Biological Availability in Animals of Iron from Common Dietary Sources

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Iron sources that are, or might be, used for fortification of feeds and foods were examined by the hemoglobin repletion technique with anemic chicks and rats. Similar results were obtained with each species. Reagent grade $FeSO_4 \cdot 7H_2O$ was used as the reference standard, and relative biological values for other iron sources were expressed as a percentage of the response to ferrous sulfate. Iron compounds studied were found to have relative values ranging from 0 to about 107. Food sources of iron were less well utilized than the more available inorganic iron sources. The influence of other dietary components was minor compared with the influence of the iron source. Provided that there was some availability, increased dietary levels of the poorly utilized iron sources were effective for the cure of iron-deficiency anemia.

Nutritional anemia is one of the most prevalent deficiency diseases in the U.S. (Finch *et al.*, 1968; Goldsmith, 1965; Gutelius, 1969; Schaefer, 1969) and throughout the world (Blanc *et al.*, 1968). The incidence is highest in young children and in women during their fertile years. Hemoglobin values below 10 g per 100 ml of blood, and hematocrit below 31.5% packed cell volume are considered abnormal by most investigators. There has been no improvement in the situation among children during the past 30 years (Gutelius, 1969). It is difficult to assess the effect of low hemoglobin and hematocrit values on the health of the affected individual. There is at least statistical evidence that anemic individuals have more frequent and more serious infectious diseases (Andelman and Sered, 1966; Bothwell and Finch, 1962; MacKay, 1928). A deficiency of dietary iron may lead to tissue depletion of iron-containing or iron-dependent enzymes and may cause secondary phenomena, including malabsorption (*Nutr. Rev.*, 1969). An iron-deficiency state may exist before reduced hemoglobin and hematocrit are apparent (Sood *et al.*, 1968).

Although anemia may result from many different causes, the form most frequently encountered is iron-deficiency anemia (Filer, 1969; Finch, 1969; Woodruff, 1969). The USDA's Agricultural Research Service (1969) estimates that, on the average, infants under 3 years of age and women under 55 years of age consume only about half of the recommended dietary allowance of iron (NAS, 1968).

Changing food habits have reduced the dietary intake of iron. Little food is cooked in iron pots that normally contribute substantial quantities of iron to the food (Peden, 1967). Another factor is the use of short extraction flour and other cereals. For example, Watt *et al.* (1963) note that while whole wheat flour contains about 33 mg of iron per kg, patent flour contains only about 8 mg. The cereal enrichment program aims to restore to the refined cereal products the whole grain levels of iron and certain B vitamins. The standards for enriched cereals (Code of Federal Regulations, 1969) state that the supplemental iron shall be a source that is harmless and assimilable. No criteria are given for determining whether or not the iron actually is assimilable.

Controversy has arisen over the effectiveness of different iron compounds that have been used for food fortification. Steinkamp *et al.* (1955) considered iron supplied as ferrous sulfate, reduced iron, ferric orthophosphate, and sodium iron pyrophosphate to be about equally effective, whereas others found more variation in the availability of iron from different sources (Ammerman *et al.*, 1967; Blumberg and Arnold, 1947; Freeman and Burrill, 1945; Hinton and Moran, 1967; Nakamura and Mitchell, 1943; Street, 1943). Hinton and Moran (1967) found considerable difference in availability of different samples of reduced iron. Harmon *et al.* (1967, 1968) found ferric ammonium citrate and ferrous sulfate to be about equally effective for preventing anemia in young pigs, but ferrous carbonate was less effective. Ammerman *et al.* (1969) showed that availability of ferrous carbonate was correlated with *in vitro* solubility. A "syrup" containing ferrous carbonate was reported to be an effective hematinic (Djafari and Kettler, 1969). In most cases where no apparent differences were found in availability of iron from various sources, the actual utilization of iron was very low by nonanemic individuals (Harrill *et al.*, 1957). This may give rise to erroneous conclusions.

