bioavailability of iron in human milk as compared with that in cow's milk (Fig. 4). In one study, McMillan et al. (49) fed 3 oz of extrinsically tagged milk to 10 adult subjects and observed a mean absorption of 13.6% (1.7–34.2%) from cow's milk containing 40 to 60 μg iron as compared to 20.8% (2.2–50.2%) from pooled human milk with the same iron content ($p < 0.02$). Extrapolating these findings to infants, the authors estimated mean absorptions of approximately 35 and 50% from formulas and breast milk, respectively. These estimates were confirmed in a later study by Saarinen et al. (56), who observed a very high mean absorption of 48.8% when a tracer dose of radioiron (FeSO$_4$) was given during breast-feeding to 11 infants 6 to 7 months old. When a tracer dose of iron was fed in the fasting state to breast-fed infants, the mean absorption was 38.1%, significantly higher ($p < 0.05$) than the 19.5% obtained with infants who were on a cow's milk diet. This technique may not have fully labeled the iron in breast milk but the differences suggest that breast milk may in some way modify conditions in the upper gastrointestinal tract so that they favor iron absorption. Whatever the explanation, it seems clear that the bioavailability of iron is greater when infants are being breast-fed.

Possible biochemical reasons for higher absorption from breast milk as compared to infant's formula have not been identified. Cow's milk contains a much higher concentration of phosphate, which is known to inhibit iron absorption under certain

![Comparison of radioiron absorption from human and unfortified cow's milk. Results are shown for studies in normal 6-month-old infants (56) and normal adults (49).](image)

**FIG. 4.** Comparison of radioiron absorption from human and unfortified cow's milk. Results are shown for studies in normal 6-month-old infants (56) and normal adults (49).
The ascorbic acid content of breast milk is substantially higher than that of cow's milk and the enhancing effect of ascorbate is well known (1). McMillan et al. (49) recently attempted to identify the factor responsible for the high iron assimilation from human milk. They found that iron absorption from human milk in adult subjects averaged 15.4% as compared with 9.0% from simulated human milk. Although absorption from the human milk was higher than that from the simulated food, the difference was not statistically significant. The addition of lactoferrin, which is present in high concentrations in human milk, to the simulated preparation depressed absorption to 4.78%. This finding indicates that it is not lactoferrin that accounts for the high bioavailability of iron in human milk and the authors speculated that a higher content of cysteine or perhaps adenine nucleotides may be of importance.

Soya-Based Formulas

It is currently estimated that soya-based formulas account for 10 to 20% of infant formula sales in the United States. Recent studies of the effect of soya on nonheme food iron from complex meals are reviewed in the following section. It is of interest here that no obvious differences have been observed between the bioavailability of iron present in milk and soya-based infant formulas. Rios et al. (57) measured radioiron absorption by whole-body counting in 4- to 7-month-old infants using three infant formulas that had been fortified with 12 mg FeSO₄/liter. The mean absorption in groups of 13 to 15 infants averaged 3.9 and 3.4% from two milk-based formulas as compared to a mean of 5.4% from the soya-based formula; the small difference was not statistically significant. The study indicates that iron availability from infant formulas is the same with cow's milk as the base as it is with soya, at least when the product is in liquid form, has a low pH, and is fortified with both iron and ascorbic acid.

Processed Cereal and Legume-Based Food

Cereals

The three major cereal grains, rice, wheat, and maize, are produced in roughly equal amounts on a worldwide basis (32). Rice is particularly important because it is the major food of over half the world's population. Since cereal grains are the major constituents of weaning foods, it is useful to consider first the bioavailability of iron from these foods. Much of the following information is derived from a recent extensive review of this subject (32).

The results of several selected studies in which the absorption of iron from cooked cereals was measured are summarized in Fig. 5. Although some of the meals contained other food items, no results are included from studies in which the meal contained food known to enhance or inhibit nonheme iron absorption. The native iron content of the test meals varied in different reports from 0.5 to 10 mg. All data were adjusted to a reference absorption of 40%. The review included
FIG. 5. Iron absorption from cereal foods. Results are summarized for published studies containing 10 or more subjects given a meal consisting only of cooked rice, wheat, or maize. All percentage absorption values have been corrected to a mean reference dose of 40%. All means are weighted means. Vertical bars represent range of reported means (32).

