# Availability of Iron in Enriched Bread

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**ABSTRACT**

The bioavailability of iron from sources commonly used in the enrichment of bread was determined in anemic rats fed enriched-bread diets containing 20 p.p.m. iron. On the basis of the amount of hemoglobin regenerated in 30 days, iron availability from the two reduced irons and from ferric orthophosphate was about two-thirds, and that from sodium iron pyrophosphate about one-third of that from ferrous sulfate. However, the earlier during the regeneration phase these availabilities are calculated, the more pronounced are the differences. For example, at day 15 the two reduced irons, ferric orthophosphate and sodium iron pyrophosphate were only 38, 28, and 6% as available, respectively, as ferrous sulfate. At much higher levels of iron intake (110 p.p.m.), the same iron sources were almost equally effective. Under simulated gastric conditions, the solubilities of test irons except ferrous sulfate correlated well with the results of bioassays. Tested in a separate experiment, ferric ammonium citrate was found to be somewhat better assimilated by anemic rats than ferrous sulfate on diets containing 20 p.p.m. iron. The process of baking appeared to increase iron availability somewhat.

The desirability of substantially raising the levels of iron in enriched bread to correct the inadequacy of iron in human diet (1) appears to necessitate a re-examination of the comparative nutritional value of iron forms commonly used in enrichment. Although there is general agreement on the role of certain factors as

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they affect iron absorption, differences of opinion exist on whether the source of iron used in enrichment has any bearing on iron absorption. In studies with man and animals, it has been reported (2–5) and contradicted (6,7) that the absorption of inorganic iron used in bread enrichment is dependent on the source of iron used. This paper reports studies on the availability of iron to growing rats fed bread enriched with commonly used iron sources.

## MATERIALS AND METHODS

Weanling male rats (Sprague-Dawley) housed in stainless-steel cages were fed a low-iron diet (Table I) for 5 weeks, or until their hemoglobin levels dropped to 6 to 7 g.%. Hemoglobin (Hb) was determined on tail blood by the cyanomethemoglobin method (8) and hematocrit (Hct) by microcapillary centrifugation.

Test irons used were from the lots intended for commercial enrichment and included the following (% iron within bracket): reduced iron, reduced electrolytically (100); reduced iron, reduced by hydrogen (100); sodium iron pyrophosphate (14.4); ferric orthophosphate (29.1); ferrous sulfate (33.4); and ferric ammonium citrate, green (15.0). Bread was enriched to contain 32 mg. of added iron per lb., a level about three times higher than currently permitted. Bread baked by the sponge-dough method and air-dried was ground to fine crumbs and used in the studies below. The iron sources, bread crumbs, and diets were analyzed for their iron content by the $\alpha, \alpha'$ dipyridyl method (9). Phytic acid in bread was determined by the method of Anderson (10).

Diet groups of six to seven rats were fed vitamin-fortified all-bread diets (iron, 110 p.p.m.) in experiment I, while in experiment II appropriate amounts of each bread were mixed with a low-iron diet (Table I) to provide test diets of 20 p.p.m. iron. Ferric ammonium citrate was tested in a separate experiment (experiment III) where the effect of baking on iron availability was also examined. Unenriched bread provided the necessary control diet in each experiment. Diet and deionized water were offered to the rats *ad libitum*. Weight gain and diet-intake records were kept on individual rats. Hb and Hct concentrations were determined at 5-day intervals. Total Hb was calculated based on blood volumes of rats (11) corresponding to their individual body weights on days 0, 15, and 30.

To determine the solubility of iron under simulated gastric conditions, 2-g. samples of bread were extracted with 25 ml. of pepsin-HCl (0.5% pepsin in 0.1N HCl) at pH 1.35 for 90 min. in a Dubnoff shaker (30 oscillations per min.) at 37°C. Total soluble iron in the filtrate was determined by the $\alpha, \alpha'$ dipyridyl method (9).

