Bioavailability of Different Iron Compounds Used to Fortify Formulas and Cereals: Technological Problems

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BIOLOGICAL AVAILABILITY OF IRON COMPONDS USED TO FORTIFY INFANT FOODS

Bioavailability of a single iron source is difficult to predict. It can vary considerably due to the enhancing or inhibitory effects of other food components on iron absorption and, especially for the less-available sources, it is strongly influenced by the physical characteristics of the iron compound itself. It is sometimes difficult for the food manufacturer or food scientist to interpret the many apparent contradictions in the literature, further complicated by the different methodologies used by different research groups and the difficulties in extrapolating from rats to humans.

Table 1 shows an advisory list of iron sources that may be used in foods for infants and young children (1). It includes the most frequent uses of these salts and an estimate of their relative bioavailability when compared to ferrous sulphate in both rats and humans. In rat studies, iron compounds have been compared to ferrous sulphate by evaluating the regeneration of hemoglobin in iron-depleted rats. In human studies, differences in iron absorption in iron-replete healthy men have been measured using radioactive isotopes. It should be remembered that the studies of Brise and Hallberg (6), which were used to calculate most of the reported values, were made by giving fasting subjects iron sources containing 30 mg elemental iron. The relative properties of different iron compounds may change when given with food. Nevertheless, it is readily noticeable from the information available that both rat and human assays give the same ranking to the bioavailability of the different salts and that, in general, iron sources added to infant formulas are more highly available than those commonly added to infant cereals.

Iron Sources Added to Formula

Ferrous sulphate is by far the most commonly added iron source to both liquid and powder formulas although ferrous ammonium citrate, ferrous citrate, ferrous gluconate, and ferrous lactate may also be added. For liquid soya-based formulas, ferric citrate and ferric gluconate are suggested by Codex (1). The relative bio-
TABLE 1. Codex advisory list (1) of iron sources that may be used for infants and young children: their common use and average bioavailability

<table>
<thead>
<tr>
<th>Source</th>
<th>Common use</th>
<th>Average bioavailability relative to ferrous sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rat\textsuperscript{a}</td>
</tr>
<tr>
<td>Ferrous carbonate stabilized</td>
<td>Liquid formula</td>
<td>4</td>
</tr>
<tr>
<td>Ferrous citrate</td>
<td>Liquid formula</td>
<td>76</td>
</tr>
<tr>
<td>Ferrous fumarate</td>
<td>Liquid and powder formula</td>
<td>95</td>
</tr>
<tr>
<td>Ferrous gluconate</td>
<td></td>
<td>97</td>
</tr>
<tr>
<td>Ferrous lactate</td>
<td>Liquid formula</td>
<td>—</td>
</tr>
<tr>
<td>Ferrous succinate</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>Liquid formula</td>
<td>107</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>Liquid soya formula</td>
<td>73</td>
</tr>
<tr>
<td>Ferric gluconate</td>
<td>Liquid soya formula</td>
<td>—</td>
</tr>
<tr>
<td>Sodium iron pyrophosphate</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Elemental iron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-reduced</td>
<td>Cereals</td>
<td>8–76</td>
</tr>
<tr>
<td>Electrolytic</td>
<td>Cereals</td>
<td></td>
</tr>
<tr>
<td>Carbonyl</td>
<td>Cereals</td>
<td></td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>Cereals</td>
<td>45</td>
</tr>
<tr>
<td>Ferric orthophosphate\textsuperscript{c}</td>
<td>Cereals</td>
<td>7–32</td>
</tr>
<tr>
<td>Ferric oxide saccharated\textsuperscript{c}</td>
<td>Cereals</td>
<td>—</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Hemoglobin repletion test from Fritz et al. (2) and Fritz and Pla (3).
\textsuperscript{b}Absorption measured using radioiron isotopes (6).
\textsuperscript{c}Not on Codex list but commonly used.
\textsuperscript{d}Absorption from bread rolls measured using radioiron isotopes (5).
\textsuperscript{e}Absorption measured using radioiron isotopes (4).

Availability of all these salts is relatively high, at least when fed alone, with the exception of ferric citrate, which is 31% as well absorbed as ferrous sulphate (6), and ferric gluconate, for which there are no published data.

Ferrous sulphate can be obtained in two forms, the heptahydrate and the monohydrate, known as dried ferrous sulphate. The reagent-grade heptahydrate (FeSO\textsubscript{4}·7H\textsubscript{2}O) has become the standard for comparison in almost all iron availability studies performed today. Its absorption by humans, however, was found to vary from less than 1 to over 50% of the initial dose (6), depending on the subject and not including other interferences due to diet composition or level of added compound. Most ferrous sulphate used in foods is the purified and dried by-product of sulphuric acid cleaning baths used in steel production (7). Despite reports by Lee and Clydesdale (7) that dried ferrous sulphate is the more commonly used form and that the heptahydrate is often unsuitable because it is difficult to grind finely and because its loosely bound water migrates into dry foods, the heptahydrate is commonly added to both powder and liquid formula.
Rios et al. (8) measured the absorption of radioactively labeled ferrous sulphate added to milk-based and soya-based formulas. The formulas contained 12 to 17 mg of added Fe per liter and were fed to 42 apparently healthy infants between 4 and 7 months old. Mean iron absorptions of 3.9% (range 0.7–23.1%) for the milk-based formula and 5.4% (range 1.0–21.9%) for the soya-based formula were recorded. It was concluded that the iron supplement in these products could essentially meet the iron needs of healthy infants (0.5 mg Fe/day at 4–6 months).

There is much less information concerning the other salts. Ferrous fumarate appears to be highly available in both humans and rats and has been used to fortify corn-soya-milk preparations used by USDA in overseas food assistance programs (7). Ferrous gluconate and ferrous lactate are also highly available and appear to maintain their high availability in thermally processed milk and soya-based infant formulas fed to anemic rats (9,10). The use of ferric ammonium citrate, which is produced as a brown or green-colored powder, is minor. When fed to anemic rats, it was equally as well used as ferrous sulphate. Bothwell et al. (11) recommend the use of ferric ammonium citrate in infant foods and they describe its bioavailability in humans as good.

The absorption of iron from fortified infant formulas has been found by several authors (8,12,13) to be in the range of 3 to 10% of the administered dose. This has been reported to be very low when compared to the 49 to 70% absorption of breast-milk iron administered to infants (13,14) or the 15 to 20% of breast-milk iron fed to adults (12,15). Saarinen and Siimes (13) measured iron absorption by changes in the calculated total body iron of infants fed breast milk, cow's milk, or an iron-supplemented infant formula containing 11 mg Fe/liter in the form of ferrous gluconate. They estimated iron absorption from breast milk to be 70% of the administered dose as compared to 30% for cow's milk iron and 10% for supplemented iron in infant formulas. McMillan et al. (12) measured absorption of iron from breast milk and iron-supplemented formulas in adults using the extrinsic tag technique. They reported a 15% absorption from breast milk as compared to 3% from infant formulas. The comparison of the percentage absorption, however, is misleading since breast milk contained 0.66 mg/liter of iron compared to 12.5 mg/liter for the iron-fortified formula. It is well known that as the amount of iron fed as a soluble salt is increased, the percentage absorption decreases (16), although the absolute amount absorbed increases. In fact, when they fed a formula containing almost the same level of iron as in breast milk (0.7 mg/liter), they reported the percentage absorbed as 9%, slightly more than half that of breast-milk iron. In absolute terms, however, there was almost four times as much iron absorbed from the fortified formula as from breast milk.

Iron absorption from breast milk appears to be considerably more efficient than that from cow's milk or infant formulas. The reason for this high bioavailability is unknown (17). It does not appear to be due to lactoferrin (12,18), but could possibly be due to chelating agents keeping the iron soluble during digestion. Ascorbic acid,
cysteine, inosine, and taurine are generally higher in human milk than in cow’s milk or formulas and have been suggested by McMillan et al. (12) as possible enhancers of absorption. Cow’s milk is also higher in phosphate, which is known to decrease absorption.

The influence of milk components on iron availability is not clear. Milk has been found to decrease iron absorption from meals with a low iron bioavailability (17). Abernathy et al. (19) reported that iron absorption was significantly increased in 7- to 9-year-old girls by removal of milk from the diet. However, in a recent study of a hamburger meal, no decrease in iron absorption was seen when water was exchanged for milk (20). In rat studies, Ranhotra et al. (21), using the hemoglobin repletion test, also found that milk and milk components did not adversely affect the bioavailability of added iron, although Amine and Hegsted (22) reported that the ferrous sulphate added to infant formulas was around 30% less well utilized than ferrous sulphate alone.

