April 16, 1975, for Implementing Requirements of Section 1.18 of Title 21, Chapter 1, Subchapter A, Part I ("Labeling of Foods in Relation to Fat, Fatty Acid and Cholesterol Content") issued June 11, 1974, Division of Nutrition, Bureau of Foods, Food and Drug Administration, Washington, D.C. 20204.
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Received for review November 29, 1977. Accepted March 17, 1978. This work was part of an interagency agreement with the National Institutes of Health.

Iron Absorption by Rats from Nonprescription Dietary Iron Supplements

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Absorption of iron from seven nonprescription dietary iron supplements was measured in both iron-replete and iron-depleted rats by use of the extrinsic label technique. Both ferrous and ferric forms of the radioactive extrinsic tracer iron were used; the iron was in the form of $^{59}FeSO_4$, $^{59}FeCl_3$, or ferrous-^{59}Fe gluconate. Of six products tested with both ferrous and ferric labels, significant differences in absorption measured by the two different labels were found for three products. This suggests that there is incomplete exchange between the intrinsic and extrinsic iron pools. Iron-deficient animals absorbed significantly more iron than iron replete animals when the ferrous label was used in both experiments. Although iron-replete animals discriminate among iron sources, absorption is always less than in iron-deficient animals. The results with iron deficient animals show the true bioavailability of the iron. The use of the extrinsic label may not always be valid when the metal in question can exist in more than one oxidation state.

The absorption of iron from various food products has been estimated by use of an extrinsic radioactive iron tracer (Björn-Rasmussen et al., 1972, 1973; Björn-Rasmussen, 1973; Monsen, 1974). The extrinsic iron exchanges with the intrinsic non-heme iron of the food and can thus be used to determine absorption. However, much of the iron in some foods, especially breads and cereals, is present as inorganic iron which is added as fortification, rather than being biosynthetically incorporated into the food. The use of supplementary iron salts in tablet form is widespread. We wished to examine the use of the extrinsic label method to measure absorption of such inorganic iron which is not necessarily in the same chemical form as the radioactive tracer. We chose to examine iron absorption from a number of over-the-counter dietary iron supplements that contained iron in various forms and varying amounts of vitamins, other minerals, binders, sweeteners, and alcohol. Absorption was measured in iron replete rats with the use of radioactive tracers in both the ferrous and ferric state and in iron-deficient animals with the use of a ferrous label.

MATERIALS AND METHODS

Iron supplements were purchased from local supermarkets and drug stores. Solubility of the iron in the nonliquid iron supplements was measured in HCl and in water. One tablet of a supplement was placed in 25 mL of 1.5 M HCl and shaken at 37 °C for 18 h. Disintegration of the tablets was checked at 5-minute intervals for 1 h, at 2 h and at 18 h. After 18 h, any large particles remaining were pulverized. One hour later the solutions were filtered, and iron was determined by atomic absorption. We calculated the percent solubility using the iron content per tablet given on the label as the 100% value. Solubility of the iron in water was determined by grinding the tablets with a mortar and pestle, quantitatively transferring the powder to a graduated cylinder, and mixing it with a known amount of water for 20 min. The iron in solution was measured by atomic absorption spectrophotometry and the percent solubility was calculated as for the HCl solutions.

To determine whether ferrous iron had been oxidized, solid samples were ground and mixed with water as in the solubility tests. Liquid samples were diluted with water to appropriate concentrations for analysis. Total iron was determined using 1,10-phenanthroline (Sandell, 1944) with hydroxylamine hydrochloride as a reducing agent. Ferrous iron was determined using 1,10-phenanthroline in assay mixtures without addition of hydroxylamine hydrochloride. Ferric iron was determined by difference.

The ferrous-^{59}Fe gluconate was prepared from $^{59}FeSO_4$ (ICN Pharmaceuticals, Inc., Chemical and Radioisotope Division). Dowex-1 anion-exchange resin, chloride form (Sigma Chemical Co.) was washed with 1 N NaOH until the addition of $AgNO_3$ to the wash showed no further elution of chloride ion. It was then washed with water until pH <9 and with 1 N gluconic acid until pH <2. It was rinsed with water until pH >4 before the sample was applied. A column 2 cm tall was packed in the lower portion of a pasteur pipet. Approximately 50 μg of ^{59}Fe as $FeSO_4$ was applied to the column and eluted with 0.05 M gluconic acid. To check that the iron was eluted as ferrous gluconate, a larger column (1 × 13.5 cm) was prepared and 0.14 g of $FeSO_4 \cdot 7H_2O$ in 0.5 mL of H_2O was applied and eluted in the same manner. The iron in the effluent gave a positive test for ferrous ion with potassium ferricyanide. A test for sulfate ion with 5% $BaCl_2$ was negative.