Eight studies with maize (189 subjects), three studies with wheat (48 subjects), and two studies with rice (149 subjects). Weighted averages were calculated for the iron content of the meal, the percentage absorption, and the absolute absorption (μg iron). A major problem that bedevils this type of analysis is the difference in iron content of the test meals. For example, the quantities of iron averaged 3.0 and 4.7 mg, respectively, in the maize and rice meals as compared with only 0.6 mg in meals containing wheat flour of high extraction. Since the percentage absorption is influenced by the iron content of the meal, a valid comparison of the relative bioavailability of iron in different cereals is not possible. It was, nevertheless, apparent that there were striking differences in the bioavailability of iron in these different cereals. Absorption from maize and rice averaged 6.3 and 3.5%, respectively, as compared with a value of nearly 30% for wheat flour of 70% extraction. When converted to the amounts of absorbed iron, the figure from the maize-based meals averaged about 100 μg iron as compared with 200 μg from both wheat and rice. It is therefore apparent that although the iron contained in wheat is highly available, its value as a source of dietary iron is lessened because of its low iron content.

An additional comparison of the absorption of iron from wheat, rice, and maize was performed recently (32). In an initial study, absorption from rolls containing approximately 1.5 mg iron averaged 11.8% when prepared with wheat flour and 3.0% when prepared with rice flour. This fourfold difference in the percentage absorption was highly significant statistically. Similar findings were obtained when rolls were prepared with starch extracted from either wheat, rice, or maize. When meals containing 1 to 2 mg iron were given, the absorption ratios for rice:wheat averaged 0.37 and for maize:wheat 0.59. Although the absorption ratios noted
with starch were somewhat different from those obtained with flour, it is apparent that the iron in white wheat is better assimilated than is the iron in rice or maize.

Sorghum is also used in infant cereals in certain developing countries although its total consumption is much less than that of the other major cereals. While there are only limited data on the bioavailability of iron in sorghum, the evidence that has been collected suggests that it is low. In one recent report, iron absorption from meals containing red sorghum, white sorghum, and maize averaged 3.6, 2.8, and 4.4%, respectively (32). In a recent study by Derman et al. (27), the geometric mean absorption of a maize and sorghum gruel was less than 2%. Fermentation of the gruel was found to enhance iron absorption, an effect that may have been due to several factors including a lower pH, a reduced content of solids, and the formation of lactic acid and alcohol. In further unpublished studies by the same workers, the geometric mean absorption of iron from whole grain sorghum porridge varied between 1.7 and 2.4% in different experiments (reference absorptions 34 and 41%, respectively).

**Legumes**

The bioavailability of iron in soya products is especially relevant to iron nutrition in infants. Soya-based infant formulas are used extensively to avoid suspected or proven allergies to milk, and soya is also a major constituent of infant protein supplements. The composition of soybean differs markedly from cereals in that more than half of the bean consists of protein and fat (40 and 20%, respectively). Soybeans are used in the preparation of a wide range of products, which are classified into three groups on the basis of their protein content. Soya flour and grits have a protein content of 40 to 50%, soya concentrates about 70%, and soya isolates between 90 and 95% (32). Another important difference between cereals and legumes is the high native iron content in the legumes. In a survey based on more than 30 samples of soya flour, the mean iron content was 8.6 mg iron/100 g product, while the iron content of 4 soya concentrates and 18 soya isolates averaged 11.9 and 15.0 mg/100 g, respectively.