Food sources of iron are less well utilized than many inorganic iron salts (Hussain *et al.*, 1965; Narula and Wadsworth, 1968; Underwood, 1962). Many factors are reported to influence the absorption of dietary iron (Brise, 1962; Brise and Hallberg, 1962; British Ministry of Health, 1968; Greenberg *et al.*, 1957; Greenberger and Ruppert, 1966; Herndon *et al.*, 1958; Kaufman *et al.*, 1966; Reddy *et al.*, 1965; Smith and Medlicott, 1944; Tucker *et al.*, 1957).

The purpose of this study was a critical examination of the biological availability of iron from various sources. Special attention was directed to iron compounds that are, or that might be, used for food fortification.

MATERIALS AND METHODS

The criteria used to judge availability of dietary iron were the repletion of hemoglobin and hematocrit in young chicks and rats made anemic on a low iron diet (Pla and Fritz, 1970). Most lots of basal diet contained about 7.2 mg of iron per kg. Reagent grade ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was used as the reference standard, and the quantity of iron furnished by the sample was compared to the quantity of iron furnished by the ferrous sulfate that was required to produce the same response in terms of hemoglobin and packed cell volume. All comparisons were made at suboptimal levels of response.

Except when reagent grade chemicals were used at their theoretical iron content, samples were analyzed for iron content by the AOAC method (1965). They were then added to the test diets in quantities required to furnish the desired iron contribution to the diet. When only small quantities of supplement were required, the test samples replaced a small portion

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Table I. Comparison of Availability of Iron to Anemic Chicks and Anemic Rats

Iron Source	Relative Biological Values[a]	
	Chicks	Rats
Ferric ammonium citrate	115	98
Ferric orthophosphate #1	18	12
Ferric orthophosphate #2	9	12
Ferric orthophosphate #3	12	30
Ferric sulfate	65	100
Ferric oxide	4	6
Ferrous carbonate #1	2	1
Ferrous carbonate #2	2	0
Ferrous carbonate #3	6	0
Ferrous carbonate #4	2	2
Fish protein concentrate	22	53
Reduced iron #1	59	34
Reduced iron #2	41	16
Reduced iron #3	66	36
Reduced iron #4	43	37
Sodium iron pyrophosphate #1	2	11
Sodium iron pyrophosphate #2	13	19
Trace mineral mix (commercial)	14	21

[a] Relative biological value = (100 × mg Fe/kg from $FeSO_4$)/(mg Fe/kg from sample) to give equal curative effect.

of the whole basal diet. When large quantities of the samples were needed to furnish the desired level of supplemental iron, the samples were added in place of dried skim milk, degermed corn meal, and glucose monohydrate (singly or in combination) to maintain protein and energy levels of the test diets at comparable levels.

Hemoglobin was determined by the method of Crosby *et al.* (1954) and hematocrit was determined as described by Cohen (1967). Where appropriate, the *t* test was used to measure significance of differences, and least significant differences were calculated (Snedecor, 1956).

In most cases, availability of the iron was expressed in terms of relative biological value, to permit comparisons between tests

$$\text{relative biological value} = 100 \times \frac{\text{mg Fe/kg from } FeSO_4}{\text{mg Fe/kg from sample}}$$

to give equal curative effect. Calculations of the actual utilization of iron for the formation of new hemoglobin were made on the assumption that 6.7% of the body weight of the rat was blood, and that hemoglobin contained 3.35 mg iron per g (Greenberg *et al.*, 1957). Various groups, on levels of supplemental iron between 5 and 20 mg per kg of diet, furnished by $FeSO_4 \cdot 7H_2O$ and corrected for the response to iron in the basal diet, utilized from 45 to 51% of the iron supplied for the formation of new hemoglobin.

RESULTS AND DISCUSSION

Comparison of Chicks and Rats as Test Animals. Day-old chicks and weanling rats were chosen as test animals because of their value in previous studies on iron absorption and metabolism, and the possibility that a similarity of response to a specific iron compound in such dissimilar species would favor their acceptability as models of the human in this respect. Accordingly, a series of iron sources were tested with anemic chicks and with anemic rats. The results are summarized in Table I.