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Table I. Solubility of Iron Supplement Tablets

	% solubility	
	H_2O[a]	1.5 M HCl[b]
Geritol tablets[c]	99 ± 26	99
Ironized yeast[d]	72 ± 5	90
Unicap plus Iron[e]	89 ± 5	99
Unicap Sr[e]	91 ± 12	95
Fem Iron[c]	0.5 ± 1.0	105
One-A-Day plus Iron[f]	13 ± 4	51
One-A-Day plus Minerals[f]	6 ± 9	81

[a] Mean ± SD, four determinations. [b] Single determination. [c] The J. B. Williams Company, Inc. [d] Glenbrook Laboratories. [e] Upjohn. [f] Miles Laboratories, Inc.

Absorption of iron in iron-replete animals was studied with male Sprague-Dawley rats, weighing 250–300 g each, that had been fed a diet of Purina Laboratory Chow (198 ppm Fe) until the time of the experiment. They were fasted overnight before the experiment. Solid supplements were ground and mixed with water, and liquids were diluted with water to a concentration of 25 μg of Fe/mL. The test solutions were labeled with $^{59}FeCl_3$, $^{59}FeSO_4$, or ferrous-^{59}Fe gluconate immediately before dosing. Each rat received by stomach tube 2 mL of the test solution containing 50 μg of Fe and 1.5 μCi ^{59}Fe. Radioactivity was determined immediately and at intervals from 5–14 days after dosing with a whole-body counter. Corrections were made for decay of the ^{59}Fe, and the percent retention was calculated with the day 0 value as 100%. Food was restored 4 h after dosing.

Absorption of iron in iron-deficient animals was determined in the same manner, with weanling male Sprague-Dawley rats who were placed on an iron-deficient diet (Teklad, 2 ppm Fe) for 7 days prior to the experiment. They were also fasted overnight before the experiment.

RESULTS

To ensure that test animals would receive a known dose of iron, we tested the solubility of the iron in the tablet-form supplements in water and in hydrochloric acid at 37 °C. The nominal iron content of the tablet as stated on the label was used as the 100% value. FDA requires that the iron content be within the range of −10 to +15% of the level stated on the label. Accordingly, compounds with solubility values within this range were considered completely soluble for purposes of calculation of iron dosages. Solubilities in water ranged from less than 1 to 100% (Table I). For three products, Fem Iron, One-A-Day plus Iron, and One-A-Day plus Minerals, solubility in water was so low that animal experiments were not performed. Solubility in hydrochloric acid at 37 °C tended to be better, ranging from 51 to 99%, as shown in Table I.

Possible oxidation of ferrous ion to ferric ion during storage was also checked. Table II shows the type of iron salt in each supplement and the percent of water-soluble iron present as the ferric form. Only a small amount (<15%) of the iron in the preparations containing ferrous sulfate had oxidized to ferric ion. Other supplements containing ferrous salts had up to 38% ferric ion. The supplement containing iron as ferric ammonium citrate showed only 33% ferric ion when assayed. The Merck Index (1968) indicates that ferric ammonium citrate is reduced to ferrous salts by light. This product carried no expiration date on the label.

The validity of the use of $^{59}FeCl_3$ as an intrinsic label to study the absorption of iron from these supplements was investigated by determining absorption with both a $^{59}Fe^{3+}$ label (ferric chloride) and $^{59}Fe^{2+}$ (ferrous sulfate or ferrous gluconate). For three of the supplements (Unicap plus Iron, Unicap Sr., and Gevrabon) no significant difference in the absorption of iron labeled with Fe^{2+} or Fe^{3+} was observed. For two supplements (Geritol tablets and Ironized Yeast), absorption was significantly ($P < 0.01$) lower with the Fe^{2+} label. For one supplement (FerInSol), absorption was significantly ($P < 0.05$) higher with the Fe^{2+} label.