The results of early studies using intrinsically labeled foods suggested that iron availability from whole soybean is relatively good. However, striking differences in availability were observed despite the fact that the soybean that was tested in the different studies had been biosynthetically tagged in the same institution (Table 1). Similar percentage absorptions of 11.0 and 12.3%, respectively, were observed by Layrisse et al. (10) and by Sayers et al. (15) from meals containing approximately 4 mg native soybean iron. On the other hand, a mean absorption of only 1.5% was observed by Bjorn-Rasmussen et al. (14), who fed a smaller meal. Low figures for iron absorption were also observed by Ashworth and March (5), even though their studies were conducted in children, many of whom had severe iron deficiency. It has been assumed that the marked differences in results that were noted were related to a number of factors, including the particular batch of soybean, the state of maturity at the time of harvest, differences in the method of preparing the food,
and perhaps also variations in the iron status of the test subjects. Incidentally, excellent agreement was observed in these studies between the absorption of the intrinsic and extrinsic radioiron tags.

Findings in more recent studies suggest that soya impairs the absorption of nonheme iron (58). In one recent study conducted in normal male volunteers, protein equivalent amounts of egg albumen, casein, and isolated soya protein were substituted in a meal containing semipurified ingredients (59). Absorption from meals containing egg albumen and casein averaged 2.5 and 2.7%, respectively, as compared to a mean figure of 0.5% with isolated soya protein. It should be noted that these meals were designed as an experimental model for assessing biochemical determinants of iron absorption and it would be hazardous to extrapolate the findings directly to iron absorption from a normal diet. Moreover, because of the low iron content of egg albumen and casein, it was necessary to add large amounts of FeCl₃ to the meals containing these protein sources in order to offset the high native iron content of the soya-based meal. In another study, full-fat soya flour, textured soya protein, and isolated soya protein were substituted in the same semipurified meal and were compared to a control meal containing egg albumen (60). The mean absorption of iron of 5.5% for the control meal fell significantly to 1.0, 1.9, and 0.4%, respectively, with the three soya products. This striking inhibition in percentage absorption by soya protein could not be explained by the method for preparing the meal. Iron absorption with isolated protein was the same whether the food was uncooked or baked, and absorption from meals containing whole soybean was the same whether the food was boiled or baked prior to serving.

**Prepared Infant Cereals**

There have been a limited number of studies in which iron availability from prepared infant formulas has been measured. It should be noted that in almost all cases the foods were fortified with relatively large amounts of iron. The percentage absorptions recorded in these studies do not therefore necessarily reflect an inhibitory effect of cereal foods on iron availability but rather the interaction between

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**TABLE 1. Absorption of iron from intrinsically labeled soybean**

<table>
<thead>
<tr>
<th>References</th>
<th>No. of subjects</th>
<th>Iron content (mg)</th>
<th>Geometric mean absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layrisse et al. (10)</td>
<td>17</td>
<td>4.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Sayers et al. (15)</td>
<td>10</td>
<td>4.6</td>
<td>12.3</td>
</tr>
<tr>
<td>Bjorn-Rasmussen et al. (14)</td>
<td>15</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ashworth and March (5)</td>
<td>16*</td>
<td>0.5</td>
<td>6.7</td>
</tr>
</tbody>
</table>

*Children.*
fortification iron and its cereal-based vehicle. It should also be noted that the fortification iron was actually tagged in only a few of these studies and in most reports it has been tacitly assumed that the added extrinsic tag underwent complete isotopic exchange with the iron fortificant.

The first isotopic study of absorption from infant cereals was performed by Schulz and Smith (52), who reported a mean absorption of 9.1% from mixed infant cereals that had been fortified with sodium iron pyrophosphate. These findings are in striking contrast to absorption values of 1 to 2% that have been observed in later studies (32). Although the exact reason for the disparity is not known, there are two possible explanations. First, the labeled iron pyrophosphate used by Smith and Schulz (51) differed in several respects from the compound presently used for fortifying infant cereals. Second, they measured iron absorption by radioiron balance, which has often contributed to falsely high values due to incomplete collection of fecal samples.