While some differences are apparent, agreement between the responses by the two species is generally good. In no instance was a compound poorly utilized by one species and well utilized by the other. Neither species gave a consistently higher relative biological value than the other.

Chickens and rats differ in many respects. In addition to the obvious differences in metabolism, they have widely differing hematological characteristics. Normal values for the chicken are hemoglobin from 7.3 to 12.9 g per 100 ml (average 10.3) and hematocrit from 24 to 43.3% packed cell volume (average 35.6). Normal values for the rat are hemoglobin from 12 to 17.5 g per 100 ml (average 14.8) and hematocrit from 39 to 53% packed cell volume (average 46) (Spector, 1956). Human levels are similar to those for rats (Devine, 1967).

The day-old chicks depleted much more rapidly than the weanling rats. Severe anemia developed within 2 weeks in the chicks and in 4 to 5 weeks in the rats. This is believed to reflect iron in the maternal diet consumed by the young rats, and not to indicate a species difference.

Preliminary studies, involving increase in plasma iron, following ingestion of test doses by human volunteers, indicate good agreement with the animal feeding results. Expressed as a percentage of the increase that resulted from the same quantity of iron furnished by ferrous sulfate, the following values were observed: ferric orthophosphate 7, ferrous carbonate 4, reduced iron 26, and sodium iron pyrophosphate 7.

Availability of Iron from Dietary Sources. Repletion tests were made on 21 iron compounds and on 14 food sources of iron. The results are summarized in Table II. These include values obtained with chicks and with rats, and include the data shown by species in Table I. Where more than one test was made on a given source, the range of relative biological values is also shown. It should be noted that this range includes both the variation between repeated tests on the same sample and variation between samples when several samples of a given material were studied.

In several cases relative biological values were rounded off to 100, either because the data calculated from hemoglobin and from hematocrit bracketed the 100 figure, or because there were no reference groups in the test that permitted numerical valuation of responses above those obtained with the sample. A typical example is the case of the feed grade ferrous sulfate. This material at a dietary level to furnish 20 mg of iron per kg of diet gave an average hemoglobin value of 10.51 g per 100 ml and an average hematocrit value of 43.9%. The comparable values for the reference reagent grade ferrous sulfate were 10.53 g per 100 ml and 42.7%, respectively.

These observations support the general view that inorganic iron compounds are better utilized than food iron.

Insufficient comparisons were made between similar salts of di- and trivalent iron to confirm the frequently held view that the former are better utilized (Brise and Hallberg, 1962; Brown, 1963). Elwood (1965) has stated that the rat uses ferric and ferrous salts equally well but that man does not. We found that ferrous chloride and ferrous sulfate were somewhat better utilized by both rats and chicks than the comparable ferric salts.

Among the food sources of iron, there is no clear distinction in availability between the animal foods and the vegetable foods. This differs from the views expressed by Blanc *et al.* (1968) and Layrisse *et al.* (1968), who considered foods of animal origin to have more of the iron available.

The iron furnished by egg yolk was fairly well utilized in these tests. This is of interest because of conflicting reports in the literature. Lowe *et al.* (1969), Moore (1965), Schulz and Smith (1958), and Woodruff (1959) found the iron in eggs to be available. In contrast, a number of workers have found