Table II. Ferric Iron Content of Iron Supplements

	Form of Fe[a]	% Fe as Fe^{3+} [b]
Geritol tablets	Ferrous sulfate	0
FerInSol[c,d]	Ferrous sulfate	0
Ironized Yeast	Ferrous sulfate	3
Unicap plus Iron	Ferrous sulfate	14
Unicap Sr	Ferrous sulfate	10
Gevrabon[c,e]	Ferrous gluconate	20
Geritol Liquid[c,f]	Ferric ammonium citrate	33
Fem Iron	Ferrous fumarate	g
One-A-Day plus Iron	Ferrous fumarate	8
One-A-Day plus Minerals	Ferrous fumarate	38

[a] According to package label. [b] Water-soluble ferric iron. [c] Liquids. [d] Mead Johnson Laboratories. [e] Lederle Laboratories. [f] The J. B. Williams Company, Inc. [g] Too insoluble to test.

Iron absorption in iron-depleted animals was also determined with a Fe^{2+} tracer to examine the effect of the physiological state of the experimental animals. Iron absorption in all four experiments approached 100% and did not differ significantly among the four supplements tested, indicating that the iron in these four supplements was equally available to animals who were depleted of iron. In all cases, absorption was significantly higher ($P < 0.01$) than in iron-replete rats where the ferrous tracer was used.

In the iron-replete animals, whose need for iron was probably minimal, we noted some differences between supplements as to the amount of iron absorbed. In tests with a ferric iron tracer, absorption from FerInSol was significantly lower, 18.1% ($P < 0.05$), than absorption from all of the other supplements tested except Gevrabon. Absorption from Gevrabon was significantly different, 25% ($P < 0.05$), from all of the other supplements except FerInSol and Unicap Sr. The other values in the ferric ion labeled experiments did not differ significantly. In the ferrous label groups of iron-replete rats, absorption from Unicap plus Iron was 51.6%; this was significantly different ($P < 0.05$) from all other supplements except Unicap Sr. Absorption from Unicap Sr. was 50.4%, significantly different ($P < 0.05$) from Ironized Yeast and Gevrabon. There were no significant differences among the other values.

DISCUSSION

It is generally assumed that foods must be broken down and rendered soluble in digestive juices if they are to be absorbed. However, attempts to correlate solubility of a substance in acid with the bioavailability of the iron it contains have not been very successful (Pla and Fritz, 1970; Jacobs and Greenman, 1969), except in the case of reduced iron (Pla et al., 1976). We examined the water solubility of the iron in these supplements to be sure that all test animals would receive the same dose of iron. While solubility in most cases was adequate, in three supplements containing ferrous fumarate (Table II), the iron was so insoluble that we did not test iron absorption from them. We also made a single determination of the acid solubility of these products by shaking them in 1.5 N HCl overnight at 37 °C. With two exceptions, the iron in all of them was completely soluble, within experimental error. Two of the

Table III. Percent of ^{59}Fe Absorbed[a]

	Replete animals			Depleted animals	
	Fe^{3+} label	p^b	Fe^{2+} label	p^b	Fe^{2+} label
Geritol tablets	60.7 ± 14.8[c] (5)	0.01	24.1 ± 9.5[d] (6)	0.0001	96.1 ± 7.2[e] (5)
FerInSol	18.1 ± 5.5 (6)	<0.05	33.9 ± 10.7 (6)	0.0001	87.9 ± 6.8 (6)
Ironized Yeast	62.4 ± 10.5 (5)	0.01	29.6 ± 12.5 (6)	<0.01	96.3 ± 3.5 (3)
Unicap plus Iron	59.4 ± 13.0 (5)	NS	51.6 ± 5.6 (5)	0.0001	89.7 ± 9.1 (6)
Unicap Sr	57.5 ± 6.2 (5)	NS	50.4 ± 15.0 (6)		
Gevrabon	25.0 ± 11.0 (6)	NS	21.5 ± 8.5[f] (6)		
Geritol Liquid	43.4 ± 16.9 (5)				