In a more complete study of the same type, iron absorption was measured in infants from a mixed grain cereal of oat flour, soft wheat flour, and barley malt flour (57). A 10-g portion of dry cereal containing 5 mg iron was mixed with the formula and fed on five successive days to 25 normal infants ranging in age from 4 to 7 months. Special care was taken in this study to prepare labeled forms of iron comparable to those actually used commercially. This was done by adhering to specifications laid down for the production of enrichment and fortification forms of iron for cereal manufacture. Particle size and solubility characteristics were rigidly controlled and were similar to those used for industrial fortification. The iron compounds that were studied included iron orthophosphate and sodium iron pyrophosphate, both of which were used extensively for cereal fortification at the time of the investigation. Two additional forms, reduced iron of small particle size (95% of the particles between 5 and 10 μm) and FeSO₄, were also studied. While FeSO₄ is not suitable for commercial use because of its tendency to cause rancidity in cereals after prolonged storage, it is highly soluble and therefore serves as a useful reference compound. Iron absorption from ferric orthophosphate and iron pyrophosphate was uniformly low, with a composite mean of only 0.9%. Comparable figures for FeSO₄ and reduced iron were 2.7 and 4.0%, respectively.

These results are very similar to those obtained when adult subjects were fed rolls fortified with the same forms of iron (20). In the adult study it was also noted that the absorption of ferrous sulfate and reduced iron could be measured with an extrinsic tag, whereas the less available forms of iron did not undergo complete isotopic exchange. As a result of these studies, there has been a reduction in the use of less soluble forms of iron for the fortification of infant cereals. Elemental iron powders of small particle size are now extensively used in the United States to fortify proprietary dry cereals. Electrolytic iron, which has not been studied isotopically, is the predominant form (45). It should be noted that it has never been established whether the absorption of different types of elemental iron powders such as electrolytic iron, hydrogen-reduced iron, and carbonyl iron is the same.
Infant Food Supplements

An important subgroup of processed infant cereals are the so-called blended foods, which are distributed to developing countries as infant protein supplements under the U.S. Food for Peace Program (32). While these foods are provided mainly for weaning infants, their use is also advocated in children, pregnant women, and lactating mothers. The most popular food of this type is corn-soya-milk (CSM), which is made up of 59% maize meal, 17.5% soya flour, 15% nonfat dry milk, and 5.5% soybean oil. In addition, a mineral premix is added, which provides, among other vitamins and minerals, 18 mg iron as ferrous fumarate and 40 mg ascorbic acid per 100 g dry food. Provided there is no degradation of the ascorbic acid, the CSM provides a molar ratio of ascorbic acid:iron of about 0.7. An intake of CSM at the recommended level of 100 g/day for a 1-year-old infant should meet the recommended dietary intake of iron for this age group.

The only study in which the absorption of iron from CSM has been measured in children was the one performed by Ashworth and March (5), who studied absorption from an extrinsically labeled test meal containing 30 g CSM, 15 g sugar, and 4.5 mg iron as ferrous fumarate. In 14 clinically healthy Jamaican children, absorption averaged 6.0% (reference absorption 63.5%), which was considered adequate by the authors. More recently, iron availability from several protein supplements was studied in iron-replete adult males (33). The foods included CSM, corn-soya blend, wheat-soya blend, wheat protein concentrate, and whey-soya drink. The protein contributions from each source are listed in Table 2. Iron absorption from the three products was compared to a reference dose of ferrous iron in two groups containing 13 and 14 volunteers. Corn-soya-milk absorption was measured in both groups as an additional study control. When the results were adjusted to a reference absorption of 60% (the mean reference absorption was 27 and 13% in the two groups), iron absorption from the five supplements ranged between 1.7 and 4.1% (Fig. 6), which is in close agreement with the results by Ashworth and March (5). After correcting to geometric means by assigning values of 0.1% to 2 subjects with zero absorption, mean absorption with CSM in the infant study was 3.1% as compared to 2.6 and 3.1% in the adult study. One limitation common to both reports is the fact that the ferrous fumarate used for fortification was not isotopically labeled and it was necessary to assume that its absorption could be measured by extrinsic tagging. However, if isotopic exchange

