Absorption of Fortification Iron by the Rat: Comparison of Type and Level of Iron Incorporated into Mixed Grain Cereal

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Under specifications agreed upon by representatives of the major U. S. A. manufacturers of iron sources for commercial enrichment and fortification, four iron supplements were prepared: ferrous sulfate; reduced iron; ferric orthophosphate; and sodium iron pyrophosphate. Relative availability of these iron supplements when incorporated into an infant cereal, in a manner meeting manufacturing procedures, was assayed in the rat. Iron was given at three dosage levels, 20, 80, and 320 μg/0.500 g of cereal. Ferrous sulfate was the most available supplement, followed by reduced iron. Ferric orthophosphate and sodium iron pyrophosphate were poorly absorbed. At the highest dosage level, considered to be beyond the normal dietary range, absorption of all supplements was poor. Current human studies show similar relative availability of the iron supplements, indicating effectiveness of the animal assay. Obligatory assessment of the availability of iron supplements should be an integral part in guidelines for food fortification.

Earlier studies by Steinkamp, Dubach, and Moore on the absorption of iron from iron-enriched bread led to the assumption that there were no significant differences in the biological availability of ferrous sulfate, reduced iron, ferric orthophosphate, and sodium ferric pyrophosphate (Steinkamp *et al.*, 1955). However, this assumption has been challenged as fortification programs utilizing these salts have not been uniformly effective (Elwood, 1968). Further, there are reasons to believe that the chemical and physical specifications of the iron compounds used by Steinkamp *et al.* differ markedly from those used by the food industry today (Cook *et al.*, 1973). Thus, as increasing attention is being given to the need for additional iron fortification, it is especially important to reexamine the assimilability of various iron forms suitable for commercial usage (Elwood, 1968; Finch and Monsen, 1972; Monsen *et al.*, 1967).

The purpose of this particular study was to test in an animal system the availability of various iron supplements after incorporation into a commercially prepared mixed grain infant cereal. Four iron compounds were assessed: reduced iron; sodium iron pyrophosphate; ferric orthophosphate; and ferrous sulfate. Commercial specifications were met in both the preparation of the iron supplements and their subsequent incorporation into the cereal.

METHODS AND MATERIALS

Experimental Animals. Rats were Sprague-Dawley weanling males, 3 weeks of age, weighing 45 to 50 g. They were maintained in the laboratory for 2 weeks prior to receiving the test meal in order to standardize living conditions and iron nutriture. Animals were individually housed in galvanized expanded metal cages. Weights of rats were taken on alternative days throughout the stabilization period. Laboratory chow (Ralston Purina Co., St. Louis, Mo.), containing at least 23% protein and 0.02% iron as ferric ammonium citrate and iron oxide, was fed *ad libitum* to the animals except during fasting and administration of the test meals. Water of negligible iron content was available at all times.

Approximately 4 days before test feeding, the animals were transferred to individual plastic cages to eliminate possible iron contamination during feeding. All rats were test fed on the 34th, 35th, or 36th postnatal day. A group of 8–10 animals was used for each treatment, with a total of 12 experimental groups. At the end of the period, hematocrits were assessed utilizing microcapillary tubes. At the time the test meals were administered, the body weight of the animals was 139 ± 8 g (mean ±SD).

Iron-Fortified Cereals. Mixed grain infant cereal was prepared and supplied by the Gerber Co., Fremont, Mich. The cereal was composed of oat flour, soft wheat flour, corn flour, barley flour, calcium phosphate (di-basic), barley malt flour, niacinamide, thiamine mononitrate, and riboflavin. The nutrient content of the cereal without iron supplementation was as follows: protein, 11.7%; fat, 4.5%; available carbohydrates, 73.0%; crude fiber, 1.1%; moisture, 7.0%; ash, 2.7%, calcium, 0.53%, phosphorus, 0.66%, and iron, 0.004%.

The mixed grain cereal was available in three forms: without iron; fortified with ^{59}Fe supplements; and fortified with nonradioactive iron supplements. The various ^{59}Fe compounds were incorporated in a manner similar to the commercial method for fabricating the cereal, which includes heating, enzymatic action, and drying after fortification. All labeled iron compounds utilized for fortification had been supplied to the cereal manufacturer by Abbott Laboratories and were prepared in a manner meeting specifications approved by a committee of representatives of the primary producers of iron compounds for enrichment and fortification (Glidden-Durkee SCM, Mallinckrodt Chemical Works, Merck and Co., Stauffer Chemicals, and Sterwin Chemicals). Sodium iron pyrophosphate was manufactured by a method furnished under a secrecy agreement to Abbott Laboratories by Stauffer Chemical Co. The particle size of each of the experimental iron supplements was similar to commercial sources with the exception of the reduced iron, which was 95% in the 5–10 μ range in the experimental product and 90% in the 25–45 μ range in the commercial material (Gerber Co., 1971). Detailed specifications are given by Cook *et al.* (1973), whose human studies employed identical iron supplements.

