Effect of Processing on Availability of Iron Salts in Liquid Infant Formula Products

Experimental Soy Isolate Formulas

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Pilot plant batches of liquid soy isolate infant formula products were prepared without added iron and with various added iron salts by standard commercial techniques. Three of the iron salts also were added without processing to lyophilized product made without added iron. Measured amounts of the lyophilized products were fed to anemic rats; hemoglobin responses were used to evaluate iron availability. Processing had little effect on the availability of ferrous sulfate, whereas it increased the relative availability of the iron of ferric pyrophosphate from 39 to 93, expressed as a percentage of reagent grade ferrous sulfate, and of sodium iron pyrophosphate from 15 to 66. The iron in a production batch of a commercially available soy isolate infant formula containing sodium iron pyrophosphate as the added iron salt had a relative availability of 77. The results indicate that the availability of iron added to foods should be determined only after normal processing.

The availability of the iron in the various iron salts used for food fortification has been a matter of controversy for many years. Nakamura and Mitchell (1943) found that the iron of sodium iron pyrophosphate and reduced iron was as well utilized as the iron of ferric chloride for hemoglobin regeneration in anemic rats. Steinkamp *et al.* (1955) reported that the absorption of iron from bread mixed with radioactive ferrous sulfate, reduced iron, ferric orthophosphate, or sodium iron pyrophosphate was similar, regardless of the form of iron added. Street (1943), on the other hand, indicated that the iron of sodium iron pyrophosphate was less than 50% as available as the iron of ferrous sulfate or ferric sulfate, whether the iron was given as such or baked into bread. Most recently, Fritz *et al.* (1970) reported that sodium iron pyrophosphate given as such had a relative availability, compared to ferrous sulfate, of 2–13% in anemic chicks and 11–19% in anemic rats.

The question of the availability of iron in infant formula products is especially important. Iron deficiency anemia is frequently found in infants and young children in the United States, especially in those from urban low income families. Anemia, defined as a hemoglobin level less than 10 g/100 ml, has been observed in 76% of infants in a Chicago study (Andelman and Sered, 1966) and in 41% (Haughton, 1963) and 25% (Hillman and Smith, 1968) of infants in two New York City studies; these infants were from low socioeconomic level families. Sturgeon (1959) found anemia in 7% of term infants from middle income families. Kripke and Sanders (1970) reported that 6% of rural Iowa infants less than 24 months old were anemic.

The Committee on Nutrition of the American Academy of Pediatrics (1969) states that the main causes of iron deficiency in infants are inadequate iron stores at birth due to prematurity and inadequate postnatal intake of iron. Due to the rapid rate of growth in the first 2 years of life, iron requirements in relation to calorie needs are greater than at any other age.

Satisfying the iron requirements of young infants can be accomplished most readily by iron fortification of infant formulas and the use of iron-enriched cereals (American Academy of Pediatrics, 1969). The two iron salts most often used to fortify infant formulas are ferrous sulfate and sodium iron pyrophosphate. Sodium iron pyrophosphate has the advantage of relative inertness from a food processing standpoint, but, as indicated above, the availability of the iron in this salt has been questioned.

Since the iron salt added to an infant formula is subjected to heat sterilization in an aqueous solution containing protein, carbohydrate, vitamins, and other minerals, the availability of this iron may be altered. To evaluate this, experimental soy isolate infant formula products were made with eight different iron salts, and the availability of the iron therein was evaluated by measuring hemoglobin regeneration in iron depleted rats. The effect of processing was assessed directly with three salts of differing availabilities as assayed in a dry state. These were sodium iron pyrophosphate, ferric pyrophosphate, and ferrous sulfate. These salts were incorporated into liquid products that were subsequently processed and lyophilized, and they also were added in the dry state to the lyophilized unfortified product.

A soy isolate formula was used for this study since Filer (1970) implied that only ferrous sulfate enrichment of a hypoallergenic protein beverage would provide appreciable amounts of absorbable iron. Moreover, when infants are changed to soy based formulas, they often have low iron stores. This is sometimes due to previous gastrointestinal blood loss because of whole cow's milk intolerance (Wilson *et al.*, 1964). The primary use of soy based formulas is the feeding of infants considered to be allergic to cow's milk protein (Owen, 1969).