[a] All values are mean ±SD. The number of animals in each group is given in parenthesis. [b] These p values refer to significance of difference between adjacent columns. [c] FerInSol is significantly different ($P < 0.05$) from all in the same column except Gevrabon. Gevrabon is significantly different ($P < 0.05$) from all in the same column except FerInSol and Unicap Sr. The other values do not differ significantly. [d] Unicap plus Iron is significantly different ($P < 0.05$) from all in the same column except Unicap Sr. Unicap Sr is significantly different ($P < 0.05$) from Ironized Yeast and Gevrabon. There are no significant differences between the other values. [e] Values in this column do not differ significantly from one another ($P < 0.05$). [f] ^{59}Fe as ferrous gluconate.

products containing ferrous fumarate, One-A-Day plus Iron and One-A-Day plus Minerals, were not completely soluble in 1.5 N HCl. These results were not surprising since Pla and Fritz (1970) reported that ferrous fumarate was only 57.7% soluble in 50 times its weight of HCl at pH 1.1 after 72 h.

The two-pool extrinsic tag method of measuring non-heme iron absorption has been demonstrated to be valid with a number of foods in both man (Björn-Rasmussen et al., 1972, 1973; Björn-Rasmussen, 1973) and rats (Monsen, 1974). Radioactive iron added to food as an extrinsic tracer exchanges with the non-heme iron in food in the gut and is absorbed in a similar fashion. The ratio of extrinsic iron absorption to intrinsic iron absorption has been very close to 1.10 in a number of studies employing widely varying conditions (Björn-Rasmussen et al., 1972, 1973; Björn-Rasmussen, 1973; Monsen, 1974). Although exchange is apparently not complete, as indicated by the $E:I$ ratio of 1.10, the consistency of the ratio makes the extrinsic label method a useful one.

We wished to investigate the effect of using extrinsic radioactive iron tracers of differing oxidation states upon the values obtained for iron absorption. This was done with dietary iron supplements containing iron in the form of various salts and of both oxidation states. The iron in these supplements is present in salt form, presumably unbound to complex organic molecules as might be the case in food.

If the extrinsic label method is valid in all cases where the intrinsic iron to be labeled is soluble, so that exchange may occur, then one would expect to obtain identical results with both ferrous and ferric iron tracers. In fact, iron absorption values measured for some supplements were dramatically different for the two tracers.

In iron replete animals, significant differences were obtained between the Fe^{2+} and Fe^{3+} labels for three of six supplements tested. For one product, FerInSol, absorption was greater with the Fe^{2+} label; for Geritol tablets and Ironized Yeast, absorption was greater with the Fe^{3+} label. All three of these supplements contain iron as $FeSO_4$. For two other supplements containing $FeSO_4$ and one containing ferrous gluconate, no significant difference in absorption between the two labels was observed, although in each case, the ferrous label gave the lower values.

Differences in absorption such as these obtained with tracers of different oxidation states cast a shadow on the validity and utility of the extrinsic label technique. Clearly, the radioactive label does not always completely exchange with and label the other, nonradioactive iron in the gut in all cases. The results demonstrate that there is imperfect interchange of iron between the two pools. Such a problem has been recognized for iron which is not soluble (Amine and Hegsted, 1974), such as metallic iron introduced in food processing or the very insoluble iron in Near Eastern breads, and for iron in the form of ferritin or hemosiderin (Martínez-Torres et al., 1977). Thein-Than et al. (1977) have suggested that, although exchange of the iron tracer with a rice meal does not take place in vitro at pH 1.8, complete exchange is completed in the duodenum. This is in contrast to the results of Björn-Rasmussen et al. (1973) who found significant differences in absorption of intrinsic and extrinsic iron from boiled polished rice. Since the iron intrinsic in food cannot be assumed to be all in one oxidation state, it is difficult to specify whether ferrous or ferric iron is better used as a tracer, or whether the extrinsic iron exchanges completely with intrinsic iron in a given case. The most reliable data will continue to be those obtained using intrinsically labeled foods. Earlier work in this area has been summarized by Layrisse and Martinez-Torres (1971).

The data from the iron-depleted animals show that the iron from various supplements is equally well absorbed, in contrast to the results in iron-replete rats. The iron-depleted animals were weanlings that had been fed an iron-deficient diet for only 7 days before the experiment. A significant change in iron absorption has been reported to occur after both adult and weanling rats have been fed iron deficient diets for 5 days (Pearson et al., 1967; Charlton et al., 1965; Bannerman et al., 1962; Pollack et al., 1964). The rapidly growing, iron-depleted animals, that need to absorb iron, absorb large amounts without discriminating between sources; apparently iron availability from the four products tested in this experiment is near 100%. The iron-replete animal discriminates among iron sources, and in no case does he absorb as much as an iron-deficient rat.