Test Meals. Analysis of the chemical iron content of each sample was performed utilizing the bathophenanthroline method following wet ashing (Bothwell and Finch, 1962). To achieve each of the desired fortified iron dosages and the standardized level of radioactivity of approximately 0.15 μCi/test meal, mixtures of the radioactive iron fortified, nonradioactive iron fortified, and unfortified cereals were made. Each test meal was composed of a total of 0.500 g of cereal mixed with 1.2 ml of noniron supplemented infant formula (Ross Laboratories, Columbus, Ohio) which had been diluted by half with deionized water to the concentration normally fed to infants. The natural iron content of the cereal, milk, and water in each test dose approximated 18 μg, with various levels of forti-

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Table I. Summary of Results of a Study of the Absorption by Rats of Four Iron Salts Incorporated into Infant Cereals at Three Different Levels. Values Reported as Mean ± SD

Iron salt	Dose, g	Number of animals	% supplemental iron absorbed	Total μg of supplemental iron absorbed	Body weight, g	Hematocrit, %	Weight gain, g/day
Ferrous sulfate	20	10	15.7 ± 4.8	3.1 ± 1.0	146.9 ± 9.0	41.1 ± 1.0	6.9 ± 0.6
	80	6	12.0 ± 3.2	9.6 ± 2.6	130.6 ± 7.0	41.1 ± 2.2	6.8 ± 0.5
	320	7	3.8 ± 1.4	12.2 ± 4.5	139.7 ± 7.2	41.3 ± 3.4	6.8 ± 0.3
Reduced iron	20	6	6.7 ± 2.8	1.3 ± 0.6	129.3 ± 10.5	43.6 ± 4.5	6.1 ± 0.8
	80	7	5.1 ± 2.0	4.1 ± 1.6	135.7 ± 16.0	40.7 ± 3.0	6.4 ± 0.7
	320	7	4.6 ± 1.7	14.7 ± 5.4	138.3 ± 14.6	43.3 ± 2.9	6.2 ± 0.5
Ferric orthophosphate	20	7	2.9 ± 0.9	0.6 ± 0.2	126.8 ± 9.6	42.9 ± 4.0	5.7 ± 1.0
	80	7	3.8 ± 1.2	3.0 ± 1.0	135.7 ± 8.6	41.1 ± 1.2	6.6 ± 0.5
	320	7	3.4 ± 1.5	10.9 ± 4.8	150.0 ± 7.2	41.6 ± 2.2	6.6 ± 0.6
Sodium iron pyrophosphate	20	7	1.0 ± 0.26	0.2 ± 0.1	134.7 ± 12.8	41.7 ± 2.2	6.1 ± 0.5
	80	8	1.0 ± 0.49	0.8 ± 0.4	146.4 ± 12.4	42.0 ± 1.6	6.2 ± 0.8
	320	7	0.6 ± 0.18	1.9 ± 0.6	151.1 ± 13.0	41.7 ± 1.5	5.6 ± 0.5

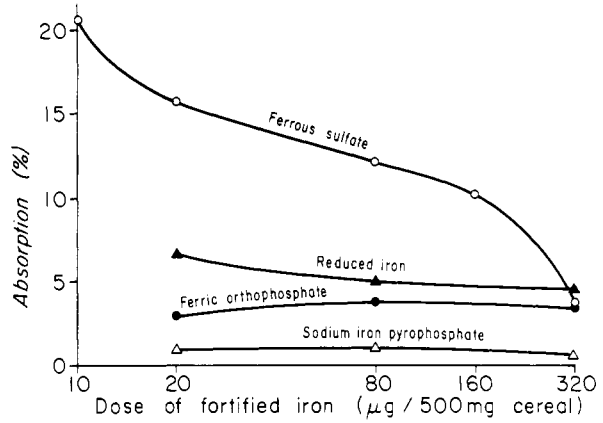

Figure 1. Absorption by rats of four iron salts incorporated into a mixed grain cereal for infants.

fied iron superimposed. Each salt was given at three levels, *viz.*, 20, 80, and 320 μg of iron/dose, as fortifying iron.

Prior to testing, rats were fasted for 16 hr; water was available during this interval. Animals were individually offered the test meal and subsequently counted in a Packard-Armac whole body counter. Normal food was replaced 2 hr after test doses had been eaten. A second total body count 7 days after ingestion of the radioactive test dose was utilized in assessing percentage absorption (Amine and Hegsted, 1970; Monsen *et al.*, 1970).

Analysis of variance (Snedecor, 1956) was performed on data for the different groups of rats and for data obtained at different dosage levels.

RESULTS

Mean values for iron absorptions, hematocrits, body weights, and weight gains for animal groups receiving each of the four salts given at the three dose levels are presented in Table I. All animals had normal hematocrits (Spector, 1956) of between 39 and 50% just prior to test feeding. The mean hematocrits of the various rat groups were not significantly different. The mean rate of growth for the 2 weeks postweaning was 6.8 g/day, normal for animals of this strain and age (Ranhotra and Johnson, 1965). No significant difference between the mean rate of growth of the various groups was observed.

