Relative Biological Value of Iron Supplements in Processed Food Products

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Relative biological values (RBV) of elemental iron and ferric orthophosphate food grade iron supplements in processed breakfast cereals and a pasta product were determined by hemoglobin repletion bioassay using rats. In a bran flake cereal RBV's of 70, 32, and 25% were obtained for electrolytic, carbon monoxide reduced iron, and hydrogen reduced iron compared to a ferrous sulfate reference standard (RBV, 100%). Particle size distribution of elemental iron was inversely correlated with RBV. The RBV's of breakfast cereals supplemented with carbon monoxide reduced iron or ferric orthophosphate, and an enriched corn–soy–wheat pasta product containing carbon monoxide reduced iron were similar, ranging from 30 to 38% compared to ferrous sulfate (100%). In vitro solubility tests of food-grade samples of ferric orthophosphate in a 10% hydrochloric acid solution were correlated with RBV's obtained by rat bioassay.

Iron enrichment of foods has been the subject of considerable controversy. Attempts to fortify selected food products with iron in order to alleviate widespread iron-deficiency anemia have been unsuccessful as evidenced by results of dietary surveys (Ten State Nutrition Survey, 1968–1970); Health and Nutrition Examination Survey, 1974) which identified major population segments having below standard hemoglobin levels.

Despite diversified efforts with human clinical testing (Cook et al., 1973; Layrisse et al., 1969) in addition to iron bioassays with animal model systems (Fritz et al., 1974; Pla and Fritz, 1970, 1971; Shah and Belonje, 1973; Amine et al., 1972; Amine and Hegsted, 1974, 1975) relatively little information is available on the bioavailability of iron contained in processed food products. Waddell (1974) emphasized the importance of determining bioavailability of iron after processing, citing research reports that processing effects, plus basal ingredients of the processed food, can significantly alter the biological value of iron in food products (Theuer et al., 1971; Demott, 1971; Bing, 1972).

The objectives of these experiments were: (1) to assess iron assimilation of commercially available elemental iron powders, differing in method of production and resultant particle size, when added during processing to a ready-to-eat bran based breakfast cereal and fed to severely anemic animals; (2) to determine iron bioavailability from breakfast cereals and a pasta product containing iron supplements when the anemic state was controlled, so that test animals would not be severely depleted of iron body stores. In the products tested, approximately 90% of the iron content was supplemental iron with the balance occurring naturally in the food product ingredients.

EXPERIMENTAL SECTION

Experiment I. Male, weanling rats (ARS, Sprague Dawley, Madison, Wis.) weighing 40–50 g were assigned to the low iron depletion diet (4 ppm of Fe) of Shah and Belonje (1973) for a period of 4 weeks. Diet and distilled water were offered ad libitum throughout the study. Rats were individually housed in raised, open mesh, stainless steel cages in a temperature and humidity controlled environment. Tail blood samples were taken at 4 weeks depletion and hemoglobin and hematocrit determinations (Pla and Fritz, 1970) performed on each rat. The criterion for assignment to iron-containing cereal diets was based upon a hemoglobin value ≤6 g % with random assignment after blocking for hemoglobin.

Elemental iron powder samples were purchased from commercial suppliers. These iron powders were reduced by either carbon monoxide, hydrogen, or electrolytic reduction with the respective product having the particle size distribution described in Table I. Each iron sample was added to bran cereal during regular production runs at the cereal processing plant.

Table I. Particle Size Distribution of Iron Powders Produced by Three Different Commercial Methods[a]

Particle size, μm	Test samples: Electrolytic iron, %	Carbon monoxide reduced, %	Hydrogen reduced, %
>40	2.2	16.3	3.1
30–40	7.8	12.5	35.2
20–30	15.7	21.2	25.7
10–20	34.8	38.6	30.6
0–10	39.5	11.4	5.4

[a] Percent by weight retentions determined by subsieve roller and Tyler sieve analyses.

In order to evaluate processing effects on iron bioavailability, the iron powders were added at the dough stage of processing, then mixed, cooked, dried, and flaked. Test diets were formulated containing levels of iron-supplemented cereal to deliver 8, 16, and 32 ppm of Fe. Ferrous sulfate, monohydrate, reagent grade (assigned reference standard) was included in the low iron basal diet at levels of 6, 12, and 24 ppm of Fe. Iron analysis was performed on the iron-supplemented cereal products and repletion diets following dry ashing of samples by atomic absorption spectrophotometry. Following 2 weeks repletion feeding, hemoglobin and hematocrit regeneration was measured and analysis of response performed using slope ratio analysis (Finney, 1964). Food intake was measured during the repletion period to obtain data on iron consumption.