Iron Sources Added to Cereals

With cereals the food manufacturer is caught in a difficult situation. Those iron compounds that exhibit the best availability reduce product quality and shelf-life. The use of the less offensive iron sources may satisfy label claims and regulations, but there is a question concerning their bioavailability. The sources that are commonly used are elemental iron, ferric pyrophosphate, and ferric orthophosphate. Saccharated ferric oxide is occasionally used. Sodium iron pyrophosphate is no longer commonly used for fortifying infant cereal. All these sources have shown a mediocre to low relative bioavailability in both rat and human assays when compared to ferrous sulphate. The bioavailability of elemental iron and orthophosphate varies greatly, depending on the physical characteristics of the product.

Elemental Iron

This is perhaps the most well-researched iron source because of its common use in infant cereal fortification in the United States and its potential use in flour enrichment. As can be seen from Table 1, there are considerable variations in the relative bioavailability of elemental iron powders. There are three basic types of elemental iron, depending on the method of manufacture: (a) reduction under hydrogen or carbon monoxide, (b) electrolytic deposition, and (c) the carbonyl process (7). Reduced iron is produced by reduction of ground iron oxide by either hydrogen or carbon monoxide. Electrolytic iron is produced by electrolytic deposition of ingot ore onto stainless steel cathode sheets. Carbonyl iron is produced by heating scrap metal with pressurized carbon monoxide to yield iron pentacarbonyl, which is then decomposed to yield iron powder. This iron powder is further reduced under hydrogen, yielding carbonyl iron, which is extremely fine (spherical particles, mean particle size about 3 μm). Reduced iron is about half the price of electrolytic or carbonyl iron but double the price of ferrous sulphate (11).
There has been considerable research into the variation of bioavailability of elemental iron with particle size, solubility in dilute acid, and reactive surface area (4,23–28). It would appear that for each iron type, bioavailability is increased with an increased solubility and a smaller particle size. Solubility in dilute HCl seems to be the best in vitro method of predicting bioavailability and this is governed more by reactive surface area than by particle size (4).

Shah et al. (28) measured the relative biological value (RBV) of eight commercial iron powders in anemic rats. The properties of four of these powders are shown in Table 2 where RBV is compared with solubility in 0.2% HCl at 37°C and particle size. The particle size distributions by number of the different types are shown in Fig. 1. These show a similar pattern to the particle size distribution values by weight given by Cocodrilli et al. (23) and Lee and Clydesdale (7) for different

### TABLE 2. Physical characteristics and relative biological value (RBV) of four commercial iron powders fed to anemic rats

<table>
<thead>
<tr>
<th>Iron source</th>
<th>Mean particle size (µm)</th>
<th>% Solubility (0.2% HCl, 37°C, 90 min)</th>
<th>RBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonyl</td>
<td>4</td>
<td>98</td>
<td>61</td>
</tr>
<tr>
<td>Electrolytic</td>
<td>8</td>
<td>91</td>
<td>32</td>
</tr>
<tr>
<td>H-reduced</td>
<td>21</td>
<td>57</td>
<td>18</td>
</tr>
<tr>
<td>CO-reduced</td>
<td>28</td>
<td>49</td>
<td>12</td>
</tr>
</tbody>
</table>

Adapted from Shah et al. (28).

![FIG. 1. Particle size distribution of different iron sources (28).](image)
commercial powders. Shah et al. (28) found that carbonyl iron had the smallest particle size, the highest solubility, and the highest bioavailability (RBV = 61). They found in general that RBV increased with increasing solubility and smaller particle size and they ranked the iron powders in the order of carbonyl, electrolytic, H-reduced, and CO-reduced, the latter having an RBV of only 12 and a mean particle size of 28 μm.

A similar relationship between particle size and RBV was shown by Fritz (29) (Table 3). He separated the different sized particles and found that, for each iron type, the finer the particle size the greater the relative bioavailability in rats. The same sized particles produced electrolytically, however, gave almost twice the RBV as those produced by CO reduction. Pennell et al. (26) similarly found in rats and in humans that electrolytic iron particles of 7 to 10 μm and 20 to 30 μm had about 1.5 and 2 times, respectively, the RBV of the same sized particles of reduced iron. According to Lee and Clydesdale (7), the electrolytic procedure produces unique fern-like crystals with a larger surface area than the irregular porous-reduced iron crystal, which presumably makes it more soluble and thus more available.

Rather surprisingly, however, Bjorn-Rasmussen et al. (4) showed commercial electrolytic iron, presumably from European suppliers, to have a low reactive surface area. The few samples they investigated, however, had no resemblance in terms of particle size distribution to the electrolytic iron described by American authors. Mean particle sizes were 3, 5, 35, and 42 μm. Using specially produced H-reduced powders, these workers showed that bioavailability in humans (13–90% of ferrous sulphate) also correlated well with in vitro solubility in dilute HCl and reactive surface area.

**TABLE 3. Influence of particle size on bioavailability of reduced iron**

<table>
<thead>
<tr>
<th>Production method</th>
<th>Particle size (μm)</th>
<th>RBV^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrolytic 1</td>
<td>7–10</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>27–40</td>
<td>38</td>
</tr>
<tr>
<td>Electrolytic 2</td>
<td>0–10</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>20–40</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>&gt;40</td>
<td>45</td>
</tr>
<tr>
<td>H reduction</td>
<td>10–20</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>&lt;40</td>
<td>34</td>
</tr>
<tr>
<td>CO reduction</td>
<td>7–10</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>14–19</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>27–40</td>
<td>13</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>&lt;4</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>4–8</td>
<td>64</td>
</tr>
</tbody>
</table>

^aHemoglobin repletion tests with rats; RBV of ferrous sulphate = 100.
Adapted from Fritz (29).
Clearly there is a wide variation in commercially available iron powders. It should be possible, however, for the manufacturer to screen different samples for solubility, particle size, and reactive surface area. Carbonyl iron would appear to be most highly available; however, it appears that, like other highly soluble iron sources, it also causes rapid organoleptic deterioration of the product. Another problem is that finely powdered iron is pyrophoric, burning to incandescence when exposed to air (7). In cereal test systems, we have found that electrolytic iron (Glidden A-131) similar to that reported by Shah et al. (28) has caused no organoleptic problems.

Shah et al. (28) have recommended specifications for the quality of elemental iron powders used by food manufacturers: (a) the iron content of the powder should be not less than 96%; (b) 95% of the particles should be less than 40 μm; and (c) at least 90% by weight of the powder should be soluble in 0.2% HCl after 90 min at 37°C. For their solubility test, they simulated gastric digestion. They took 100 mg iron powder in 250 ml 0.2% HCl (pH about 1.2) at 37°C and shook for 90 min on an orbital shaker. Other workers have used very different methods of measuring solubility (4,23,27). These methods differ in the quantity of powder taken, the concentration and volume of HCl, the temperature and time of shaking, and the method of shaking. Although all methods may rank the solubility of iron sources in a similar way, some standardization is required before a meaningful comparison with bioavailability can be made.

**Ferric Orthophosphate**

The material used in foods is light in color and is hydrated with two or four molecules of water. Like elemental iron, its bioavailability appears to vary considerably from batch to batch. Fritz et al. (2) evaluated four samples in the hemoglobin repletion test with rats and reported RBVs from 7 to 32 (Table 1). Harrison et al. (30) similarly found that the RBVs of five commercial samples of ferric orthophosphate varied from 6 to 46 (Table 4) and they also demonstrated that it was strongly influenced by particle size and solubility in 0.1 N HCl. Shah et al. (31) found the RBVs of ferric orthophosphate added to cereals varied from 33 to 60.

![Table 4](image)

**TABLE 4. Physical characteristics and relative biological value of five commercial samples of ferric orthophosphate**

<table>
<thead>
<tr>
<th>Particle size (μm)</th>
<th>% Solubility (0.1 N HCl, 3 hr)</th>
<th>RBV (Ferrous sulphate = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>11.6</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td>11.6</td>
<td>7</td>
</tr>
<tr>
<td>&lt;1</td>
<td>41.9</td>
<td>33</td>
</tr>
<tr>
<td>&lt;1</td>
<td>45.5</td>
<td>33</td>
</tr>
<tr>
<td>&lt;1</td>
<td>63.4</td>
<td>46</td>
</tr>
</tbody>
</table>

Modified from Harrison et al. (30).
Hallberg (17) reported that the bioavailability of ferric orthophosphate in humans also varied considerably between different commercial preparations.