## RESULTS

The results of Hb and Hct regeneration studies in experiments I, II, and III are summarized in Tables II, III, and IV, respectively. Each value, which is a group average, represents a gain (or loss) of Hb or Hct over the starting values. Since 20 p.p.m. iron in the diet, though marginal, has been reported to induce satisfactory Hb regeneration in anemic rats (7,12,13), permitting an accurate evaluation of iron sources, the bioavailability of iron was calculated based on the results of experiment II only (Table V). In this experiment the food intake, and therefore the iron intake as well as body-weight gains of rats, showed a significant ($P<0.01$)

TABLE I. COMPOSITION OF LOW-IRON DIET

| Ingredients | % |
|---|---|
| Dried skimmilk[a] | 30.0 |
| Vitamin diet fortification mixture[a] | 2.0 |
| Corn oil (Mazola) | 4.0 |
| Sodium chloride | 1.0 |
| Calcium carbonate | 1.0 |
| Monosodium phosphate | 1.0 |
| Trace mineral mixture[b] | 0.43 |
| Sucrose | 60.57 |
| | 100.00 |
| Iron, p.p.m. | 2.0 |
| Protein (N X 6.25) | 10.0 |

[a]Obtained from Nutritional Biochemical Corp., Cleveland, Ohio.
[b]Contained the following (mg.): $MgSO_4 \cdot 7H_2O$, 414; $MnSO_4 \cdot H_2O$, 15; $CuSO_4$, 1.3; $ZnCl_2$, 2.5 and K I, 0.024.

TABLE II. GAIN OF HEMOGLOBIN AND HEMATOCRIT (OVER STARTING ANEMIC LEVELS[a]) IN RATS FED ENRICHED BREAD[b]
(Experiment I; 15 Days)

| | Iron Source | | | | | |
|---|---|---|---|---|---|---|
| | None | Reduced iron (electrolytic) | Reduced iron (by hydrogen) | Sodium iron pyrophosphate | Ferric orthophosphate | Ferrous sulfate |
| Body weight, g. | | | | | | |
| Initial | 139.9±9.5 | 130.0±6.8 | 138.4±21.2 | 141.8±13.4 | 136.0±8.1 | 138.9±22.8 |
| Gain | 29.1±3.5 | 29.6±4.1 | 25.0±3.9 | 22.9±6.4 | 21.6±2.6 | 25.1±8.4 |
| Diet intake[c], g./day | 13.6±0.4 | 13.3±0.3 | 13.4±0.4 | 13.6±0.3 | 13.3±0.4 | 13.4±0.5 |
| Iron in diet, p.p.m. | 9.0 | 109.5 | 109.0 | 108.0 | 112.0 | 111.5 |
| Gain of Hb, g.% | | | | | | |
| 0–5 days | 3.23±1.29 | 5.26±1.05 | 6.02±0.90 | 4.83±0.72 | 5.83±1.19 | 6.68±0.91 |
| 0–10 days | 3.52±1.35 | 8.67±1.37 | 9.55±1.17 | 8.41±0.57 | 9.41±1.00 | 9.49±1.74 |
| 0–15 days | 4.22±0.88 | 9.60±1.54 | 10.24±1.12 | 9.90±1.21 | 9.94±1.74 | 10.82±1.14 |
| Gain of Hct, % | | | | | | |
| 0–5 days | 0.1±1.8 | 11.3±3.8 | 12.2±3.3 | 10.9±3.5 | 10.1±4.2 | 13.4±4.5 |
| 0–10 days | 1.7±1.8 | 23.5±5.3 | 24.1±4.2 | 21.9±3.3 | 22.6±4.2 | 22.6±5.1 |
| 0–15 days | 2.8±2.2 | 25.0±6.0 | 25.9±4.4 | 23.3±3.8 | 25.7±4.0 | 26.2±4.1 |

[a]Starting anemic levels: Hb, g.% 5.67±1.62; Hct, % 26.4±3.6.

[b]Each value represents an average of seven rats ± standard deviation.

[c]Rats were fed an all-bread diet. Unenriched bread provided the necessary control.

TABLE III. GAIN OR LOSS OF HEMOGLOBIN AND HEMATOCRIT (OVER STARTING ANEMIC LEVELS[a]) IN RATS FED ENRICHED BREAD[b] (Experiment II; 30 Days)