Experiment II. A similar protocol described for experiment I was used, except that the degree of anemia was controlled by regulating the length of the depletion period and test animals were assigned to repletion diets when hemoglobin was 8.8–9.0 g % Hb. The biological value of iron from commercially produced bran-based flaked cereal, oat-based flaked cereal, and an enriched macaroni product was measured using the bioassay described by Pla and Fritz (1970). The low iron basal diet containing 8 ppm of Fe was purchased from Teklad Mills, Madison, Wis. Repletion diets contained iron fortified products added to the low iron basal diet to deliver 20 ppm of Fe from the test product.

In Vitro Solubility Test. Two-gram aliquots of ferric orthophosphate and ferrous sulfate were placed in a 10% HCl solution and agitated for 10 min on a Burrell wrist

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Table II. Estimation of Relative Biological Value of Test Diets by Slope-Ratio Analysis Based on Hemoglobin Regeneration

Test diets	Regression coeff	Slope-ratio	Std error	95% confidence limits Lower	Upper
Reference standard, reagent grade ferrous sulfate monohydrate	1.054	1.000			
Bran cereal (BC) + electrolytic iron	0.860	0.816	0.039	0.739	0.893
BC + hydrogen reduced iron	0.379	0.359	0.036	0.288	0.430
BC + carbon monoxide reduced iron	0.359	0.341	0.029	0.283	0.398

action unit. Each sample was centrifuged and filtered through a tared Gooch crucible followed by three washings with distilled water. The crucible was dried for 1 h at 100°C, cooled, and weighed and the percent acid insolubles calculated.

RESULTS AND DISCUSSION

Experiment I. Biological value of iron from the bran-based breakfast cereal supplemented with three different commercial elemental iron powders was measured using a hemoglobin depletion–repletion type bioassay in rats. Figure 1 presents the regression lines fitted by slope ratio analyses following 2 weeks repletion feeding plotting iron intake against hemoglobin increment. The bran flake cereal diet containing the fine particle sized electrolytic iron powder was significantly higher in biological value ($P < 0.01$) as measured by hemoglobin regeneration than cereal diets 2 and 3 containing the coarser particle sized hydrogen and carbon monoxide reduced irons. Table II presents the relative biological values of test diets determined by measuring hemoglobin regeneration response in anemic rats using slope–ratio analysis. Table III gives the average value and standard errors for hemoglobin, hematocrit, weight gain, and biological value expressed from the slope ratios for each of the iron supplemented diets. As expected, hematocrit values following 2 weeks repletion feeding correlated highly ($r = 0.869$) with hemoglobin regeneration values. Cook et al. (1973) showed excellent absorption in humans of iron reduced by hydrogen and milled to an extremely fine particle size (5 to 10 μm). Our experiments support the concepts that biological value of elemental iron powder is highly correlated to fineness of particle size and method of production. Furthermore, it appears that neither processing effects nor the bran cereal matrix results in significant changes in the expected biological values of the added iron sources. In an earlier study when similar samples of elemental iron powder were assayed in semipurified diets, relative biological values were 65% for electrolytic, 23% for carbon monoxide, and 25% for hydrogen reduced iron samples. These values are not significantly different from those observed when the iron powders were commercially processed into bran-based cereals. In fact, these data suggest that at least for bran-based cereal, the combination of iron inherent in the bran plus added supplementary iron results in a trend toward higher biological value than for the iron samples alone. It would appear that future food fortification practices with elemental iron powders should relate to a meaningful standard based upon particle size distribution and method of production for the commercially available sources.