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Table I. Composition of the Low Iron Basal Diet

Ingredient	g/100 g diet
Glucose[a]	61
Casein A.N.R.C.[b]	20
Corn oil[c]	10
Salt mixture[d]	4
Fiber[e]	4
Vitamin mixture[f]	1

[a] Cerelose 2401, Corn Products Co., New York, N.Y. [b] Sheffield Chemical Co., Union, N.J. [c] Mazola, Corn Products Co., New York, N.Y. [d] Bernhart and Tomarelli (1966), with ferric citrate omitted. [e] Nonnutritive cellulose, General Biochemicals, Inc., Chagrin Falls, Ohio. [f] AOAC (1965b) with choline bitartrate *in lieu* of choline chloride.

MATERIALS AND METHODS

Preparation of Soy Isolate Formulas. A batch of experimental concentrated (130 kcal/100 ml) soy isolate infant formula was separated into nine portions for addition of various iron salts, as shown below, and each was processed by standard commercial techniques in a pilot plant. Each batch of product was sterilized and filled aseptically into 240-ml (8 fl oz) cans. The average proximate composition of the formula was, in g per 100 ml: solids, 24.5; protein, 3.5; fat, 6.6; ash, 0.96; and carbohydrate by difference, 13.4 (sucrose, 8.0, and corn syrup solids, 5.4). A sufficient amount of each product was subsequently lyophilized and incorporated into one or more experimental diets.

Iron salts incorporated into each portion were as follows: Product O, no added iron salt; Product A, ferrous sulfate ($FeSO_4 \cdot 7H_2O$); Product B, ferric pyrophosphate; Product C, sodium iron pyrophosphate; Product D, ferrous citrate; Product E, ferric citrate; Product F, ferric gluconate; Product G, ferrous lactate; and Product H, ferric glycerol phosphate. Experimental Product O (with no added iron) contained 8.6 mg of iron per l. of concentrated formula. The experimental products with added iron provided 25 to 35 mg of iron per l. of concentrated formula. Samples of each salt were retained for later use.

A small amount of a regular commercial production batch of ProSobee Concentrated Liquid (Mead Johnson Laboratories, Evansville, Ind.) was lyophilized and also used in this study. The proximate composition of this product, was in g per 100 ml protein, 5.0; fat, 6.8; ash, 1.0; and carbohydrate, 13.5. It contained 22 mg of iron per l. of concentrated formula. Sodium iron pyrophosphate is the added iron salt.

Animals and Diets. Male weanling rats obtained from Laboratory Supply Company, Indianapolis, Ind., were housed individually in galvanized screen-bottomed cages and fed a basal diet (Table I) *ad libitum*. This diet contained by analysis 0.07 mg of iron per 10 g. After 32 days, hemoglobin levels averaged 4.6 g/100 ml. Rats with hemoglobin levels from 3.5 to 5.6 g/100 ml were then selected for the hemoglobin regeneration portion of this study.

Food intakes during the last week of the iron depletion period for all animals selected were greater than 10 g daily. Therefore, each animal was fed a weighed 10 g portion of diet daily in the hemoglobin regeneration portion of the study. For animals receiving standard supplements of ferrous sulfate, 0, 0.05, 0.10, 0.20, or 0.30 mg of iron as $FeSO_4 \cdot 7H_2O$ was added to 10 g of the basal diet. The animals in the other groups received 8 g of basal diet and 2 g of lyophilized test products. These were Products O, the eight products containing iron added before processing, or Product O into which three of these iron salts were dry blended after lyophilization.

The iron-supplemented rations were fed each day for 4 weeks. Blood was drawn from the orbital sinus at the first, second, and fourth week for hemoglobin and hematocrit determinations. After 4 weeks, the rats were sacrificed. The gastrointestinal tracts were removed and separated into four sections for dry weight determination (Fischer, 1957) to determine the effect of dietary iron on gastrointestinal tract weight. The iron content of the test diets was determined by a slight modification of the AOAC method (1965a). Hemoglobin was measured by the direct photometric method (Hawk *et al.*, 1954) and standardized by the method of Connerty and Briggs (1962). Hematocrit was determined after centrifugation in 1 mm diameter heparinized capillary tubes. The significance of differences was determined by analysis of variance or "t" test; honest significant differences were calculated where appropriate (Steel and Torre, 1960).