| | Iron Source | | | | | |
|---|---|---|---|---|---|---|
| | None | Reduced iron (electrolytic) | Reduced iron (by hydrogen) | Sodium iron pyrophosphate | Ferric orthophosphate | Ferrous sulfate |
| Body weight, g. | | | | | | |
| Initial | 115.8±10.8 | 127.8±14.5 | 117.5±15.4 | 115.2±10.2 | 117.5±10.5 | 125.7±7.1 |
| Gain | | | | | | |
| 0–15 days | 14.0±9.2 | 47.7±5.2 | 45.3±6.0 | 37.0±6.6 | 46.0±7.5 | 59.8±3.3 |
| 0–30 days | 39.3±21.5 | 105.5±14.0 | 102.5±12.3 | 90.8±14.9 | 102.0±14.5 | 126.7±8.4 |
| Diet intake[c], g./day | | | | | | |
| 0–15 days | 9.8±0.5 | 12.6±0.7 | 12.8±0.5 | 12.1±0.5 | 12.2±0.2 | 14.6±0.5 |
| 0–30 days | 8.3±1.4 | 13.8±0.7 | 13.4±1.1 | 12.4±1.4 | 13.0±0.6 | 16.2±0.8 |
| Iron in diet, p.p.m.[c] | 3.2 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Gain or loss of Hb, g.% | | | | | | |
| 0–5 days | −2.92±0.89 | −2.08±0.60 | −2.07±0.94 | −2.45±0.80 | −2.38±0.65 | −0.05±0.81 |
| 0–10 days | −2.98±1.35 | −1.33±1.41 | −2.02±1.03 | −2.35±1.00 | −1.89±0.72 | 1.83±1.22 |
| 0–15 days | −2.96±1.25 | 0.47±0.38 | 0.63±1.02 | −1.12±1.10 | −0.06±0.87 | 4.17±1.82 |
| 0–20 days | −4.07±1.13 | 0.95±0.45 | 0.33±1.48 | −1.10±1.20 | −0.35±0.93 | 4.54±0.77 |
| 0–25 days | −3.93±1.20 | 3.53±1.39 | 2.48±1.33 | 0.20±1.47 | 2.67±1.42 | 6.90±2.03 |
| 0–30 days | −4.28±1.13 | 2.81±0.84 | 2.76±1.70 | 0.13±1.46 | 2.36±1.77 | 6.30±1.86 |
| Gain or loss of Hct, % | | | | | | |
| 0–5 days | −1.2±1.2 | 3.3±2.1 | 2.4±2.0 | −0.4±1.2 | 2.2±1.9 | 9.1±1.7 |
| 0–10 days | −2.3±2.1 | 6.8±2.0 | 5.1±5.5 | 1.3±2.5 | 3.2±2.3 | 14.8±1.9 |
| 0–15 days | −2.8±1.5 | 8.7±1.5 | 7.6±3.1 | 2.1±1.8 | 6.9±2.6 | 19.6±2.6 |
| 0–20 days | −2.3±1.0 | 13.8±1.2 | 10.5±2.2 | 5.0±2.5 | 8.9±2.8 | 24.0±3.2 |
| 0–25 days | −3.6±1.4 | 16.3±1.9 | 13.3±3.1 | 5.5±2.3 | 11.9±2.0 | 26.8±3.7 |
| 0–30 days | −3.0±1.4 | 17.2±2.7 | 16.0±4.4 | 7.5±3.3 | 13.0±2.8 | 28.0±4.4 |

[a] Starting anemic levels: Hb, g.% 7.07±1.14; Hct, % 18.5±2.1.

[b] Each value represents an average of six rats ± standard deviation. Negative sign before values indicates a loss.

[c] Bread was mixed with low-iron diet to obtain diets containing 20 p.p.m. iron. Unenriched bread provided the necessary control.

variation between diets. The relative availabilities of test irons were thus calculated based on the net gain of Hb in 15 and 30 days in response to a unit (mg.) rather than total intake of iron (Table V). Relative iron availabilities in experiment III were calculated as in experiment II and the results are included in Table IV. Results on the solubility of bread iron under simulated gastric conditions are presented in Table V. Preliminary studies showed that aqueous extraction of bread failed to liberate iron into solution and that extraction with HCl alone, compared to pepsin-HCl, was low. Iron solubility could be increased by using greater agitation but not by longer times or larger volumes of extractant than used here.