**Ferric Pyrophosphate**

This white powder, although widely used in Europe to fortify infant cereals, is less utilized in the United States and consequently has been less well investigated. It may be added as the ferric pyrophosphate alone; as a coprecipitate of ferric pyrophosphate and ferric citrate, containing about half citrate by weight; or as a coprecipitate of ferric pyrophosphate (1 mole) and ammonium citrate (2 moles). It has been called a mediocre source of available iron and had an RBV of 45 in the studies of Fritz et al. (2). We could not find an RBV on the coprecipitates, although Pla et al. (27) have reported an RBV of 96 for stabilized ferric pyrophosphate, which they described as ferric pyrophosphate solubilized with sodium citrate.

**Sodium Iron Pyrophosphate**

This salt has shown a somewhat variable but always low relative availability in both rats (RBV 2–22) and humans, and it would seem unwise to use it for infant food fortification. Shah et al. (31) found the RBV of sodium iron pyrophosphate added to cereals to vary from 14 to 40.

**Saccharated Ferric Oxide**

This compound comes as an amorphous brownish red powder containing from 3 to 10% iron. It is very soluble in water and an aqueous solution has been used in intravenous injection to treat anemic patients (32). It is made from ferrous chloride, sodium carbonate, sucrose, and sodium hydroxide. It has no fixed formula but it is reported to be a mixture of ferric oxide and saccharose. It is used only to a limited extent and there is little information on its bioavailability, although because it is highly soluble it should theoretically have a fairly good availability. When this compound was fed to anemic rats, we obtained an RBV of 90.

**Bioavailability for Infants**

Rios et al. (8) added radiolabeled sodium iron pyrophosphate, ferric orthophosphate, hydrogen-reduced elemental iron, and ferrous sulphate at the level of 50 mg Fe/100 g to mixed grain infant cereals. Iron absorption was measured in infants 4 to 6 months of age. Rios et al. (8) showed that sodium iron pyrophosphate and ferric orthophosphate were poorly absorbed (<1.0%), and that reduced iron and ferrous sulphate were 4.0% and 2.7% absorbed, respectively. The sodium iron pyrophosphate and the ferric orthophosphate were of similar particle size and solubility as would be used in industrial fortification. These authors concluded that these materials were not dependable sources of iron to meet the nutritional needs of infants. The absorption of sodium iron pyrophosphate and ferric orthophosphate relative to ferrous sulphate was 37 and 26%, respectively, and was similar to that
predicted by rat assays. Reduced iron, on the other hand, had 148% the absorption of ferrous sulphate. This was a hydrogen-reduced product of a very small particle size containing 95% of the particles in the 5- to 10-μm range. This is similar to carbonyl iron but much smaller than other sources of elemental iron currently available. Electrolytic iron A-131 from Glidden (Durkee Industrial Foods) has 40% by weight of the particles less than 10 μm; an electrolytic iron used by Shah et al. (28) had 63% of the particles by number less than 10 μm. It is evidently possible, therefore, to have an elemental iron source of the same bioavailability as ferrous sulphate. This has been confirmed by Cook et al. (5) and Björn-Rasmussen et al. (4); however, these powders are apparently unsuitable for addition to cereal products because they lead to the same discoloration and reduced shelf-life as reported for the soluble iron salts (5). This remains to be confirmed.

Cook et al. (5) baked the same iron sources as used by Rios et al. (8) into dinner rolls and measured their bioavailability in humans as compared to ferrous sulphate. The results of the two studies were similar. Cook et al. (5) found mean absorption ratios relative to ferrous sulphate of 5% for sodium iron pyrophosphate (mean absorption 0.3%), 31% for ferric orthophosphate (mean absorption 1.1%), and 95% for hydrogen-reduced iron (mean absorption 8.6%). The absorption of ferrous sulphate varied widely from 0.9 to 48.9% of the administered dose and the mean absorption, 3.9, 6.6, and 9.1% in the different assays, was roughly one-quarter of that found when feeding ferrous sulphate as a solution of inorganic iron.

**General Conclusion**

It would appear that commercial sources of elemental iron, ferric orthophosphate, and ferric pyrophosphate vary considerably in their bioavailability. In order to assist the food manufacturer in the control of supplies, there is a need for more information on the relationship between a standardized *in vitro* solubility test and particle size with relative biological availability in rats and humans for all three iron sources. However, considering current information, even with careful control, it is doubtful whether food manufacturers can find a suitable source for addition to infant cereals which has more than 30 to 50% the bioavailability of ferrous sulphate. Elemental iron powders, both hydrogen-reduced and carbonyl, have been reported with higher bioavailability but they have also been reported to cause organoleptic problems. Much information is still lacking on the bioavailability in humans of saccharated iron oxide and of the different forms of iron pyrophosphate. Fortunately, there are other ways of improving the bioavailability of iron in cereals which appear promising. Firstly, there is encapsulation of a highly available iron source such as ferrous sulphate to isolate it from the food environment. This should prevent fat oxidation problems while hopefully still maintaining high bioavailability. Secondly, and maybe the simplest approach, is to add an absorption enhancer such as ascorbic acid along with a mediocre iron source. Ascorbic acid, by way of its reducing and chelating properties, can significantly increase iron absorption (33).
FACTORS INFLUENCING IRON ABSORPTION FROM INFANT FOOD

Chemistry of Iron

This subject has been extensively reviewed by Forth and Rummel (34), Spiro and Saltman (35), and Lee and Clydesdale (7). To understand the behavior of iron in foods and during digestion, and the influence of various factors on its bioavailability, however, one must have some knowledge of its chemical properties.

Oxidation States

Iron has several oxidation states ranging from Fe$^{6+}$ to Fe$^{2-}$, depending on its chemical environment. Ferrous (Fe$^{2+}$) and ferric (Fe$^{3+}$) are the only states that occur naturally in foods since they are the only ones that are stable in an aqueous environment. Elemental iron is rarely found in biological systems but it does occur as a common food additive. Ferrous iron is rapidly oxidized to ferric iron in the presence of oxygen. Alternatively, ferrous iron may be formed by the reduction of ferric iron or the oxidation of elemental iron, i.e., Fe$^{3+} + e^- \rightleftharpoons Fe^{2+}$; Fe$^{2+} + 2e^- \rightleftharpoons Fe^0$.

Solubility of Ferrous and Ferric Iron

Ferrous iron is far more soluble than ferric iron, especially as the pH of the system is increased. In acidic aqueous solutions, ferrous and ferric iron do not occur in the free state but are hydrated as Fe(H$_2$O)$_6^{3+}$ and Fe(H$_2$O)$_6^{2+}$. As the pH is raised, they split off protons, in a process known as hydrolysis, to form the corresponding iron hydroxides: Fe(OH)$_2$ and Fe(OH)$_3$. These hydroxides become more insoluble with increasing pH and tend to be irreversible. Spiro and Saltman (35) indicate that the ferrous ion has a solubility of about $10^{-11}$ M at pH 7 and $10^{-3}$ M at pH 8, whereas the ferric ion has a solubility of about $10^{-3}$ M at pH 2 and only $10^{-18}$ M at pH 7. This is of considerable nutritional importance since, although both ferrous and ferric ions are readily soluble in the acid conditions of the stomach (about pH 2), the ferric ion is not soluble in the slightly alkaline conditions of the small intestine where the iron absorption takes place.

Formation of Complexes

In general, a metal ion can react with more than one ligand. The number of possible bonds with the ligands depends on the coordination number of the metal, which in the case of iron is six. If six ligands form one bond each with one iron atom, it would be a monodentate complex. Complexes formed by the participation of two or more ligand atoms from the same complexing agent are called chelates. Thus three ligands each forming two bonds would give a bidentate chelate and two ligands each forming three bonds would give a tridentate chelate and so on. Different ligands may also react with the same metal atom to form mixed complexes.
During digestion, the stability of the iron-ligand complex is governed by its thermodynamic and kinetic stability constants and by interfering reactions with other ligands and other metals. In general, the stability of the metal-ligand complex increases with the concentration of the ligand. Chelates are the most stable complexes and multidentate chelate has a much greater stability than a complex formed from several monodentate ligands.

Figure 2 summarizes the physicochemical factors that influence iron availability. Solubility of the iron source is extremely important; as some fortification iron sources are insoluble in gastric juice, they never enter the common iron pool. Most iron, however, is released from the iron complexes in the food by the acid pH of the stomach and enters the common iron pool. Ferric iron is reduced to ferrous iron (75–98%), depending on the presence of reducing agents (34). It is at this stage that reducing agents, such as ascorbic acid, would appear to have a big influence on iron availability. The elemental iron which dissolves in the gastric juice is transformed into ferrous iron.