<table>
<thead>
<tr>
<th>Soya</th>
<th>Cornmeal</th>
<th>Milk</th>
<th>Wheat</th>
<th>Whey</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn-soya-milk</td>
<td>45</td>
<td>27</td>
<td>28</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Corn-soya blend</td>
<td>63</td>
<td>37</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat-soya blend</td>
<td>55</td>
<td>45</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat-protein-concentrate blend</td>
<td>61</td>
<td>39</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whey-soya drink</td>
<td>74</td>
<td>26</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Iron availability from infant foods

The data derived from studies in two groups of iron replete adult males, which accounts for the fact that there are two observations with corn-soya-milk. Absorption values have been adjusted to a reference absorption of 60% (33).

Iron Absorption (%)

had in fact been incomplete, iron absorption from these foods would have been even lower.

These recent findings suggest that although relatively large amounts of iron are added to infant food supplements, they may still fall short of iron requirements. For example, the recommended iron intake for a 1-year-old infant is 15 mg/day, which assumes a 10% absorption of dietary iron. The study by Morck et al. (33) indicates that the iron-deficient child may only absorb between 0.3 and 0.7 mg iron from 100 g of supplement, the amount recommended by WHO/UNICEF, which would not meet the daily iron requirements of 0.7 to 1.0 mg. This disparity is probably of little importance in industrialized countries where the diet contains several iron sources, but it may be a matter for concern in developing countries where fulfillment of iron requirements may be heavily dependent on blended cereals.

Ascorbic Acid Supplementation

The ability of ascorbic acid to enhance the absorption of nonheme iron has been discussed previously. In view of the low bioavailability of iron added to infant formulas and cereals, one alternative to the addition of more iron to the diet is to fortify with iron plus ascorbic acid. This approach, which is common with proprietary formulas in developed countries, has proven effective in Chile, where 80% of the infants are now fed an iron-fortified milk powder (48). In pilot studies, iron absorption averaged only 3 to 4% in iron-deficient infants who were fed 100 g low-fat (12%) milk powder containing 15 mg iron as FeSO₄. In a field trial in which milk was supplied until the age of 15 months, 12% of infants receiving the iron-fortified milk had anemia, as compared with 34.6% in the control group. Although the difference was statistically significant, anemia was still present in the group receiving iron-fortified formula. In a further pilot study it was possible to show that the addition of 100 to 200 mg ascorbic acid to 15 mg iron increased absorption by a factor of 2 to 3 and, in a second field trial using milk fortified with both iron and ascorbic acid, anemia at 15 months was reduced to less than 2%. While this
series of studies provides clear evidence of the efficacy of ascorbic acid in promoting absorption of fortification iron, the cost effectiveness of the approach still needs to be established in a national intervention program.

Further convincing evidence of the efficacy of ascorbic acid fortification was obtained recently by Derman et al. (24) from studies in iron-deficient adult subjects. Absorption was measured from an iron-enriched infant milk formula, a protein supplement, and three cereals differing mainly in the type of iron fortification (Fig. 7). The results with cereal-based foods were of particular interest. Thirty grams of cereal containing 5 to 9 mg iron were fed with or without 20 to 50 mg ascorbic acid, which was added immediately prior to serving. A highly significant enhancement in iron absorption with ascorbic acid was noted in all studies regardless of the iron fortification compound (ferric ammonium citrate, FeSO₄, or iron pyrophosphate). The relative increase in iron absorption from cereals ranged from 1.4 to 12.9% with corresponding molar ratios of ascorbate to iron of 1.1 to 3.2. These results indicate that a molar ratio of about 1.5 can cause a two- to threefold increase in iron absorption and suggest that the molar ratio of ascorbic acid to iron should be placed well above the value of 0.7 currently used for infant protein supplements. It has recently been calculated that if a molar ratio of 1.5 were used, it would increase the cost of ascorbic acid per ton from $5.85 to $8.27, which would affect the total cost of the food less than 2% per ton (32).