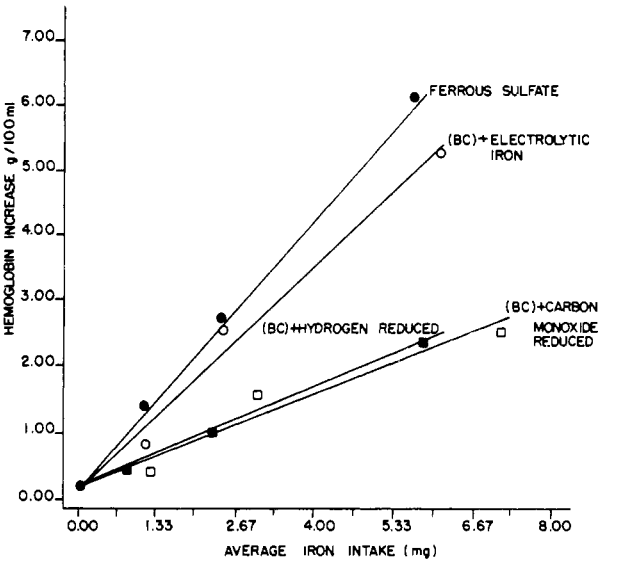

Figure 1. Slope-ratio dose-response lines observed with bran-based breakfast cereal (BC) containing three different elemental iron powders.

Experiment II. One of the objectives of this experi-

Table III. Mean Values for Hemoglobin, Hematocrit, Weight Gain, and Relative Biological Value for Each Iron Supplemented Cereal Diet following Two Weeks Repletion Feeding

Test diets	Iron dose, ppm	Two-week repletion data: Hemoglobin, g/100 ml	Hematocrit, %	Body wt gain, g	Rel biol value, %
Reference standard reagent grade ferrous sulfate monohydrate	6	6.4 ± 0.2[a]	30.0 ± 1.3	45 ± 2	100
	12	7.8 ± 0.2	34.9 ± 1.2	54 ± 2	
	24	11.3 ± 0.2	46.2 ± 1.3	58 ± 2	
Bran cereal (BC) + electrolytic iron	8	5.9 ± 0.3	29.0 ± 1.1	44 ± 3	82 ± 3.9
	16	7.6 ± 0.3	29.1 ± 0.9	55 ± 2	
	32	10.5 ± 0.2	39.4 ± 0.9	70 ± 2	
BC + hydrogen reduced iron	8	5.4 ± 0.2	25.5 ± 1.1	44 ± 2	36 ± 3.6
	16	6.0 ± 0.2	26.8 ± 0.7	49 ± 4	
	32	7.5 ± 0.2	33.5 ± 1.0	57 ± 1	
BC + carbon monoxide reduced iron	8	5.5 ± 0.2	25.2 ± 1.2	47 ± 2	34 ± 2.9
	16	6.7 ± 0.2	30.4 ± 0.6	58 ± 2	
	32	7.7 ± 0.3	35.6 ± 1.9	63 ± 3	
Low iron test diet	0	5.3 ± 0.2	24.6 ± 0.8	35 ± 2	

[a] Mean ± standard error.

Table IV. Relative Biological Value of Iron Supplements in Ready-to-Eat Breakfast Cereals and Pasta Product

Test diets	Iron dose, ppm	Two-week repletion data: Hemoglobin, g/100 ml	Two-week repletion data: Hematocrit, %	Rel biol value, %
Bran based flakes + ferric orthophosphate	20	11.0 ± 0.291[a]	31 ± 0.56	38
Oat based flakes + reduced iron[c]	20	10.7 ± 0.579	32 ± 0.92	37
Enriched corn-soy-wheat pasta + reduced iron[c]	20	10.5 ± 0.361	32 ± 1.25	30
Reference standard, reagent grade ferrous sulfate heptahydrate	20	13.5 ± 0.191	42 ± 0.86	100[b]
Low iron test diet	0	8.8 ± 0.307	25 ± 1.03	

[a] Mean ± standard error. [b] Reagent grade reference standard was included in diet at 10, 20, and 30 ppm of iron with subsequent hemoglobin regeneration response assigned a relative value of 100. [c] Carbon monoxide reduced iron.

Table V. Comparison of in Vitro Solubility Tests with Biological Values Assigned from Rat Bioassay