As the common pool of ferrous and ferric iron plus the ligands leave the stomach and enter the intestine, there is a rise in pH to between 7 and 8. This favors a reformation of the complexes and there is competition between the different ligands

**FIG. 2. Physicochemical factors influencing the absorption of food iron.***
to complex iron. Bioavailability of iron is then determined by the solubility of the 
complexes which form and the strength of the affinity of the ligand for iron. If it 
is too strong, iron will not be released for absorption. In general, ferric iron binds 
with ligands more strongly than ferrous iron.

There are three competing phenomena in the intestine. The influence of hydrox-
ide itself can precipitate the ferric iron. It can also dissociate the ligands, and 
which ligand then combines with iron depends both on its concentration and the 
magnitude of its affinity for iron. Absorption inhibitors are those ligands that 
chelate iron to form insoluble complexes or complexes of very high affinity so that 
iron is not released from the chelate for absorption. Examples are phosphates, 
xylate, dietary fiber, tannins, and phytate.

Absorption enhancers are those ligands that form soluble chelates with iron 
(especially Fe$^{3+}$) and prevent its precipitation. Iron is released from the chelates 
for absorption. Amino acids, citrate, and ascorbate are in this category. It should 
be added that any ligand that forms stable chelates at the more acid pH of the 
stomach—ascorbic acid, lactoferrin, and ethylenediaminetetraacetic acid (EDTA) 
are possible examples—would complex iron before competing with other ligands 
in the intestine.

Inhibitors of Absorption

Hallberg (17) has reviewed the major inhibitors of nonheme iron absorption. The 
two major components of infant foods that contain inhibitors are cereals and soya 
products. The latter are often mixed with cereals as a weaning food or used to 
replace the milk protein in formulas. The exact nature of the inhibition is still 
unclear, although phytate, phosphates, and dietary fiber have been suggested for 
cereals. A less important inhibitor could be polyphenolic substances such as the 
tannins of chocolate or sorghum.

Cereals

The iron from cereal products is poorly available for absorption by humans. 
Various inhibitors have been suggested, including phytate and dietary fiber. Data 
concerning the role of phytate are confusing, with some studies finding an inhibitory 
effect and others not. According to Rotruck and Luhrsen (36), all studies which 
have shown an inhibitory effect have used purified sodium phytate, whereas those 
studies which have failed to show an effect have investigated naturally occurring 
phytate. The synthetic compound should more easily ionize and more readily form 
insoluble complexes with iron from the common pool.

The inhibitory effect of wheat bran on iron absorption was thought to be due to 
phytate; however, bran has been reported to maintain its inhibition of iron absorp-
tion even after its phytate had been destroyed (37), although the phosphate was 
not removed. In addition, monoferric phytate, which is the major form of iron in 
wheat bran, has been shown to be highly available to rats (38), dogs (39), and 
humans (37). It would now seem that the influence of bran or cereals on iron
absorption is at least partly due to its fiber component (40). Absorption studies with various constituents of dietary fiber, including pectin and cellulose, have yielded negative results, however (41).

One recent finding is that the magnitude of the inhibitory effect is different for different cereals (41). Iron absorption is highest from wheat, intermediate from rice, and lowest from maize. These differences appear to be due in part to the influences of the different starches and to the different levels of phytate.

Soya

Many reports on the bioavailability of iron from soya products have appeared in literature. Most studies with iron-deficient rats or iron-deficient humans have demonstrated a reasonably good bioavailability. Recent studies (42,43), however, using the extrinsic tag method with iron-replete adults, differ considerably from the earlier results and indicate that soya products may be a strong inhibitor of iron absorption.

Cook et al. (42) reported that when egg albumin in a semisynthetic meal was replaced with full fat soya flour, texturized soya flour, or soya isolate, the iron absorption fell from 5.5 to 1.0, 1.9, and 0.4%, respectively. The iron of the same soya isolate was found to have 59% of the availability of ferrous sulphate when fed to anemic rats (44).

Morck et al. (43) measured the iron absorption from an infant food supplement containing a mixture of soya with either wheat or maize. Forty-five to 74% of the protein content of the supplements was from soya protein. They were fortified with ferrous fumarate at the level of 15.6 mg/100 g and the level of ascorbic acid relative to iron was approximately 2:1 when fed. In iron-replete males, the iron absorption was only 0.6 to 1.4% which, when related to the reference dose absorption, was calculated to represent an absorption of 1.7 to 4.1% for iron-deficient infants. It was concluded that if these supplements were fed as recommended, they would supply only half of the daily requirements of iron-deficient children between 6 months and 3 years of age. These results, suggesting that the presence of soya is at least partially responsible for the poor iron availability, are in direct contrast to a previous study on infant formula by Rios et al. (8). Using the radioactively labeled compounds, these workers showed that the absorption of ferrous sulphate in a soya isolate formula was 5.4% as compared to 3.9 and 3.4% for two milk-based formulas.

The influence of soya on iron availability has recently been reviewed (41). It was concluded that soya-based infant formulas appeared to be adequate as a source of iron for infants under 1 year of age. The differences between the availability of iron from soya-based formulas and cereal-soya blends were suggested to be at least partly due to a higher level of ascorbic acid in the formula which is also more stable during storage of the product in hermetically sealed containers.

There are clearly some contradictory results in the literature on the influence of soya on iron absorption. These differences could be due to methodology; iron-
replete adults may also not be a good model for young infants. However, as well as its content of other antinutrients (trypsin inhibitors, hemaglutinins, goitrogens) it now appears that soya may also have an iron-binding factor. The exact nature of the proposed inhibitor is as yet unknown; it has been observed with soya flours (~50% crude protein) and soya isolates (~90% crude protein). It does not appear to be due to phytate (~2% in the isolate) (20) nor to dietary fiber or carbohydrates since the highest inhibition was observed in the isolate (42), which contains very little or none of these substances. This inhibition was marginally reduced by baking at 200°C for 1 hr (iron absorption increased from 0.6 to 1.3%) and partially overcome by the addition of ascorbic acid (25:1, ascorbic acid:iron, wt/wt) when absorption was increased from 0.6 to 3.2%. One explanation could be that an insoluble iron-binding peptide is produced during the partial hydrolysis of soya protein in the stomach.

Tannins

One of the most potent inhibitors of nonheme iron absorption is Indian tea (45). When Indian tea was fed with a variety of meals there was a marked reduction in iron absorption which was shown to be due to the formation of soluble iron tannates (46). Although tea is not normally added to infant foods, other possible sources of polyphenols such as cocoa and sorghum may be, and could similarly inhibit iron absorption.

Enhancers of Iron Absorption

Ascorbic Acid

The ability of ascorbic acid to enhance nonheme iron absorption in humans has long been recognized. When ingested with a highly available iron salt such as ferrous sulphate, an increase in absorption of 33% was observed when 200 mg or more was given with 30 mg iron (6). When added to foodstuffs, the effect is even more dramatic because ascorbic acid also lessens the inhibitory effect of other ligands. Layrisse et al. (47) reported an almost sixfold increase in iron absorption (1.4–7.9%) by adult peasants in Venezuela, from 100 g of cooked maize containing 2.8 mg iron when 70 mg of ascorbic acid was added. Crystalline ascorbic acid or natural ascorbic acid from papaya had the same absorption-promoting effect. The enhancing effect of ascorbic acid augments with increasing doses. Using radioiron absorption tests, Cook and Monsen (48) measured the iron absorption in young men fed a semisynthetic meal containing dextrimaltose, corn oil, ovalbumin, and 4.1 mg iron. The increase in iron absorption (0.77–7.1%) was directly proportional to the amount of ascorbic acid added over the range of 25 to 1,000 mg (Fig. 3).

The effect of ascorbic acid on iron absorption seems to be related to both its reducing effect, preventing the formation of insoluble ferric hydroxide, and to its effect of forming soluble complexes with both ferrous and ferric ions at low pH, which then preserves iron solubility at the more alkaline duodenal pH. It also
competes with other inhibitory ligands and has improved absorption of iron in the presence of inhibitors from cereals and soya (33,47,49) and the tannins from tea (45). Iron absorption is improved although inhibition is not removed completely. Morck et al. (33) fed young men meals of isolated soya protein containing 4 mg Fe; adding 100 mg ascorbic acid increased the iron absorption from 0.6 to 3.2%. This effect of ascorbic acid in the presence of inhibiting ligands could be explained by the fact that ascorbic acid complexes soluble food iron at a lower pH than do inhibitory ligands. Iron from the common nonheme pool complexes with ascorbate in the stomach and passes into the intestine as an iron-ascorbate complex, thus eliminating the influence of the inhibitory ligands which bind iron at the more alkaline intestinal pH.