TABLE IV. THE EFFECT OF BAKING ON THE AVAILABILITY OF IRON TO ANEMIC RATS[a,b]
(Experiment III; 15 days)

| | Iron Source | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ferrous sulfate Mixed with bread ingredients | | | Mixed with | | Ferric ammonium citrate |
| | None | Ingredients | Dough (fermented) | Bread | Low-iron diet | Baked bread | Bread |
| Body weight, g. | | | | | | | |
| Initial | 133.3 ±15.9 | 139.2 ±20.8 | 132.5 ±18.1 | 122.0 ±9.6 | 132.7 ±10.4 | 126.3 ±19.0 | 113.0 ±17.7 |
| Gain | 10.6 ±10.9 | 69.3 ±7.9 | 71.8 ±9.8 | 72.0 ±10.9 | 64.2 ±6.4 | 73.2 ±9.1 | 69.7 ±7.2 |
| Diet intake, g./day | 10.6 ±3.1 | 15.4 ±3.0 | 15.7 ±3.0 | 15.5 ±2.3 | 15.1 ±2.0 | 15.2 ±1.6 | 14.8 ±1.8 |
| Iron in diet[c], p.p.m. | 3.2 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Gain or loss[d] of Hb, g.% 0–15 days | −1.83 ±1.00 | 4.60 ±1.10 | 5.27 ±0.84 | 5.20 ±1.23 | 5.14 ±0.98 | 5.02 ±1.05 | 5.57 ±1.00 |
| Gain or loss[d] of Hct, % 0–15 days | −4.2 ±2.5 | 17.0 ±2.1 | 19.3 ±2.4 | 20.8 ±1.8 | 20.7 ±1.8 | 21.4 ±3.0 | 22.6 ±1.7 |
| Hb gain[e], g./mg. iron intake | ... | 0.186 ±0.030 | 0.201 ±0.029 | 0.198 ±0.028 | 0.201 ±0.034 | 0.196 ±0.030 | 0.207 ±0.024 |
| Relative iron[e] availability, % | ... | 93.9 | 101.5 | 100.0 | 101.5 | 99.0 | 104.5 |
| Phytic acid phosphorous, mg./100 g. diet | ... | 6.2 | 3.2 | 4.6 | ... | ... | ... |

[a] Starting anemic levels: Hb, g.% 5.84±0.95; Hct, % 20.2±1.9.

[b] Each value represents an average of six rats ± standard deviation. Negative sign before values indicates a loss.

[c] Appropriate amounts of bread, dough, or ingredients were mixed with low-iron diet to obtain diets containing 20 p.p.m. iron. Unenriched bread provided the necessary control.

[d] Over starting anemic levels.

[e] Calculated as in Table V.

## DISCUSSION

At the high level of iron intake in experiment I, source of dietary iron appears not to affect appreciably the rate of regeneration of Hb and Hct in anemic rats. In just 5 days, Hb increased by 5 to 6 g.% over the starting anemic levels of about 6 g.%, and thus approached values normal for rats. This probably cannot be taken to mean, as the results of experiment II show, that iron availability between sources

TABLE V. RELATIVE AVAILABILITY (BASED ON RESULTS OF EXPERIMENT II) AND EXTRACTABILITY OF IRON IN BREAD ENRICHED WITH DIFFERENT IRON SOURCES[a]

| | Iron Source | | | | |
|---|---|---|---|---|---|
| | Reduced iron (electrolytic) | Reduced iron (by hydrogen) | Sodium iron pyrophosphate | Ferric orthophosphate | Ferrous sulfate |
| | AVAILABILITY | | | | |
| Iron consumed, mg.[b] | | | | | |
| 0–15 days | 3.787±0.221 | 3.836±0.160 | 3.616±0.186 | 3.674±0.065 | 4.394±0.145 |
| 0–30 days | 7.998±0.441 | 7.744±0.639 | 7.167±0.780 | 7.572±0.346 | 9.374±0.445 |
| 15–30 days | 4.211±0.391 | 3.908±0.513 | 3.551±0.613 | 3.898±0.199 | 4.980±0.393 |
| Total Hb gain, g.[c] | | | | | |
| 0–15 days | 0.243±0.070 | 0.238±0.071 | 0.036±0.052 | 0.173±0.077 | 0.731±0.145 |
| 0–30 days | 0.767±0.172 | 0.729±0.146 | 0.367±0.120 | 0.713±0.188 | 1.304±0.218 |
| 15–30 days | 0.524±0.161 | 0.491±0.101 | 0.331±0.090 | 0.540±0.173 | 0.573±0.191 |
| Hb gain[d], g./mg. iron consumed | | | | | |
| 0–15 days | 0.064±0.015 | 0.062±0.019 | 0.010±0.011 | 0.047±0.021 | 0.166±0.034 |
| 0–30 days | 0.096±0.018 | 0.094±0.021 | 0.051±0.017 | 0.094±0.025 | 0.139±0.027 |
| 15–30 days | 0.124±0.013 | 0.126±0.022 | 0.093±0.015 | 0.139±0.020 | 0.115±0.029 |
| Relative iron[d] availability, % | | | | | |
| 0–15 days | 38.6 | 37.3 | 6.0 | 28.3 | 100.0 |
| 0–30 days | 68.9 | 67.6 | 36.8 | 67.6 | 100.0 |
| 15–30 days | 107.8 | 109.6 | 80.9 | 120.9 | 100.0 |
| | EXTRACTABILITY (SOLUBILITY) | | | | |
| Iron in 2 g. bread crumbs, μg | 219.0 | 218.0 | 216.0 | 224.0 | 223.0 |
| Solubility in pepsin-HCl[e], % | 44.0±0.3 | 44.4±0.3 | 23.7±0.5 | 28.7±0.3 | 45.0±0.6 |