Table II. Relative Biological Value of Iron from Various Dietary Sources

Iron Source	No. Samples	Relative Biological Value[a] Average	Range[b]
Iron Compounds			
EDTA, dihydrogen ferrous salt	1	99	97–100
Ferric ammonium citrate	1	107	98–115
Ferric choline citrate	1	102	
Ferric chloride	1	44	26–67
Ferric citrate	1	73	70–76
Ferric glycerophosphate	1	93	86–100
Ferric pyrophosphate	1	45	38–52
Ferric orthophosphate	4	14	7–32
Ferric oxide	1	4	0–6
Ferric sulfate	1	83	65–100
Ferrous ammonium sulfate	1	99	99–100
Ferrous carbonate	5	2	0–6
Ferrous chloride	1	98	
Ferrous fumarate	1	95	71–133
Ferrous gluconate	1	97	
Ferrous sulfate ($FeSO_4.7H_2O$)[a]	1	100	
Ferrous sulfate, anhydrous	1	100	
Ferrous sulfate, feed grade	1	100	
Ferrous tartrate	1	77	70–83
Reduced iron	6	37	8–66
Sodium iron pyrophosphate	3	14	2–23
Food and Feed Ingredients			
Biscuits with ferrous sulfate	1	89	77–100
Blood meal	1	35	
Corn meal enrichment mix[c]	1	46	
Corn germ	1	40	
Egg yolk	1	33	
Fish protein concentrate	2	28	8–53
Enriched breakfast cereal[c]	1	43	
Enriched flour[c]	1	32	
Oat flour	1	21	
Smectite-vermiculite	1	11	3–17
Soybean protein (isolated)	2	97	70–125
Trace mineral mix (commercial)[d]	2	12	0–21
Wheat germ	1	53	

[a] See footnote a, Table I. [b] Lowest and highest values are shown where more than one availability test was made. Note that this reflects variation both between samples and between repeated determinations on the same sample. [c] Fortified with reduced iron. [d] Fortified with ferrous carbonate.

that the iron in eggs is unavailable and that the presence of eggs in the diet interferes with the utilization of iron from other sources (British Ministry of Health, 1968; Elwood, 1968; Elwood *et al.*, 1968; Narula and Wadsworth, 1968).

A biscuit mix was fortified with enough ferrous sulfate to furnish 176 mg of iron per kg, prior to baking. The relative biological value of the iron in the resulting baked biscuits was 77 and 100, respectively, in two tests with chicks. This compared favorably with the arbitrary value of 100 when the ferrous sulfate was added directly to the test diet.

Attention is also directed in Table II to the relative biological values found for the various foods fortified with reduced iron. In all cases these values were within the range found for reduced iron when this material was added separately to the test diet.

Influence of Dietary Protein. In their review articles, Coons (1964) and Layrisse *et al.* (1968) noted that low protein diets interfered with iron uptake. A test was made to compare diets with 10% protein and 20% protein fed to anemic rats. The data are summarized in Table III. With either a well-utilized compound (ferrous sulfate) or a poorly utilized compound (ferrous carbonate), hemoglobin and hematocrit increased numerically when the diet contained the higher protein level. The effect seemed to be greater in the case of the better utilized iron source, but the differences were not quite statistically significant.

Table III. Effect of Dietary Protein on Response of Rats to Supplemental Iron

Supplemental Iron Source	mg Fe/kg	Protein %	Hemoglobin (g/100 ml)	Hematocrit (% P.C.V.)
None	0	10	5.40	25.0
Ferrous sulfate	10	10	7.94	37.0
Ferrous sulfate	10	20	8.67	39.7
Ferrous sulfate	20	10	11.36	47.6
Ferrous carbonate	20	10	5.08	25.0
Ferrous carbonate	20	20	5.48	25.2

Table IV. Effect of Ascorbic Acid on Utilization of Iron by Chicks

Iron Supplement Source	mg Fe/kg	Ascorbic Acid (200 mg/kg)	Hemoglobin (g/100 ml)	Hematocrit (% P.C.V.)
None	0	–	2.82	16.8
Ferrous sulfate	5	–	4.76	24.3
Ferrous sulfate	10	–	6.75	28.1
Ferrous sulfate	15	–	6.96	28.7
Ferrous sulfate	20	–	7.79	30.9
Smectite-vermiculite	20	–	3.05	18.8
Smectite-vermiculite	20	+	3.80	21.7
Ferrous carbonate	20	–	3.29	19.9
Ferrous carbonate	20	+	2.90	18.1
Ferric oxide	20	–	2.78	18.0
Ferric oxide	20	+	3.29	18.6