From the work of Derman et al. (50), the absorption-enhancing effect of ascorbic acid is clearly demonstrated both in infant formulas and infant cereals. These workers measured the influence of ascorbic acid addition on the absorption of various radioactive iron sources added to infant formulas and infant cereals. Their results, summarized in Table 5, show that ascorbic acid similarly enhanced the absorption of the more soluble ferrous sulphate or ferrous ammonium citrate and the less soluble ferric pyrophosphate. They concluded that, irrespective of the iron source added, the absorption of iron from infant formulas or cereals can be increased threefold by an ascorbic acid to iron ratio of 5:1 (wt/wt) and sixfold by a 10:1 ratio. The influence of ascorbic acid on the absorption of elemental iron powders remains to be determined. The recommended ratios of ascorbic acid to iron of Derman et al. (50) compare favorably with the ratios of 5:1 normally found in infant formulas and in infant cereals when ascorbic acid is added. They are somewhat lower than what would be expected from other studies (47,48).

One potential problem with ascorbic acid addition is its instability during processing and storage. An overaddition by the manufacturer compensates for processing losses. Its instability during the storage of infant formulas is also easily
### TABLE 5. Influence of added ascorbic acid on iron absorption by multiparous women from infant formula and cereals

<table>
<thead>
<tr>
<th>Iron source</th>
<th>Fe (mg)</th>
<th>Added ascorbic acid (mg)</th>
<th>Ratio ascorbic acid to Fe (wt/wt)</th>
<th>% Fe absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant formula (100 ml)</td>
<td>Ferrous sulphate</td>
<td>1.27 (1.27)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>6.2</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>19.7</td>
<td>55.7</td>
</tr>
<tr>
<td>Infant cereal (30 g)</td>
<td>Ferrous ammonium citrate</td>
<td>5</td>
<td>6.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>7.6</td>
<td>10.3</td>
</tr>
<tr>
<td>A</td>
<td>Ferrous sulphate</td>
<td>6.9</td>
<td>8.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>2.9</td>
<td>3.7</td>
</tr>
<tr>
<td>B</td>
<td>Ferric pyrophosphate</td>
<td>3.75</td>
<td>8.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.5</td>
<td>1.2</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.8</td>
<td>2.2</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37.5</td>
<td>4.4</td>
<td>7.5</td>
</tr>
</tbody>
</table>

From Derman et al. (50).
overcome by gassing the tins with nitrogen before closing; there is, then, virtually no loss under normal storage conditions. In stored cereals, however, ascorbic acid could be severely diminished during storage, depending on time, temperature, moisture content, and oxygen tension. One possible way of overcoming this may be the use of ethylcellulose-coated ascorbic acid, which costs only 3% more than the uncoated material (41).

Meat, Fish, Amino Acids, and Cysteine

The enhancing effect of meat and fish on iron absorption is well known. Unlike other enhancers, meat and fish increase the absorption of both heme and nonheme iron. The mechanism of absorption enhancement is not necessarily the same. For nonheme iron it does not appear to be due to protein per se, as egg albumin has no promoting effect. It could, however, be linked to amino acid composition. In human studies feeding black beans, Martinez-Torres and Layrisse (51) reported that the addition of either fish or an equivalent amount of synthetic amino acids almost doubled iron absorption. Absorption did not appear to be enhanced by any other amino acids except cysteine alone or in the presence of methionine. In rat experiments, however, histidine and lysine have also been reported to increase iron absorption (52).

The action of cysteine is presumably linked to its chelating and reducing powers, although its enhancing effect would seem to be far less than that of ascorbic acid. Martinez-Torres et al. (53) report that when 210 mg of cysteine was added to maize, soybean, or bean diets containing 2 to 3 mg Fe, it increased the absorption of the intrinsic iron 1.5 to 2-fold. The absorption of fortification iron (3 mg/Fe as ferrous sulphate, or ferric chloride) was similarly increased by 630 mg cysteine. The addition of cysteine to infant formulas or cereals appears impracticable because of its unpleasant smell; the addition of meat or fish similarly cannot be considered. As with ascorbic acid, cysteine loses its enhancing activity during cooking (53), presumably due to its conversion to cystine, or to other oxidation reactions.

Other Organic Acids

Moore (54), measuring the iron absorption from eggs, showed that whereas ascorbic acid, and to a lesser extent cysteine, increased absorption, citric acid, tartaric acid, and lactic acid were without effect. Succinic acid, on the other hand, appears to have powerful absorption-enhancing properties, at least in pharmaceutical preparations. Brise and Hallberg (6) (Fig. 4) added 30 to 500 mg succinic acid, 30 mg of iron as ferrous sulphate, and 10 mg ascorbic acid. They reported an increase in iron absorption from less than 5 to more than 50%. Other organic acids had no effect. Hallberg (17) reported that the addition of 150 mg of succinic acid to a hamburger meal increased nonheme iron absorption by 35%. Succinic acid is a chelator but has no reducing properties. It would appear to be much less active than ascorbic acid in enhancing iron absorption from meals.
Succinic Acid

The influence of succinic acid on iron absorption in humans (6). Succinic acid was administered with 30 mg Fe as ferrous sulphate and 10 mg ascorbic acid.

Fructose

The influence of sugars and fructose in particular has been much studied (7). Fructose, like other sugars, forms complexes with iron. Pollack et al. (55) found that fructose but not glucose or galactose increases iron absorption. The addition of large amounts of fructose to infant foods is not a practical proposition although the addition of the iron source ferric fructose, which contains about 20% iron, could be considered. In pharmaceutical preparations containing ferrous sulphate, sorbitol (about 12 mg/mg Fe) almost doubled the absorption of iron (56). In other studies on oral iron therapy (57), it was shown that sorbitol, mannitol, and xylose were the only carbohydrates with an absorption-promoting effect.

Synthetic Chelating Agents

These agents are not exactly absorption enhancers but more a way of adding iron in a reasonably strong complex which is little influenced by the inhibitory ligands present in some foods. Ethylenediaminetetraacetic acid is the most commonly used chelator, although others such as nitrilotriacetic acid (NTA) and lactobionate have also been used. The disodium salt of EDTA is a food additive permitted in the United States. It is used primarily as a preservative and in multivitamin preparations as a stabilizer for vitamin B12 (7). A highly stable iron chelate is formed by EDTA and direct feeding of NaFe EDTA showed impaired iron absorption when compared to ferrous sulphate (6). In a meal, however, more iron was absorbed from NaFe EDTA than from ferrous sulphate (58). MacPhail et al. (59) showed that the inhibitors of absorption present in cereals had less influence on the absorption of iron from NaFe EDTA, which was more than twice as well absorbed as ferrous sulphate when added to maize porridge. The subsequent addition of bran reduced the absorption of ferrous sulphate 11 times but had no influence on the absorption of iron from NaFe EDTA. The tannins of tea, a more potent inhibitor, did, however,
cause some reduction in absorption and, as would be expected, ascorbic acid also had a lower absorption-enhancing effect. The use of NaFe EDTA seems very promising for populations subsisting largely on cereal-based diets where a high incidence of iron deficiency is due to a poor absorption of iron from foodstuffs. Its addition to infant cereals or soya-containing products could also prove extremely useful if it could be shown to be safe in long-term toxicology trials. It is interesting to note that ferric chelates of EDTA, NTA, and lactobionate produced significantly less lipid peroxidation than ferrous sulphate when added to liquid milk (60), indicating that they may cause less organoleptic problems.

**Vitamin E**

Vitamin E is a reducing agent which could help reduce ferric to ferrous iron in the stomach. In animal trials (2,61), vitamin E has been shown to enhance the effect of ascorbic acid, although its influence on iron absorption in humans has never been reported.

**Food Processing and Storage**

Food processing as well as product composition can affect the final bioavailability of iron in food. This seems to be due mainly to a transformation of ferric salts into the ferrous state.

Theuer et al. (9,10), using the hemoglobin repletion test in rats, determined the availability of iron salts in liquid soya or milk-based infant formulas, before and after commercial sterilization. Their results are shown in Table 6. In both types of formulas, processing substantially increased the RBVs of ferric pyrophosphate and ferric sodium pyrophosphate but had little influence on the RBV of ferrous sulphate. It is interesting to note that RBVs were lower in the soya products than in the milk products. Wood et al. (62) confirmed the beneficial effects of processing on ferric pyrophosphate and sodium iron pyrophosphate in liquid systems but found no effect on ferric orthophosphate or ferrous sulphate.