[a] Values represent mean of six rats ± standard deviation.

[b] In diet consumed in first 15, entire 30, and last 15 days.

[c] Calculated based on blood volume of rats and their Hb concentration (g.%) as measured initially (day 0) and at days 15 and 30 of feeding enriched bread diet.

[d] Calculated based on Hb gain expressed as g./mg. iron consumed.

[e] Mean of triplicate determinations ± standard deviation.

tested did not differ, but rather that despite this, because of high iron intakes, sufficient iron became available even from the otherwise poor iron source to permit a rapid regeneration of Hb and Hct. This observation illustrates that the question of iron availability assumes importance only when the dietary supply is limiting relative to physiological needs. Thus in experiment II, where dietary iron was just adequate to promote satisfactory Hb regeneration, differences between iron sources became apparent. These results are in contrast to the findings of Steinkamp et al. (6) who considered iron supplied as ferrous sulfate, reduced iron, ferric orthophosphate, and sodium iron pyrophosphate to be equally effective in healthy

and anemic human adults. Nakamura and Mitchell (7) also reported equal effectiveness of ferric chloride, sodium iron pyrophosphate, and reduced iron in curing anemia in rats. On the other hand, these results agree well with most other reports, particularly with those of Blumberg and Arnold (4) and of Fritz et al. (5) who, working with anemic rats, showed sizable differences in iron absorption from sources including those tested here.

During the first 5-day period in experiment II, anemia actually became more severe in all groups except the one receiving ferrous sulfate-enriched bread, perhaps reflecting a better assimilability of iron from ferrous sulfate. The group receiving ferrous sulfate also showed a faster regeneration of Hb and Hct over the subsequent periods and achieved Hb values in the normal range by about day 20. Hb levels were still below normal with the other irons when the experiment was terminated on day 30. Although regeneration was initially slower with ferric orthophosphate than with either of the two reduced irons, it was most rapid in the later periods, so that the overall availabilities were essentially equal from these three sources. Regeneration rates with bread containing sodium iron pyrophosphate were consistently slower than with other test irons.

The differences in effectiveness of sources observed in experiment II cannot be explained entirely by differences in the amount of iron consumed. Although total iron intake varied greatly among test diets, it is apparent from Table V that differences still remain when the data are expressed on the basis of "Hb gained per mg. iron consumed," particularly during the first 15 days of recovery. Evidently at marginal iron intakes and especially during the initial stages of recovery, it is the extent of availability more than total intake which determines the rate of response.

In the present study, the relative order of extractability of iron from breads containing reduced irons, ferric orthophosphate, and sodium iron pyrophosphate is in agreement with the order noted for Hb regeneration. The superior physiological response to ferrous sulfate, however, was not accompanied by a higher solubility in pepsin-HCl. It may be that additional factors, such as degree of ionization and valence state, are also important in determining the assimilability of an iron source.

The results of experiment III (Table IV) revealed a somewhat better iron availability from bread enriched with ferric ammonium citrate than from ferrous sulfate. Ferrous sulfate, whether added to low-iron diet, baked into bread, or added to baked bread, revealed no differences in iron availability. However, fermentation-baking did cause some increase in iron availability. As suggested by Leichter and Joslyn (14), this could be due to a breakdown of phytic acid during the baking process or to a denaturation of iron-binding sites, or both.

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