An important question with regard to the use of ascorbic acid is the length of time the vitamin remains active after its addition to various infant foods. This may be a particular problem in developing countries where foods are exposed to higher

![Graph](image)

**FIG. 7.** The improvement in the absorption of iron fortificants due to increasing the ratio of ascorbic acid to iron. The ratio of the amount of iron absorbed from the fortified foodstuff to the amount absorbed from a standard dose of ferrous ascorbate was used as a measure of iron absorption, since the ratio corrects results for individual variations in nutritional status (24). Circles, infant milk formula; triangles, infant cereal B; squares, infant cereal C.
and more humid temperatures. Studies under controlled laboratory conditions suggest that degradation of the vitamin may be fairly rapid and, on this basis, Bookwalter et al. (61) recently suggested the use of a more stable ethyl cellulose-coated ascorbic acid preparation. The proposal has now been accepted and will be used for the formulation of CSM and wheat-soya blend (32). The additional cost of the stabilized preparation is negligible and it may well have a wide application. However, there is clearly a need for more studies to determine the efficacy of coated ascorbic acid under prevailing conditions of preparation, distribution, and consumption of infant cereals.

**Solid Foods**

Solid foods are progressively introduced during the second 6 months of life so that the diet of children over the age of 1 year approaches that consumed by the rest of the household. While attention in the past has been directed to the iron content of different foods, it is now apparent that the percentage absorbed from different foods varies over a very wide range (1). Iron is well absorbed from meat and foods containing ascorbic acid and is poorly absorbed from cereals and legumes. In passing, it should be emphasized that not all animal foodstuffs enhance iron absorption. The iron in dairy products is much less available than that in meat (22). The relevance of individual foodstuffs lies not only in the relative bioavailability of the iron contained within them but also in their overall effects on the absorption of iron from a mixed meal. For example, meat and ascorbic acid enhance the absorption of iron from the entire meal. With data currently available it is possible to construct a model that provides an estimate of the availability of iron in different diets. These estimates are based on the amount of heme and nonheme in the meal and on the content of both animal tissue (meat, poultry, or fish) and ascorbic acid. On the basis of these variables, meals are then classified as being of low, medium, or high iron bioavailability (Fig. 8). A meal of low bioavailability contains less

![Graph](image)

**FIG. 8.** The percentage absorption of nonheme iron by individuals with no body iron stores from three different types of diet (62).
than 30 g animal tissue and less than 25 mg ascorbic acid. A meal of medium bioavailability contains 30 to 90 g meat, poultry, or fish or 25 to 75 mg ascorbic acid. A meal is considered to be of high bioavailability if the figures for either of these components are greater than this. The model is obviously an oversimplification since it ignores the possible enhancing effects of dietary constituents such as organic acids, and also the many inhibitors that can be present in mixed diets. Nevertheless, it provides a more rational basis for determining the adequacy of dietary iron intake than do traditional calculations that are based solely on total iron content of the diet. In addition, it suggests strategies by which iron nutrition in later infancy might be improved. For example, the addition of orange juice to a meal is sufficient to double the amount of iron absorbed from the entire meal (63).

**SUMMARY AND CONCLUSIONS**

Much of our current knowledge concerning iron nutrition is based on radioisotopic studies. It has been established that heme iron in food is well absorbed regardless of the composition of the diet. Nonheme food iron is generally less bioavailable. During digestion most of the nonheme iron enters a common intraluminal pool so that its bioavailability is determined by the relative concentrations of the various enhancing and inhibiting ligands present in the meal. The dietary factors that actively promote nonheme iron absorption include certain animal tissues including meat, poultry, and fish, and a number of organic acids, the most important of which is ascorbic acid. Milk and cheese are relatively “neutral” in terms of their effects on iron absorption since they exert neither a strong enhancing nor inhibiting effect. Cereals and legumes are poor sources of bioavailable iron, presumably due to the presence within them of variable amounts of tannins, phytates, and bran. However, the inhibitory effects of these can be overcome, in part at least, if cereals and legumes are fed together with adequate amounts of animal tissue or ascorbic acid.