**TABLE 6. Effect of processing on the availability of iron added to infant formulas**

<table>
<thead>
<tr>
<th>Iron source</th>
<th>RBV* before processing</th>
<th>RBV* after processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya formulas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>90</td>
<td>106</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>39</td>
<td>93</td>
</tr>
<tr>
<td>Ferric sodium pyrophosphate</td>
<td>15</td>
<td>66</td>
</tr>
<tr>
<td>Milk-based formulas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>126</td>
<td>129</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>78</td>
<td>125</td>
</tr>
<tr>
<td>Ferric sodium pyrophosphate</td>
<td>42</td>
<td>60</td>
</tr>
</tbody>
</table>

*Ferrous sulphate = 100.
From Theuer et al. (9,10).
It seems probable that the increase in bioavailability of these ferric salts on processing is due to their transformation to the ferrous state. Lee and Clydesdale (63) reported that elemental iron, ferric orthophosphate, and ferric EDTA were solubilized to ferrous forms to a greater or lesser extent in an acid-type fruit beverage, with or without ascorbic acid undergoing ambient storage, spray-drying, or freeze-drying. Three days' storage at 25°C with added ascorbic acid converted 17% of the iron from ferric orthophosphate and 90% from elemental iron to the ferrous state. Hodson (64) similarly found that iron from ferric orthophosphate was converted to the ferrous form after 6 months' storage of a liquid dietary. The reduction potential of a food system affects the chemical state of the iron present (65) and conversion of ferric to ferrous iron is increased by the addition of reducing agents such as ascorbic acid (66) and by lowering the pH.

Processing poorly available iron salts into cereal products such as bread rolls (5) or mixed infant cereals (16), however, does not appear to increase their bioavailability. Similarly, Lee and Clydesdale (67) found that both soluble and relatively insoluble iron sources were largely insoluble after baking into biscuits.

It would appear that heat processing offers little assistance to the infant food manufacturer to improve the iron availability of the products. There is no improvement of the iron availability in cereals and, in most infant formulas, iron is added in the highly available forms that are largely unaffected by processing. There is some indication that processing of cereals does transform ferric to ferrous iron, since iron salts, wet-mixed with cereals and then heat-processed, often cause more organoleptic deterioration on storage than do the same iron sources dry-mixed with the final product. Although poorly available iron sources do probably improve in bioavailability on heat treatment in liquid products, if the products do not deteriorate organoleptically during storage then highly available ferrous salts presumably could have been added in the first place.

Other ways of improving iron availability from infant foods by processing are difficult to envisage unless there is some progress in identifying more clearly the inhibitory factors in cereals and soya so that selective extraction, destruction, or modification procedures could be developed. Phytates, for instance, could be largely removed. It is possible that different processing methods may change the way in which iron is bound within the product. Camire and Clydesdale (68), for example, showed that in vitro at pH 5 to 7, more iron was bound to wheat bran and lignin after toasting or boiling. It has never been demonstrated, however, whether this has any significance on its bioavailability. Once in the stomach, food iron may be released from its food-bound forms into a common iron pool where all food or endogenous ligands compete to complex it.

**Increased Iron Level**

One of the easiest ways of increasing the amount of iron absorbed would at first sight seem to be the addition of more iron. This is why infant formulas contain 12 mg Fe/liter as opposed to the 0.4 mg/liter present in breast milk. However, there
are two problems. There is a question concerning the gastrointestinal tolerance of iron-fortified formulas. Iron plays an important role in the growth of microorganisms and it has been suggested that fortified formulas might increase the risk of gastrointestinal infection (69). Recent studies, however, indicate that supplemented iron does not provoke excessive gastrointestinal problems in infants (70). More importantly, iron has a number of demonstrated mineral-mineral interreactions with other divalent cations. A competitive reaction between iron and copper has been demonstrated in experimental animals (71). The inhibition of copper absorption by iron is believed to have been a contributory factor in a case of clinical copper deficiency on a premature infant fed an iron-fortified formula (72). Similar competitive reactions between zinc and iron have been shown in rodents (73) and in humans (74). The latter workers measured the influence of both heme and nonheme iron on the absorption of zinc in human subjects by measuring the change in plasma zinc concentration. These results demonstrated that zinc absorption was already greatly inhibited by inorganic iron at an iron to zinc ratio of 2:1. Heme iron had little influence on zinc absorption.

Iron-fortified formulas (about 12 mg Fe/liter) normally have an iron to zinc ratio of about 2:1, as has been recommended by Codex (75). Cereals, however, for which Codex makes no recommendations, are often fortified with iron (6–20 mg Fe/100 g) but seldom fortified with zinc. The significance is difficult to ascertain as cereals are not intended as a complete food but as a supplement to diets of infants and children. It would seem unwise, however, to increase the level of iron unconditionally in either formulas or cereals without considering the influence on zinc and copper absorption.

TECHNOLOGICAL PROBLEMS

Technological problems are not specific to iron but also concern other minerals commonly added to foods. The main problems concern color and off-flavor production. Other difficulties include the physical problems of precipitation of insoluble salts in liquid products and the even distribution of salts in dry products. Control of mineral fortification is also an important consideration as the consumer has the right to expect the product to contain the amount of nutrient claimed on the label. As part of the mineral is normally provided by the other ingredients of the foodstuff, natural variation in their composition is a complicating factor when formulating a product. Although expensive to set up and maintain, an in-house analytical capability is essential to detect under- or overadditions. This should be coupled with a sound sampling procedure for nonhomogenous products.

Color

As one might expect, many color problems result from the addition of iron to foods (76). In general, the more soluble the salt, the greater the problem. The addition of ferrous sulphate to liquid milk or soya products darkens their color and the higher the level of fortification the darker the product (77). Similarly, when we
added ferrous sulphate or other soluble iron salts to infant cereals, a dark gray or green color developed when the products were made into a pap with milk. Douglas et al. (78) have reported that ferrous sulphate, ferrous lactate, ferrous gluconate, ferrous fumarate, ferric citrate, ferric choline citrate, and ferric ammonium citrate produced initial and persistent off-color when added to a chocolate milk drink.

Flavor

The addition of soluble iron sources to milk products can also cause changes due to fat oxidation (60). However, initial off-flavors have been shown to decrease after a storage period (78) and ferrous sulphate is often chosen as the fortification iron for milk-based formulas. In contrast, the addition of ferrous salts to cereal products, because of their higher level of readily oxidizable unsaturated fatty acids, can rapidly result in unacceptable off-flavor production (30).

The extent of the flavor problem depends mainly on the solubility of the iron salt; the less soluble salts give fewer problems. As an example, J. Burri (personal communication) added different iron salts to whole wheat flour (15 mg Fe/100 g) in aqueous suspension. The slurry was cooked by steam injection, roller-dried, packed into aluminum cans, and stored at 37°C for up to 7 weeks or for 1 year at 20°C. The extent of fat oxidation was estimated by measuring the level of pentane in the headspace. The double unsaturated fatty acids are known to be a major source of pentane during lipid oxidation. Off-flavors and odors were measured by an experienced taste panel. The results are shown in Figs. 5 and 6. Reduced iron, ferric pyrophosphate, iron saccharate, and ferric citrate generated less pentane than ferrous sulphate or ferrous gluconate. This correlated well with the taste panel who found that ferrous sulphate and ferrous gluconate gave unacceptable off-flavors after 4 to 6 weeks' storage. Ferric citrate was also judged unacceptable. Wheat flour fortified with reduced iron, pyrophosphate, or saccharate was judged to be similar to the unfortified control. A similar pattern of results was obtained after 1 year at 20°C. It is possible, of course, that some of the elemental iron or ferric iron could have been transformed to ferrous iron during the heat treatment, but this seems unlikely to have occurred to any great extent in view of the results. The duration and temperature of the heat processing, however, could play an extremely important role in regulating the shelf-life of the cereal products fortified with elemental iron or ferric salts before processing.