The failure of the iron-deficient animal to discriminate among foods has been cited as a reason for not using a deficient animal to determine iron bioavailability (Monsen, 1974). However, the fact that the iron-replete animal does discriminate between foods is a result of his iron status; it is not a function of the true bioavailability of the iron in that food. An animal that really needs the iron, that is, an iron deficient animal, absorbs larger amounts of iron and the presence or absence of ascorbate or other substances that enhance absorption in the replete animal make little or no difference. Thus the iron deficient animal is the more suitable for measuring bioavailability in various foods. Results obtained with iron replete animals are befuddled by the exchange of extrinsic, isotopic iron with iron which is sloughed off from the gut and subsequently reabsorbed, as well as with the pool of iron intrinsic to the

food being tested. The amount of exchange of extrinsic tracer iron with endogenous intestinal iron and intrinsic food iron will not necessarily be the same. Thus, a distinction must be made between the *amount of iron absorbed* by an individual of given iron status from a food and the *bioavailability* of the iron from the food, which can only be measured in an iron-deficient individual. This distinction has not always been fully and carefully made in the past. The data in Table III indicate clearly the need for such a distinction.

While some interesting differences in iron absorption were found among the various supplements in iron-replete animals (Table III), they are probably of little significance to the consumer. For a person with adequate iron stores the variations among brands would be unimportant; for an iron-deficient individual the differences among brands would probably not exist.

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Received for review February 21, 1978. Accepted May 1, 1978. This work was supported in part by the U.S. Department of Agriculture Cooperative Agreement No. 12-14-3001-294. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

Elemental Content of Tissues and Excreta of Lambs, Goats, and Kids Fed White Sweet Clover Growing on Fly Ash

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White sweet clover found voluntarily growing on a deep bed of soft coal fly ash was found to contain high concentrations of a number of elements including selenium, bromine, molybdenum, rubidium, strontium, and others. The clover was harvested and fed as 23.5% of a dry pelleted ration to lambs and pregnant goats for up to 173 days. High concentrations of selenium were found in 11 tissues, blood, goats' milk, and excreta of lambs, goats, and newborn kids. Molybdenum in liver, strontium in bone, and bromine and rubidium in animal tissues were also elevated over those in the corresponding tissues of animals fed an identical ration containing control clover grown on soil. No gross or histologic lesions were present in any of the animals.

Fly ash is trapped in electrostatic precipitators of soft coal-burning electric power-generating plants. It has been estimated that up to 36 million tons of the material will be produced in the United States by 1980 (Brackett, 1970). This estimate may be conservative owing to the recent renewed interest in coal as an energy source. Whereas a small percentage of the fly ash produced is used as a road base material or in concrete products, the bulk of it is disposed of in landfills.

Some studies have been made of the use of fly ash as an alkaline amendment to reclaim coal mine spoils (Adams et al., 1972). It has also been incorporated into soil to correct plant deficiencies of boron, phosphorus, zinc, potassium and molybdenum (Martens, 1971; Martens et al., 1970). Fly ashes may also contain toxic elements which are available to growing plants. Vegetables and millet cultured on potted soil containing 10% by weight of fly ash showed higher concentrations of a number of elements including boron, molybdenum, and selenium (Furr et al., 1976a). The extent of absorption of selenium was roughly proportional to the rate of application of fly ash. An analytical survey of 45 elements in fly ashes from 21 states was conducted (Furr et al., 1977). Cabbage grown on soil containing 7% by weight of 16 of these fly ashes absorbed arsenic, boron, molybdenum, selenium, and strontium to an extent that showed a high degree of correlation with the total content of the elements in the respective fly ashes (Furr et al., 1977).

Toxicologically, the use of fly ash in agriculture requires a knowledge of the magnitude of transfer of toxic elements in it to plants and finally to farm animals consuming these plants. Yellow sweet clover (*Melilotus officinalis*) found

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0021-8561/78/1426-0847$01.00/0