These various points have special relevance to iron nutrition in infancy because milk and cereals represent staple foodstuffs at critical stages of development. There are three overlapping periods in infancy. During the early part of infancy when the infant’s needs are lowest, dietary iron is largely derived from milk and milk products. Weaning or transitional foods, which are mostly processed cereals, supplement and then gradually replace milk in the diet during a period when requirements are high. Solid foods, including meat, fruit, and vegetables, are introduced in the latter part of infancy and the diet progressively approaches that of the rest of the family.

Although the reasons are not clear, there is good evidence that the iron in breast milk is significantly better absorbed than is the iron in cow’s milk. However, fortified cow’s milk is an adequate source of iron in that it does not exert a significant inhibitory effect on iron absorption. Although the overall absorption rate from infant cereals is low, the relatively large amounts of fortification iron they currently contain should be able to meet the needs for dietary iron of healthy
infants. In circumstances where iron requirements are greater, it is possible to increase iron bioavailability in cereals by raising the level of ascorbic acid fortification. Concern about the low bioavailability of the iron in soya-containing protein supplements, which are widely used as weaning foods in developing countries, has led to the recommendation that the molar ratio of ascorbic acid to iron fortification be increased from 0.7 to 1.5 and that the more stable preparation ethyl cellulose-coated ascorbic acid be used.

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Discussion

Dr. Hurrell: Could you give some details of the in vitro system that you have just discussed.

Dr. Cook: This is a system that involves adding extrinsic radioiron to a homogenized meal, incubating at pH less than 2 in the presence of pepsin, readjusting the pH to about 5, and finally determining the proportion of radioiron remaining in the supernatant after centrifugation. There are actually several in vitro methods of this type described in the recent literature and I believe they all give fairly similar results.

Dr. Chandra: Two questions: firstly, what was the impact of the content of other minerals and trace elements within different foods on availability of iron?

Dr. Cook: This is an important question and I have no information whatsoever about it.

Dr. Chandra: Secondly, have you considered the amount of different cereals eaten in an average meal as being a determinant of the total amount of iron absorbed?

Dr. Cook: This hasn't been taken into account in the data that I showed to you.

Dr. Chandra: You showed the results as percentage absorption of iron rather than the total amount of iron taken in an average meal.

Dr. Cook: We have not taken into account the amount of iron that would be ingested in the average meal. All these studies were done with isolated food sources, such as wheat alone or rice alone, rather than with a complete meal.
Dr. Stekel: Regarding the effect of milk on iron absorption, I think this is just a matter of opinion and we could be discussing forever what it is; my opinion is that cow's milk is a relatively poor iron source. One reason for this opinion is that, when one compares it with human milk, cow's milk iron has a much lower absorption. A second consideration, which is of very practical importance, is what is the absorption of iron from different foods in relation to their caloric content. I think that in this respect Dr. Hallberg's concept of bioavailable nutrient density is a very useful one because it relates the bioavailability of your nutrient, in this case iron, to the total caloric intake. Infants may be fed only cow's milk for long periods of time which will fulfill their total caloric needs but not their iron needs, thus I would consider cow's milk a poor iron source. Also, the effect of milk on nonheme iron absorption is marked. Studies in our laboratory indicate that ferrous sulphate in 6- to 18-month-old infants is absorbed about 35 to 40% when given in water, but is absorbed only about 3 to 4% when given with milk. I would consider this a marked inhibitory effect.

Dr. Cook: The effect you describe seems more pronounced than that reported in other published studies. In the studies which I reviewed in our manuscript, the difference in absorption between water and milk was on the order of two- to threefold, not as dramatic as in the studies you describe.

Dr. Hallberg: In our experience the effect of milk on nonheme iron absorption depends on the experimental conditions used. In a meal composed only of wheat bread we observed a reduction of the iron absorption when serving milk instead of water. However, with a hamburger meal the nonheme iron absorption was the same when the drink was water or milk.