Influence of Ascorbic Acid and Vitamin E. Many workers have reported that ascorbic acid improves the absorption and utilization of iron (Apte and Venkatachalam, 1965; Banerjee and Chakrabarty, 1965; Brise and Hallberg, 1962; Greenberg *et al.*, 1957; McCurdy and Dern, 1968). Brise and Hallberg (1962) showed that the effect was in the digestive tract, and that intravenous ascorbic acid had no effect. Greenberg *et al.* (1957) reported that the combination of ascorbic acid and vitamin E improved the utilization of dietary iron more than did either vitamin alone. A few laboratories have reported no such effects. Chaney and Barnhart (1964) found that addition of ascorbic acid, sorbitol, and vitamin E did not increase iron absorption by baby pigs. Similarly, in studies with chicks, Hill and Starcher (1965) observed that ascorbic acid had no effect on hemoglobin with or without supplemental iron.

Two chick tests were made to study the influence of ascorbic acid and DL-α-tocopheryl acetate on the utilization of iron. Table IV summarizes the data on availability of iron from three poorly utilized compounds when 200 mg of ascorbic acid was added per kg of diet. There were no significant differences due to the addition of ascorbic acid to the diet. Table V summarizes results on the relative biological values for ferrous sulfate and for ferric orthophosphate in the presence and absence of added ascorbic acid and vitamin E, alone and in combination. Hemoglobin response increased ($P = .01$) when the combination was fed with ferrous sulfate, but there was no improvement in response to either vitamin alone when ferrous sulfate was fed, or to any supplement when the iron source was the poorly utilized ferric orthophosphate.

Table V. Effect of Dietary Ascorbic Acid and Vitamin E on Utilization of Supplemental Iron

Iron Supplement Added to Diet	Vitamin E (60 mg/kg)	Ascorbic Acid (200 mg/kg)	Relative Biological Value of Iron[a]
None	−	−	0
Ferrous sulfate	−	−	100
Ferrous sulfate	+	−	97
Ferrous sulfate	−	+	82
Ferrous sulfate	+	+	133[b]
Ferric orthophosphate	−	−	9
Ferric orthophosphate	+	−	7
Ferric orthophosphate	−	+	9
Ferric orthophosphate	+	+	10

[a] See footnote a, Table I. [b] Significantly greater than group 2 (ferrous sulfate without added ascorbic acid or vitamin E). Other values do not differ significantly from corresponding control group.

Table VI. Effect of Miscellaneous Foodstuffs on the Utilization of Supplemental Iron

Material Added to Diet or Treatment of Sample Before Mixing into Diet	Relative Biological Value[a] Ferrous Sulfate	Ferric Orthophosphate
Direct addition of iron salt	100	9
$FeSO_4$ mixed with biscuit mix and baked biscuits tested	89	
$FeSO_4$ dissolved in evap. milk	110	
$FeSO_4$ dissolved in skim milk	95	
10% cellulose	92	38[b]
10% lactalbumin	83	
10% soy protein #1	88	
10% soy protein #2	79	
10% gelatin	89	12
5% dried whole egg	100	
15% dried egg white	79	

[a] See footnote a, Table I. [b] Significantly greater than the corresponding control group. Other differences were not significantly different from appropriate control group.

Table VII. Effect of Increased Dietary Levels of Poorly Utilized Sources of Supplemental Iron

Iron Supplement Compound	mg Fe/kg	Hemoglobin (g/100 ml)	Hematocrit (% P.C.V.)
First Test, with Chicks, on Ferrous Carbonate with Relative Biological Value = 0			
None	0	3.44	19.8
Ferrous sulfate	5	4.76	24.1
Ferrous sulfate	10	6.19	27.6
Ferrous sulfate	15	7.65	31.1
Ferrous carbonate	15	3.29	18.9
Ferrous carbonate	150	3.40	20.0
Second Test, with Chicks, on Reduced Iron with Relative Biological Value = 43			
None	0	2.84	15.4
Ferrous sulfate	5	4.66	22.3
Ferrous sulfate	10	5.48	27.0
Ferrous sulfate	15	6.58	27.5
Ferrous sulfate	20	7.68	28.9
Reduced iron	20	5.50	27.8
Reduced iron	40	6.86	28.8
Reduced iron	80	7.32	30.3
Third Test, with Rats, on Ferric Orthophosphate with Relative Biological Value = 15			
None	0	4.98	26.0
Ferrous sulfate	10	7.88	36.4
Ferrous sulfate	20	9.82	44.4
Ferrous sulfate	50	13.10	52.0
Ferric phosphate	20	5.66	29.6
Ferric phosphate	40	5.83	31.0
Ferric phosphate	80	11.05	45.2
Fourth Test, with Rats, on Sodium Iron Pyrophosphate with Relative Biological Value = 19			
None	0	4.76	27.0
Ferrous sulfate	10	6.91	36.1
Ferrous sulfate	20	8.62	42.2
Sodium iron pyrophosphate	20	5.67	30.8
Sodium iron pyrophosphate	40	6.50	32.2
Sodium iron pyrophosphate	80	7.18	36.0