The availability of the total iron or the added iron in each formula product was determined relative to that of ferrous sulfate. Relative iron availability was calculated as the reciprocal of the ratio of the quantity of iron provided by the product or by the added iron salt to the quantity of iron as standard ferrous sulfate required to produce the same hemoglobin increase, and was expressed as percent of ferrous sulfate. Hematocrit increases gave similar availabilities as hemoglobin increases, so the latter measure was used in reporting relative availabilities.

RESULTS AND DISCUSSION

Response to Standard Ferrous Sulfate. Hemoglobin increases at the four low levels of iron supplementation closely approximated a straight line when plotted against iron intake (Table II). The hemoglobin response at the highest level of standard ferrous sulfate supplementation exceeded that of any other group, confirming that all evaluations of relative iron availability were made at suboptimal hemoglobin response levels. Hematocrit responses were similar to hemoglobin responses.

Increasing the level of ferrous sulfate supplementation of the basal diet significantly ($P < 0.01$) decreased total gastrointestinal weight. Small intestine weight was most affected, being 10 to 15% higher in the most anemic animals. Increased small intestine weight in iron-deficient animals has not been previously reported. Kimber and Weintraub (1968) found decreased cytochrome oxidase and lactase levels in the small intestinal mucosa of chronically iron deficient dogs. Naiman *et al.* (1964) described abnormal small bowel functions in some iron-deficient children. However, Hoffbrand and Broitman (1969) found no difference in the histological appearance of the intestinal mucosa between iron-deficient and control dogs.

Processing and Iron Availability in Infant Formulas. The effect of processing was evaluated with three salts of widely divergent availabilities: ferrous sulfate, ferric pyrophosphate, and sodium iron pyrophosphate (Table III). Product O was not fortified with iron but contained a significant amount of iron which came primarily from the protein source, soy protein isolate. The relative availability of this iron was 86%. This agreed with the findings of Fritz *et al.* (1970), who reported relative biological values of 70 and 125% for iron in isolated soybean protein.

Processing had a slight effect on the availability of the iron of ferrous sulfate in this experimental product (Table III). The hemoglobin response of anemic rats fed Product A, containing processed ferrous sulfate, was significantly ($P < 0.05$) greater than that of rats fed the iron-free Product O

Table II. Hemoglobin and Hematocrit Responses and Gastrointestinal Organ Weights of Anemic Rats Fed Graded Levels of Iron as Ferrous Sulfate

Added iron, mg/day Total iron intake, mg/day	0.00 0.07	0.05 0.12	0.10 0.17	0.20 0.27	0.30 0.37	F[a]	Honest Significant Difference P < 0.05	 P < 0.01
Hemoglobin increase, g/100 ml	1.1 ± 0.6[b]	2.4 ± 0.7	4.1 ± 1.3	7.4 ± 0.9	8.9 ± 0.9	134.13	1.2	1.4
Hematocrit increase, % P.C.V.	9 ± 2	13 ± 3	17 ± 4	27 ± 3	29 ± 3	81.20	4	5
Gastrointestinal organ weights[c]								
Stomach, mg	356 ± 44	324 ± 28	329 ± 27	323 ± 28	334 ± 24	1.88	40	48
Small intestine, mg	1382 ± 140	1275 ± 131	1213 ± 92	1228 ± 125	1182 ± 79	4.52	148	180
Cecum, mg	154 ± 46	138 ± 32	160 ± 36	130 ± 27	138 ± 21	1.44	42	52
Colon plus rectum, mg	252 ± 47	205 ± 30	263 ± 57	209 ± 25	252 ± 56	3.67	57	70
Final body weight, g	214 ± 18	223 ± 18	224 ± 22	230 ± 15	230 ± 18	1.32	23	28

[a] Statistical significance is as follows: F > 2.58; P < 0.05; F > 3.77; P < 0.01. [b] Mean given with standard deviation. [c] Dry basis.