The availability of the four iron salts in the mixed grain cereal is compared in Figure 1. Ferrous sulfate was the best absorbed salt when given at normal dietary levels ($p < 0.01$). The hydrogen-reduced iron tested here was found to be the next most available salt for the test animals, being absorbed 43% as well as $FeSO_4$ at the levels of 20 and 80 μg of iron/0.500 g of cereal.

Ferric orthophosphate and sodium iron pyrophosphate were poorly absorbed at all levels. Mean percentage absorption for the ferric orthophosphate at the 20-μg level was 18% of the comparable $FeSO_4$-supplemented cereal. The sodium iron pyrophosphate exhibited the poorest absorption. At the 20- and 80-μg iron level, sodium iron pyrophosphate was 6 and 8% as well absorbed as $FeSO_4$.

At the highest dietary level, 320 μg of iron/0.500 g of cereal, the mean absorptions of all salts were below 5.0%. An analysis of variance showed the absorption of $FeSO_4$ significantly declined with increased level of iron supplementation ($p < 0.01$). Differences shown in the absorption curves of the other salts were not significant.

DISCUSSION

In comparing the availability of various iron salts, both the chemical and physical characteristics of the salts play critical roles. Utilizing a short-term radioisotopic technique in rats, the present study confirms ranking of iron availability: ferrous sulfate > reduced iron > ferric orthophosphate > sodium iron pyrophosphate. This pattern appears independent of the technique employed, as much of the data reported by other investigators was obtained by assessment of hematopoiesis in the rat over a long period of time (Amine *et al.*, 1972; Blumberg and Arnold, 1947; Fritz *et al.*, 1970; Hinton and Moran, 1967; Ranhotra *et al.*, 1971). Species differences in iron absorption (Bothwell and Finch, 1962) eliminate the possibility of direct application of results with rats to human situations; however, there is evidence that the rat absorbs various forms of iron with the same predisposition to order as does man (Cook *et al.*, 1973; Elwood, 1968; Fritz *et al.*, 1970; Hoglund and Reizenstein, 1969; Layrisse *et al.*, 1968). Of particular importance are the data confirming rank order of availability issuing from the human study by Cook *et al.* (1973), which assessed the absorption of identical iron supplements manufactured to commercial specifications when these supplements were baked into rolls. As similar relationships were observed from the animal model data and the parallel human study, the further use of the animal model is recommended, especially in ways which may either decrease the need for human experimentation or pinpoint areas of critical concern for human study.

Aside from the chemical characteristics of individual iron salts, differences in the physical parameters of the specific salts themselves appear to affect absorption. Several physical forms of sodium iron pyrophosphate, ferric ammonium citrate, and reduced iron have been mentioned in studies of iron availability (Elwood, 1968; Fritz *et al.*, 1970; Hinton and Moran, 1967; Ranhotra *et al.*, 1971). Two important factors contributing to physical variation are the method of preparation and the particle size (Elwood, 1968). With regard to particle size, Hinton

et al. (1967) observed a relationship between particle size and solubility. The reduced iron used in this study was of a smaller particle size than the reduced iron used in the commercial product (Gerber Co., 1971). To the degree that larger particle size decreases availability, it would be assumed that the iron absorbed from the commercial reduced iron-supplemented product would be lessened.

It is obvious that each form of iron used for fortification should be standardized. Indeed, where inorganic iron is incorporated into food, the specific chemical and physical form of iron must be identified before one can gain an idea of its availability. Such acknowledgment is seldom given on commercial products and, even more unfortunately, such information is too frequently omitted from research reports, making comparisons impossible (Steinkamp *et al.*, 1955).

Of the iron salts which would be classed as available, an inverse relationship is seen between per cent absorption and level of fortification. Although the absolute amount of iron absorbed was greater when larger doses were ingested, it was accompanied by a decreased efficiency of utilization. This has been reported by others for rats (Bannerman, 1965; Bannerman *et al.*, 1962; Forrester *et al.*, 1962) and human infants (Garby and Sjolin, 1959; Schulz and Smith, 1958). In this study, when the dose of ferrous sulfate was increased 300% from 80 to 320 μg, only a 33% increase was observed in the absolute amount of iron absorbed. Thus, a well absorbed salt could be incorporated at a low level to furnish the iron required by an organism.

Certain aspects of iron absorption illustrated in this study should be taken into account by nutritional science and the food industry in dealing with human dietary iron problems and establishing guidelines to provide for effective food fortification. Specifically, variations in availability of various iron salts, further documented here, must be recognized. The value of an iron-containing food ought to be assessed as to its availability and not simply on the basis of total iron content. Guidelines requiring fortifying iron to be of types utilized efficiently should eliminate the addition of large amounts of unusable iron. Additionally, preparation of salts used in fortification should be standardized with regard to particle size as well as other key physical and chemical characteristics. As research yields data regarding food factors which facilitate iron absorption and verify the relative availability of various iron compounds to humans, guidelines should be revised to allow for practical implementation in terms of increasingly beneficial fortification programs.

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