Effect of Miscellaneous Foodstuffs. Brise (1962) reported that food generally interfered with the utilization of supplemental iron given simultaneously. The results of a series of tests in which various foodstuffs were mixed with the iron salt, or added to the basal diet, are summarized in Table VI. In those cases where a significant quantity of iron was present in test substance, the same quantity of the material was added to the basal diet and to the test diet that contained the iron supplement.

There was no significant difference when ferrous sulfate was (1) added directly to the basal diet; (2) incorporated into a biscuit mix, baked, and then fed in the form of the biscuits; (3) dissolved in evaporated milk before addition to the test diet; or (4) dissolved in skim milk before addition to the test diet. Leichter and Joslyn (1967) reported that iron in bread was found largely in the ferric state, regardless of the form in which it is added before baking. Their *in vitro* tests did not show reduced availability.

Dissolving ferrous sulfate in either evaporated milk or skim milk did not influence its availability to anemic chicks. This agrees with the report by Woodruff (1959) that infants absorb ferrous sulfate added to milk just as efficiently as when the ferrous sulfate is given alone.

Additlon of high protein foodstuffs to the diet did not have much effect on the availability of iron added in the form of ferrous sulfate. The results were essentially the same when lactalbumin, soy protein, gelatin, dried whole egg, or dried egg white was used. In most cases, the relative biological values tended to be lower in the presence of the high protein foodstuff. Kuhn *et al.* (1968) have reported that chelates interfere with the absorption of food iron. Most of the amino acids are effective chelating agents. Of the high protein foodstuffs, only gelatin was tested with ferric orthophosphate and it had little or no effect on the utilization of iron from this source.

Addition of 10% cellulose to the diet had little effect on availability of iron from ferrous sulfate, but it did improve the utilization of iron furnished by ferric orthophosphate. Inclusion of the cellulose may have slowed the rate of passage of food through the intestinal tract and thereby increased the period of gut exposure to the iron supplement. Schade *et al.* (1969) reported that anything that slowed passage increased absorption of dietary iron.

Effect of Increased Dietary Levels of Poorly Utilized Sources of Supplemental Iron. Since so many of the iron compounds used to fortify feeds and foods were poorly utilized, it was of interest to determine if increased dietary levels of these compounds would cure iron-deficiency anemia. A series of four tests (two with chicks and two with rats) were made with the commonly used iron compounds that are not well utilized. The results are summarized in Table VII. An increased level of ferrous carbonate was not effective. Harmon *et al.* (1969) also observed that ferrous carbonate which they used was in-

effective as an oral hematinic for swine even at levels much in excess of the suggested requirement level.

When increased levels of reduced iron, ferric orthophosphate, or sodium iron pyrophosphate were used, these compounds were effective for the cure of iron-deficiency anemia in our experimental animals. The effectiveness was in the order of magnitude that would be expected from the relative biological values established in earlier tests with these materials. These observations indicate that iron sources with at least some minimal availability can be used for food fortification provided enough of the source is used to furnish the needed quantity of available iron. In some applications, technological problems (rancidity; discoloration) may make it impractical to use iron compounds that have maximum availability. These observations provide an alternate mode for food fortification.

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Received for review March 2, 1970. Accepted May 8, 1970.