Table III. Effect of Processing on Iron Availability in Soy Isolate Infant Formula Products

Iron salt added	None	Ferrous Sulfate		Ferric Pyrophosphate		Sodium Iron Pyrophosphate		
Experimental formula fed[a]	Product O	Product A	Product O[b]	Product B	Product O[b]	Product C	Product O[b]	Commercial Product[c]
Processed with added iron	...	Yes	No	Yes	No	Yes	No	Yes
Total formula iron, mg/day	0.051	0.216	0.224	0.201	0.231	0.239	0.249	0.151
Iron from added iron salt, mg/day	None	0.165	0.173	0.150	0.180	0.188	0.198	...[c]
Four-week hemoglobin increase, g 100 ml	1.9 ± 0.5[d]	7.6 ± 0.6	7.0 ± 0.7[e]*	6.6 ± 0.2	4.3 ± 0.4**	6.0 ± 0.9	2.9 ± 0.9**	4.2 ± 0.8
Four-week hematocrit increase, % P.C.V.	11 ± 2	27 ± 2	26 ± 2	23 ± 6	18 ± 4*	23 ± 2	13 ± 4**	17 ± 3
Relative iron availability[f]								
Total formula iron	86	101	89	92	49	71	28	77
Iron from added iron salt	...	106	90	93	39	66	15	...[c]

[a] Two grams of lyophilized product were fed daily. [b] The added iron salt was dry-blended into lyophilized Product O. [c] ProSobee Concentrated Liquid, Mead Johnson Laboratories, Evansville, Ind. The precise distribution by source of the iron in soy isolate infant formula products is not calculable from label information and is not given. However, it can be estimated for this particular sample as 0.07 mg/day from endogenous sources (soy protein isolate) and 0.08 mg/day from sodium iron pyrophosphate. [d] Mean given with standard deviation. [e] Asterisks indicate that the difference between the means for rats fed Product O with iron and rats fed the Product processed with added iron was statistically significant by "t" test (Steel and Torre, 1960); *, P < 0.05; **, P < 0.01. [f] Percent of ferrous sulfate.

with dry blended ferrous sulfate, even though the former rats had a slightly smaller iron intake. Hematocrit was not affected. The relative availability of the added iron in processed Product A was 106, and that of the same ferrous sulfate dry blended into Product O was 90, or substantially the same as that of the ferrous sulfate standard. Woodruff (1959) found that infants absorb ferrous sulfate added to milk as well as ferrous sulfate administered alone. Fritz *et al.* (1970) found the relative biological value of the iron of ferrous sulfate was 110 when ferrous sulfate was dissolved in evaporated milk, and 95 when ferrous sulfate was dissolved in skim milk.

Product B contained ferric pyrophosphate as the added iron. Rats fed the supplement of lyophilized Product B had significantly (P < 0.01) greater hemoglobin increases and significantly (P < 0.05) greater hematocrit increases than rats fed lyophilized Product O to which ferric pyrophosphate was added in the dry state (Table III). Processing increased the relative availability of the iron of ferric pyrophosphate from 39 to 93. The availability of the total formula iron was similarly affected. The relative iron availability of 39 for ferric pyrophosphate agreed closely with the relative biological value of 45 (range 38–52) reported by Fritz *et al.* (1970) for this salt.

Sodium iron pyrophosphate is a source of iron commonly used in flour enrichment (Steinkamp *et al.*, 1955) and in infant formula products. Product C was processed with sodium iron pyrophosphate as the iron source. Anemic rats fed a supplement of this product had an average hemoglobin increase of 6.0 g/100 g (Table III), compared to an increase of only 2.9 g/100 ml when anemic rats were fed Product O into which sodium iron pyrophosphate was dry blended. The difference was statistically significant (P < 0.01). Processing increased the relative iron availability of sodium iron pyrophosphate from 15 to 66 and the relative availability of total formula iron from 28 to 71.