Attempts to Overcome Organoleptic Problems

Because of flavor and color problems, infant food manufacturers are forced to add poorly available iron compounds such as ferric pyrophosphate or reduced iron to their products instead of the more highly available ferrous sulphate. Attempts have thus been made to overcome the pro-oxidant nature of the highly available iron compounds while still maintaining their bioavailability.
**Iron Casein Complexes**

Phosphoproteins such as milk caseins are strong natural chelators of transition metals such as iron. Their high affinity for metals is attributed to the presence in casein of clustered phosphoseryl residues (79). Iron binds more strongly than calcium so that, when added to a casein solution, it will replace calcium from the high-affinity binding sites. Forni et al. (80) prepared iron (ferric) caseinate by adding ferric lactobionate to a sodium caseinate solution and stirring for 1 hr at room temperature before concentrating and freeze-drying. The complex contained 0.31% iron. It was added at 6 mg Fe/100 g to a milk-based infant formula and 12 mg Fe/100 g to a precooked wheat flour. These products were placed in aluminum cans, stored at 37°C, and the lipid oxidation was followed by the analysis of pentane in the headspace. In the infant formula, pentane production as expected was very low with both iron sources. The levels of pentane generated by the stored cereal were much higher, even in the unfortified control sample, and although the iron casein generated less pentane than ferrous sulphate, it was still judged to be relatively pro-oxidative.
FIG. 6. Taste-testing of precooked whole wheat flour containing various iron sources (15 mg Fe/100 g) after 6 weeks' storage at 37°C. (J. Burri, personal communication.)

A preliminary bioavailability study with rats using radioactive $^{59}$Fe showed that incorporation of $^{59}$Fe from the iron-casein complex was 72% of that of iron from ferrous sulphate (F. Forni, M. J. Arnaud, and J. Hildago, personal communication). In the hemoglobin repletion test, the RBV was 90. The low iron content would mean that around 3% would need to be added to a cereal product. A possible advantage is that the absorption of iron from the iron-casein complex, like that from heme iron and ferric Na EDTA, would be less inhibited by other dietary constituents such as fiber or tannins. This still has to be investigated.

Encapsulated Ferrous Sulphate

In collaboration with Durkee Industrial Foods group, we have encapsulated anhydrous ferrous sulphate (~2H$_2$O) with a variety of partially hydrogenated vegetable oils. We tested bioavailability and investigated organoleptic problems in a cereal flour. The products contained 40% capsule and 60% active substance. The coating materials, their melting points, and the relative bioavailability of the encapsulated ferrous sulphate compared to ferrous sulphate itself is shown in Table 7. The hemoglobin repletion test was used to measure bioavailability.

Encapsulation had little influence on the bioavailability of ferrous sulphate. The products encapsulated with partially hydrogenated soybean oil, partially hydrogenated palm oil, and mono- and diglycerides performed particularly well. In contrast, electrolytic iron and pyrophosphate had only about half the bioavailability of ferrous sulphate. The electrolytic iron (Glidden) was described as an extra fine powder (<44 μm) with a relative bioavailability of 72%.

When dry-mixed with a pre-cooked wheat flour (10 mg Fe/100 g) and stored at 37°C for 3 months, the encapsulated products did not cause any off-flavor problems.
TABLE 7. Relative bioavailability of encapsulated ferrous sulphate, ferric pyrophosphate, and electrolytically reduced iron as compared to ferrous sulphate

<table>
<thead>
<tr>
<th>Iron source</th>
<th>Melting point (°C)</th>
<th>Relative* bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encapsulated ferrous sulphate 2H₂O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>partially hydrogenated soybean oil</td>
<td>65–66</td>
<td>101, 115</td>
</tr>
<tr>
<td>partially hydrogenated palm oil</td>
<td>57–58</td>
<td>95</td>
</tr>
<tr>
<td>mono- and diglycerides</td>
<td>54–55</td>
<td>101, 1116</td>
</tr>
<tr>
<td>partially hydrogenated soybean and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51–52</td>
<td></td>
<td>79</td>
</tr>
<tr>
<td>cottonseed oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>maltodextrin</td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>Ferrous sulphate 2H₂O</td>
<td>—</td>
<td>102</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>—</td>
<td>64, 45</td>
</tr>
<tr>
<td>Electrolytically reduced iron</td>
<td>—</td>
<td>40</td>
</tr>
</tbody>
</table>

*As measured in the hemoglobin repletion test (ferrous sulphate 7H₂O = 100).

TABLE 8. Discoloration of iron-enriched dried cereal-milk mixtures on addition of hot milk

<table>
<thead>
<tr>
<th>Iron source</th>
<th>Color reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrolytic iron</td>
<td>No discoloration</td>
</tr>
<tr>
<td>Iron pyrophosphate</td>
<td>No discoloration</td>
</tr>
<tr>
<td>Ferrous chloride</td>
<td></td>
</tr>
<tr>
<td>Ferric chloride</td>
<td></td>
</tr>
<tr>
<td>Ferrous citrate</td>
<td></td>
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<tr>
<td>Ferric citrate</td>
<td></td>
</tr>
<tr>
<td>Ferrous acetate</td>
<td></td>
</tr>
<tr>
<td>Ferric acetate</td>
<td></td>
</tr>
<tr>
<td>Ferrous sulphate 2H₂O</td>
<td>Gray at low concentration</td>
</tr>
<tr>
<td>Ferrous sulphate 7H₂O</td>
<td>Green at high concentration</td>
</tr>
<tr>
<td>Ferrous sulphate 2H₂O</td>
<td>Reactions similar to ferrous sulphate but slower</td>
</tr>
<tr>
<td>encapsulated with partially</td>
<td></td>
</tr>
<tr>
<td>hydrogenated soybean oil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at 50°C—no discoloration</td>
</tr>
<tr>
<td></td>
<td>at 60°C—slow discoloration</td>
</tr>
<tr>
<td></td>
<td>at 70°C—rapid discoloration</td>
</tr>
</tbody>
</table>

or excessive fat oxidation as measured by taste panel and pentane generation, respectively. They performed equally as well as electrolytic iron and the unfortified control. Unwanted color formation was investigated with the hydrogenated soybean oil capsule only. This capsule had the highest melting point (65°C) but provided little protection against unwanted color formation when added to the cereal (Table 8). The encapsulated product and a series of other iron salts at 7.5 and 40 mg/100 g were dry-mixed with a dried cereal-milk weaning food. The color problem did not occur during storage but during the preparation of the product for consumption. As with ferrous sulphate, various shades of gray and green appeared
when hot milk was added. The color formation depended on the temperature of the milk and appeared at 60°C and above. This was perhaps to be expected as the capsule had a melting point of 65°C. Other soluble iron salts also exhibited green color formation, whereas electrolytic iron and pyrophosphate caused no discoloration.

We plan to investigate capsules of higher melting points; carnauba wax (melting point 82°C) and zinc stearate (melting point 121°C) are possibilities. Such capsules may well overcome color problems but their influence on bioavailability remains to be determined. For the moment, elemental iron, ferric pyrophosphate, and perhaps ferric orthophosphate and iron saccharate appear to be the most suitable iron sources with which to fortify cereals from an organoleptic viewpoint.

GENERAL CONCLUSIONS

The iron sources added to infant formulas are generally ferrous salts of high relative bioavailability. The amount absorbed from milk-based formulas would appear to be in the region of 4%, which is considered sufficient to meet the iron requirements of infants (81). The absorption of iron from soya-based infant formula would appear to be adequate (41).

Because of organoleptic problems, ferrous salts cannot be added to cereal products. Encapsulation of ferrous sulphate to prevent organoleptic deterioration would seem a worthwhile approach, provided a more heat-stable coating can be found which does not reduce bioavailability. The iron sources normally added to infant cereals are elemental iron, ferric pyrophosphate, and ferric orthophosphate. Another iron source which looks promising, but has been little investigated, is saccharated iron oxide.

As iron sources differ greatly in quality from batch to batch, the food manufacturer needs more precise information on the relationships between in vitro tests and bioavailability, so that an effective control system may be implemented. However, even with good control, it is doubtful whether the relative bioavailability of these sources will be much more than half that of ferrous sulphate. One exception may be carbonyl iron, but that too may cause organoleptic problems. Adding higher levels of iron sources in order to increase the absolute absorption must be balanced against a possible adverse effect on the absorption of copper and zinc.

In addition to being fortified with the less available iron sources, infant cereals also contain inhibitors of iron absorption. If the precise nature of the inhibitors in cereals (or soya) could be clearly identified, it might be possible to remove them technologically. Other technological treatments to modify the nature of food-bound iron may also be possible, although their influence on bioavailability is uncertain. For the moment, the addition of ascorbic acid seems to be the best and the simplest way to overcome inhibition partially and to produce satisfactory absorption values. The optimum ratios of ascorbic acid to iron, however, need to be confirmed or determined for the different iron sources commonly used. The use of other reducing agents such as vitamin E or chelating agents such as succinic acid also merits further investigation. By far the most promising chelator would appear to be EDTA.
Iron bound as ferric sodium, EDTA is much less influenced by inhibitors than other iron salts and would appear to be an extremely useful iron source for addition to infant cereals or even soya products, provided that it could be shown to be harmless.