Dr. Cook: There is an inhibitory effect on iron absorption of adding almost any food to water.

Dr. Florentino: Would you comment on the relative effects of dietary ascorbic acid compared to supplementary ascorbic acid.

Dr. Cook: I know of no data to indicate any difference in the enhancing effect of dietary ascorbic acid as opposed to ascorbic acid supplement, assuming that the ascorbic acid in the food source has not been destroyed by heating or during preparation of the food.

Dr. Hallberg: Our studies indicate that the absorption-promoting effect of ascorbic acid is the same when the same amount is given in pure chemical form or, for example, as orange juice, as a mixed vegetable salad, as cabbage, as broccoli, or as papaya.

Dr. Fomon: Could we have some discussion of availability of contamination iron? In most instances in developing countries, the iron intake of a breast-fed infant will be appreciably greater from dirt than from human milk.

Dr. Hallberg: Iron in dirt is partially bioavailable for humans. We have not made any extensive studies but the results we have so far indicate that in a few samples of clay, iron has a relative bioavailability of around 30 to 40%. In a few other soil samples that we have studied, the bioavailability is much lower; the iron in the abyssinian red soil found on teff had a relative bioavailability of about 2%. Iron in some other red soils was not available at all. Considering the high iron content in many soils, however, the intake of soil iron must be considered in human nutrition, especially in the diets in developing countries where the intake of soil iron can be quite considerable.

Dr. Cook: The inhibitory effect of soya on iron absorption is presumably of little importance in relation to soya formulas because of the large amounts of iron that are commonly added. There is nothing in the literature to suggest a difference in the effect of fortification iron added to soya formulas as opposed to a milk-based formula. We have studied the question of soya in relation to an infant food such as corn-soya milk containing corn, soya, and maize. When we deleted each of these components serially, we found no effect when the maize or milk was deleted but about a twofold increase absorption when soya was eliminated. I don't consider this a dramatic effect and would suggest that the inhibitory effect of soya is of little consequence in regard to iron-fortified formulas.
Dr. Stekel: Are there any data on the iron status of infants who have been fed soya formulas?

Dr. Fomon: Sales of soya protein isolate formulas account for about 20% of total sales of infant formulas in the United States. However, some of the soya formulas are consumed by children beyond infancy or by adults and the best estimate is that about 15% of formula-fed infants receive soya protein isolate formulas. These formulas are fortified with 1.8 mg iron/100 kcal and include, in addition, substantial amounts of native iron. Iron nutritional status of infants fed such formulas appears to be adequate, but there has been no specific, well-designed, large-scale study.

Dr. Hallberg: It should be remembered that when soya flour is added to a meal there may be a significant increase in the iron content of the meal as soya flour products have a high iron content. In a recent study we compared the effect of adding soya flour to a simple Latin American-type meal composed of black beans, maize, and rice with the addition of the same amount of protein as meat and the same amount of iron as ferrous sulphate. We reached about the same level of amount of iron absorbed. The addition of soya protein to a simple diet to improve the protein nutrition will thus also improve the iron nutrition.

Dr. Guirriec: Textured vegetable protein is being promoted as a meat substitute; I should like to know what is its iron availability?

Dr. Hallberg: The effect of soya protein on nonheme iron absorption depends on the basis of comparison. When soya is added as a meat extender (to make a bigger hamburger), the amount of iron absorbed is increased as there is a considerable increase in the amount of iron ingested due to the high iron content of soya protein products. The percentage of iron absorbed, however, is somewhat decreased for unknown reasons. When soya is used as a substitute for meat, thus replacing a certain part of the meat, there is a decrease in the total amount of iron absorbed, due to a reduction in the heme iron content of the hamburger and a reduction of the enhancing effect of meat on nonheme iron absorption. This has been explained in greater details in the INACG report "The effect of cereals and legumes on iron availability," 1982 [see the chapter by Hurrell, ref. 41, this volume].