The relative iron availability of 15 for unprocessed sodium iron pyrophosphate was very similar to relative biological values of 11 and 19 in anemic rats reported by Fritz *et al.* (1970). The relative iron availability of 77 found for the iron in the commercial product made with sodium iron pyrophosphate confirmed that processing an infant formula markedly improves the availability of the iron of sodium iron pyrophosphate.

Availability of Iron in Formulas Made with Other Salts. The hemoglobin and hematocrit responses of anemic rats fed supplements of lyophilized Products D through H are

Table IV. Availability of Iron in Soy Isolate Infant Formula Products made with Organic Iron Salts

Iron salt added	Ferrous Citrate	Ferric Citrate	Ferrous Gluconate	Ferrous Lactate	Ferric Glycerol Phosphate
Experimental formula fed[a]	Product D	Product E	Product F	Product G	Product H
Total formula iron, mg/day	0.292	0.325	0.287	0.280	0.255
Four-week hemoglobin increase, g/100 ml	7.3 ± 1.0[b]	7.9 ± 1.1	6.5 ± 1.3	7.8 ± 0.9	6.5 ± 1.3
Four-week hematocrit increase, % P.C.V.	28 ± 4	26 ± 3	24 ± 5	27 ± 2	24 ± 5
Relative iron availability[c]					
Total formula iron	89	87	79	100	92
Iron from added iron salt	89	87	78	104	95

[a] Two grams of lyophilized product were fed daily. [b] Mean given with standard deviation. [c] Percent of ferrous sulfate.

presented in Table IV. Ferrous citrate and ferric citrate and the experimental product made with each had similar iron availabilities: 87–89% of ferrous sulfate. In contrast, Brise and Hallberg (1962) found that ferric citrate was absorbed half as well as ferrous citrate when fed as such.

Fritz *et al.* (1970) reported a relative value of 97 for ferrous gluconate. The added iron in Product F, made with ferrous gluconate, had a relative availability of only 78. As a source of iron, ferrous lactate had a relative availability of 104, which compares very favorably with the finding of Brise and Hallberg (1962) that ferrous lactate is absorbed 106% as well as ferrous sulfate. Ferric glycerol phosphate had a relative availability of 95; Fritz *et al.* (1970) found it had a relative biological value of 93 (range 86–100).

The improvement in availability of some forms of iron when processed in this soy isolate formula could be due to a number of factors, including the other ingredients in the formula and the heat applied during sterilization. The experimental and commercial formulas contained sucrose and dried corn syrup (corn syrup solids). Corn syrup is partially hydrolyzed corn starch and is composed of glucose, maltose, maltotriose, and higher dextrins. Iron can form higher stable complexes with simple sugars and polyols (Charley *et al.*, 1963). Fructose sequesters ferric iron most strongly, but glucose, maltose, lactose, and sucrose also sequester iron. The iron in the fructose-ferric iron complex is almost twice as absorbable as the iron of ferrous sulfate (Stitt *et al.*, 1962). Modifying or eliminating the carbohydrate component of liquid formula could markedly affect the availability of iron from iron salts such as sodium iron pyrophosphate.

Processing conditions may also affect the extent to which the availability of the iron in a salt utilized poorly *per se*, such as sodium iron pyrophosphate, is increased by processing. The commercial formula examined in this study was previously shown by Theuer and Sarett (1970) to have a much lower trypsin inhibitor content than other commercial soy isolate formulas. The levels of trypsin inhibitor in a soy product is an index of the adequacy of heat treatment (VanBuren *et al.*, 1964). The experimental products studied here were processed in the same way as this commercial product; it remains to be seen whether lesser degrees of heat treatment have different effects on iron availability.

It is generally accepted that food iron is only 16–20% as available as the iron in a ferrous salt (NAS-NRC, 1968). Since the iron in the experimental soy isolate formulas processed with various iron salts was about 70 to 100% as available as ferrous sulfate, and the iron in the commercial soy isolate infant formula product had a relative availability of 77, it appears safe to assume that soy isolate infant formulas such as these, which supply more than the recommended dietary allowance of iron for young infants (NAS-NRC, 1968), provide the infant with generous levels of absorbable iron. Similar studies are now in progress on milk-based formulas processed with various iron salts.

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