REFERENCES


DISCUSSION

Dr. Dallman: I understand that some forms of fortification iron can settle to the bottom of the package with products such as cereals, so that the iron is unevenly distributed. One might expect that carbonyl iron, which is spherical, would be more likely to settle out than something like electrolytic iron, which has a rough surface. Do you know of any studies on this?

Dr. Hurrell: As far as I know there have been no published studies on this phenomenon, but I agree that carbonyl iron would be more expected to fall through to the bottom of the package than electrolytic iron, which could be more easily held by its flat, jagged structure.

Dr. Hallberg: All flour in Sweden has been fortified with carbonyl iron for many years. The sedimentation of the iron particles has been studied by the milling industry and as far as I am informed it is no practical problem.

Dr. Dallman: I think that this problem may apply to infant cereals.

Dr. Fomon: I had been under the impression that the electrolytic iron powder currently used for fortification of instant cereals in the United States was of small particle size (I believe 95% passes through a 325-mesh sieve) and therefore of high bioavailability. From Dr. Hurrell's discussion, I suspect that bioavailability of this iron may actually be rather low. I wonder what is known about the bioavailability of this form of electrolytic iron powder.

Dr. Hallberg: I don't think that particle size distribution is a good parameter of the bioavailability of reduced iron. We found that surface area per unit weight and rate of dissolution were better related to the bioavailability in humans.

Dr. Stekel: One aspect that concerns me is that most data are based on experience with foods that are available in developed countries. For instance, you say that we have no problems with milk formulas because we fortify the formula with ferrous sulphate with adequate results, but these formulas are sophisticated in their composition and especially in their packaging. They come in sealed cans, oxygen free, and so on. When one considers taking the regular milk powder available in a developing country and fortifying it with iron, which would be in principle a simple matter and not very expensive, the problem of packaging comes up and this may be a limiting factor. A factor that then needs to be discussed is what are the alternatives to good packaging conditions. I think that one alternative might be to protect the vehicle in some way from interaction with the iron salt. Would you like to comment on this?

Dr. Hurrell: Do you still use ferrous sulphate in Chile even though the products go rancid?

Dr. Stekel: We have told the manufacturers that when the iron fortification program starts they have to switch to oxygen-tight containers, but that is the problem. This has been one of the limiting factors in going from a successful pilot study to a national program.

Dr. Hurrell: Packaging is something that I did not consider in my talk although it is undoubtedly important. All soluble iron salts would presumably promote rancidity if the milk powders were not packed in air-tight containers. In a nonair-tight container I suppose one could consider using one of the less soluble iron salts, encapsulated iron or maybe iron saccharate, which may well overcome the organoleptic problems. Of course, the problem may then be one of bioavailability.

Dr. Stekel: I think that when you make calculations of the costs involved, in a program like the one in Chile where about 13 million kg of dry milk are distributed each year to
infants, it turns out that to switch from unfortified to a fortified milk means an increase in price of about 13 to 14%. Of this, about 1% is ferrous sulphate and the ascorbic acid and 13% is the increased cost of packaging. So, this really becomes a very important limiting factor.

**Dr. Hurrell:** I agree, but air-tight packaging is also necessary to protect certain vitamins, such as A, C, and folic acid, in the product. It is not just for organoleptic reasons but also for nutritional reasons.

**Dr. Stekel:** I can see that, but you must realize that the product that is currently used is aimed primarily at the prevention of protein-calorie malnutrition, so that it is really dry milk without any vitamins or minerals added to it.

**Dr. Hallberg:** There is a great variation in the bioavailability of the iron phosphates. Even among ferric orthophosphate preparations on the market, the variation in rate of dissolution and in bioavailability is very marked. We produced ferric orthophosphate preparations with different physicochemical properties and found that preparations with the highest rate of dissolution had a relative bioavailability of about 40% compared with ferrous sulphate.

The main requirements of a reduced iron preparation are that the relative bioavailability in humans is known, including the effect of food preparation (e.g., baking), that it is fairly high and does not vary from one batch to another.

**Dr. Hurrell:** I should like to ask how much iron we can add. If we add more iron we can increase the amount of iron absorbed, but just how high can we go from a nutritional or toxicological point of view, until we influence the absorption of other trace elements such as zinc, for instance. Increasing the amount of iron would seem to be by far the simplest method of getting more iron absorbed.

**Dr. Fomon:** In the United States a product fortified above a specified limit becomes officially designated as a supplement and may no longer be advertised as a food. For children under 4 years of age this limit for iron is 7.5 mg in a serving of average size. A serving is declared (by the manufacturers) to be 15 g of dry cereal. After dilution, this will amount to about 100 g or more of cereal as fed: a very large serving for a small infant. I doubt that manufacturers would like to advertise their infant cereals as nutritional supplements rather than cereals so I do not believe that the level of iron fortification will be increased unless the declared size of an average serving is reduced.

**Dr. DeMaeyer:** When planning a fortification program, one has also to take into consideration the cost and the commercial availability of the iron compounds that may be used in the fortification process. It would be interesting to have some information on the subject.

**Dr. Hurrell:** Ferrous sulphate is by far the least expensive and the most easily available.

**Dr. DeMaeyer:** Could you comment on the different forms of reduced iron?

**Dr. Hurrell:** As I mentioned, there are four types of elemental iron: hydrogen reduced, carbon monoxide reduced, electrolytic, and carbonyl. I believe that the hydrogen-reduced form is the most widely available and the least expensive. Electrolytic is perhaps the least easily obtained. Carbonyl iron is available in Europe from manufacturers of magnetic tape (such as BASF). I cannot comment more on the relative cost. I think that as far as price is concerned, Dr. Stekel has pointed out that the price of the iron compound itself is always a very small part of the overall price of the product.

**Dr. Stekel:** Dr. Hurrell, I wonder whether you would like to comment on the use of hemoglobin as a food fortificant.

**Dr. Hurrell:** I think the main problem with hemoglobin is that it contains only 0.3% iron, so that one would have to add 5% of hemoglobin to have 15 mg of iron. That would certainly turn the product a different color. You said yourself that you could only add it to chocolate-colored biscuits because that was the only place where the additional color was not too important. Also, I think ethical problems of adding blood to infant foods will be
difficult to overcome, and there would be microbiological problems. I don't think one can foresee the addition of hemoglobin to commercially produced infant foods.

Dr. Stekel: I think you are right that the iron concentration is very low, but you have to consider that you are using not only the iron but also the protein.

Dr. Hurrell: Another problem with hemoglobin which should be borne in mind is that it contains little isoleucine, although it does have a high level of lysine.

Dr. Stekel: It is short on isoleucine, but when you add it to products that are cereal based—instant cereals, for instance, or wheat cookies—because of the high amounts of lysine and other essential amino acids in hemoglobin, and because of the low amino acid scores of these vegetable products, despite the fact that it is short in isoleucine, the amino acid score of the final product is increased. I agree that the microbiological and other problems are such that we probably do not have many reliable sources of hemoglobin available at this time, but I think it is certainly something that can be shown to work and it should perhaps be considered.

Dr. Hallberg: I think that dried blood (hemoglobin iron) can be used to fortify foods under certain conditions. Attempts were made in Sweden to fortify bread with dried blood; the bioavailability was fairly good, but there were problems with the shelf-life of the bread and it is no longer used. I think the best use of dried blood as an iron fortificant is in meat products such as sausages and hamburgers, especially when soya protein has been used as a partial substitute for meat and therefore the heme iron content of the product is reduced. The reason for using dried blood in meat products is that meat for unknown reasons promotes the absorption of heme iron; it is about 25% in meals containing meat but only 10% in other meals.

Dr. Garby: What about heme? That would solve the problem of the low iron content, but is heme difficult to handle chemically?

Dr. Hallberg: I think there are two patented processes I know about which both produce hemoglobin iron with good bioavailability and good shelf-life. In most meat products I think that the blood can be used as such.

Dr. Stekel: It is not always the case that purified heme is poorly absorbed. If you do the studies in a water solution this is the case, but we have some studies adding heme to milk, for instance, where absorption was very good. Apparently, the protein media in the milk had a protective effect on the formation of the large heme macromolecules, thus the heme in the milk was as well absorbed as the hemoglobin.