Sample	In vitro test: % soluble	In vitro test: % insoluble	Bioassay rel biol value, %
Ferrous sulfate heptahydrate reagent grade	100	0	100
Ferric orthophosphate #1	95.6	4.4	44
Ferric orthophosphate #2	99.0	1.0	33
Ferric orthophosphate #3	8.6	91.4	9
Ferric orthophosphate #4	5.8	94.2	4

ment was to determine bioavailability of commercial iron supplements from fortified ready-to-eat breakfast cereals and a pasta product when test animals were not severely depleted of hemoglobin. A criticism of the depletion–repletion bioassay method for iron bioavailability has been that severely anemic animals may exhibit an abnormal iron absorptive capacity, therefore, biological values assigned test iron supplements may be erroneous. Table IV lists the hemoglobin regeneration response, hematocrit, and relative biological values expressed for each product compared to a ferrous sulfate reference standard. Ferric orthophosphate and reduced iron (carbon monoxide) samples processed into the food products resulted in similar biological values. Processing did not decrease the relative biological value of the carbon monoxide reduced iron based on results from an earlier experiment in which we obtained an RBV score of 23 in a semipurified diet. The carbon monoxide reduced iron sample was a coarse particle sized material compared to the electrolytic grade iron included in experiment I. No significant differences in growth, hemoglobin, or hematocrit were found between rats fed processed food products containing carbon monoxide reduced iron or ferric orthophosphate supplements.

Identical products were not evaluated for iron bioavailability, in the two experiments. Therefore, a valid comparison cannot be made between the two different bioassay methods. However, we recommend the slope–ratio bioassay for a more precise estimation of iron RBV whereas the single point determination has utility for screening purposes.

Ferric orthophosphate has been used extensively in the fortification of food products. However, biological ratings assigned to ferric orthophosphate show high variation ranging from 0 to 50% of the potency of ferrous sulfate. In our laboratory we have found in vitro solubility tests of inorganic iron phosphate sources correlated rather well with biological values observed in subsequent animal tests. Table V shows a definite positive relationship between solubility of commercial samples of ferric orthophosphate in a 10% HCl solution and biological value. Recently Ammerman et al. (1974) have found a similar situation occurring with respect to ferrous carbonate. It is possible that a series of in vitro tests can be developed to estimate the biological values of inorganic iron compounds such as ferric orthophosphate. Also, it should be recognized that the wide variability in biological values assigned to iron phosphate by many experimentors may be due in part to ease of solubility of the compound in the acid medium of the upper portion of the gastrointestinal tract.

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LITERATURE CITED

Amine, E. K., Hegsted, D. M., *J. Agric. Food Chem.* **22**(2), 470 (1974).
Amine, E. K., Hegsted, D. M., *J. Agric. Food Chem.* **23**(2), 204 (1975).
Amine, E. K., Neff, R., Hegsted, D. M., *J. Agric. Food Chem.* **20**(2), 246 (1972).
Ammerman, C. B., Standish, J. F., Holt, C. E., Houser, R. H., Miller, S. M., Combs, G. E., *J. Anim. Sci.* **38**(1), 52 (1974).
Bing, F., *J. Am. Diet. Assoc.* **60**, 114 (1972).
Cook, J. D., Minnich, V., Moore, C. V., Rasmussen, A., Bradley, W. B., Finch, C. A., *Am. J. Clin. Nutr.* **26**, 861 (1973).
Demott, B. J., *J. Dairy Sci.* **54**, 1609 (1971).
Finney, D. J., "Statistical Method in Biological Assay", 2nd ed, Hafner Publishing Co., New York, N.Y., 1964.
Fritz, J. C., Pla, G. W., Harrison, B. N., Clark, G. A., *J. Assoc. Off. Anal. Chem.* **57**(3), 513 (1974).
Health and Nutrition Examination Survey, United States DHEW Publication No. (HRA) 74-1219-1, National Center for Health Statistics, Rockville, Md., Jan, 1974.
Layrisse, M., Cook, J. D., Martinez-Torrez, C., Roche, M., Kuhn, I. N., Walker, R. B., Finch, C. A., *Blood* **33**, 430 (1969).
Pla, G. W., Fritz, J. C., *J. Assoc. Off. Anal. Chem.* **53**, 791 (1970).
Pla, G. W., Fritz, J. C., *J. Assoc. Off. Anal. Chem.* **54**, 13 (1971).
Shah, B. G., Belonje, B., *Can. Inst. Food Sci. Technol. J.* **6**, 37 (1973).
Ten-State Nutrition Survey, United States DHEW Publication No. (HSM) 72-8134, Center for Disease Control, Atlanta, Ga., 1968–1970.
Theuer, R. C., Kemmerer, K. S., Martin, W. H., Zoumas, B. L., Sarret, H. P., *J. Agric. Food Chem.* **16**, 555 (1971).
Waddell, J., *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **33**(7), 1779 (1974).

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