United States originally arose from a suggestion by H. Ted Huddleston, Jr.

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A Comparative Study in Rats of Iron Bioavailability from Cooked Beef and Soybean Protein

John T. Rotruck* and Kenneth R. Luhrsen

Two variations of the basic depletion–repletion method were used to measure iron bioavailability from a soybean protein isolate, a processed soybean protein isolate, and cooked beef. The depletion phase was the same for all animals; however, the repletion phase compared the effects of restricted feeding to ad libitum feeding. Slope-ratio analyses of the increase in hematocrit, hemoglobin, and total hemoglobin gave the best statistical fit for the data. Although all methods of analyses gave comparable results, calculation based on total hemoglobin provided the best statistical fit to the slope-ratio analysis (i.e., linearity of response). The results showed that iron from the soybean protein isolate and iron from the processed soybean protein isolate had bioavailabilities 82–100% of ferrous sulfate. Iron from cooked beef had a bioavailability of 26–55% of ferrous sulfate. The results of these animal experiments are compared to iron absorption studies conducted in anemic and normal humans.

The replacement of beef with analogues containing soybean protein raises many nutritional questions. Since beef supplies a substantial part of the iron in the human diet, it is one of the important nutritional questions when considering replacement foods. Although separate studies of iron bioavailability from beef (Pye and MacLeod, 1976; Oldham, 1941; Mahoney et al., 1974; Sherman et al., 1934; Rose et al., 1934) and soybean protein preparations (Fritz et al., 1970; Theuer et al., 1971) have been conducted, measurements of iron bioavailability from these two sources of iron have not been compared in the same study. In addition, the methodologies used to determine the bioavailability of iron from beef were different from those used for soybean protein preparations. Furthermore, the methods used to determine bioavailability in these foods are different from the procedure which is currently being recommended as the official method for determining iron bioavailability (Fritz et al., 1975). The objectives of this study were to use the same method to compare the bioavailabilities of these two food sources of iron in rats and to evaluate various methods of determining and calculating iron availability from foods.

Fritz et al. (1975) have recommended that their method be adopted as the official method for determining iron bioavailability. Although this method can yield acceptable results, there are some potential problems with it. The method first requires that rats be depleted of iron by feeding an iron-deficient diet, then the relative bioavailability of the iron source is determined by comparing the increase in hemoglobin concentration or hematocrit elicited by the unknown iron source to the increase in these parameters elicited by ferrous sulfate. Three dietary levels of the iron source are recommended as a means of increasing the sensitivity and reliability of the assay.

The first problem with the method as recommended by Fritz et al. (1975) is found in analysis of the data. There are two possible statistical methods of analyzing the data: the parallel-lines method and the slope-ratio method. The parallel-lines method requires that a plot of log of dose vs. response yield a linear response and parallel lines for the different treatments. The slope-ratio method requires that a plot of dose vs. response yield a linear response with a common intercept at the zero dose level. Both Fritz et al. (1975) and Waddell (1973) have recommended that the parallel-lines technique be used to calculate the relative bioavailabilities of the iron sources. However, Finney (1964), in his book on bioassays, pointed out that the appropriateness of a parallel-lines analysis or slope-ratio analysis depends upon the range of doses investigated. Thus one analysis is not appropriate for all experiments, suggesting that each experiment should be assessed by both statistical methods to determine which method might be applicable for the particular experiment. The problems of statistical analysis are discussed in more detail by Amine and Hegsted (1974).

In addition to the question of statistical analysis, an additional question about the method of Fritz et al. (1975) has been raised by Mahoney et al. (1974). The work of Mahoney et al. (1974) suggested that rating the bioavailability of iron sources according to hemoglobin concentration could lead to erroneous conclusions. This is because increases in hemoglobin concentration fail to take

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45247.

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into account differences in the amount of iron consumed or differences in weight gained resulting from differences in food intake by groups of rats in the same experiment. If the diets containing the various sources of iron have inherent differences in palatability, protein quality, or caloric density, the amounts of food consumed could be quite different. These attributes could influence iron consumption and thus weight gain. Changes in growth may significantly alter the animal's iron requirement. And finally, changes in body size would result in blood volume changes. Mahoney et al. (1974) have aptly pointed out that these considerations can invalidate calculations of iron bioavailability when based on hemoglobin concentration. It has been our experience that calculations of total hemoglobin and the efficiency of iron conversion into hemoglobin are better reflections of iron bioavailability.

The current method of Fritz et al. (1975) does not consider the possible influence of variability in food consumption and thus the influence of iron consumption on growth. We have therefore modified the method in two ways to take these variables into account. The first method employed the basic procedures of Fritz et al. (1975), and in addition to using hemoglobin concentration and hematocrit determinations we also used total hemoglobin to calculate iron bioavailabilities. All of these parameters were analyzed as a function of dietary iron level and of dietary iron consumed: total hemoglobin formation was employed to account for the differences in iron consumption. For the second method we modified the procedure of Fritz et al. (1975) such that, rather than allowing the animals to consume the food ad libitum, we restricted the food intake of all animals to a constant amount. This would of course eliminate any difference in iron consumption between treatment groups and also minimize much of the difference in weight gain due to treatment.

These experiments have allowed comparisons of iron bioavailability from soybean protein, a processed soybean protein, and cooked beef. We have also compared our results from these animal experiments to results other investigators have obtained using similar diets fed to human subjects.

MATERIALS AND METHODS

Two hundred and thirty weanling male Sprague-Dawley rats were fed the iron-deficient diet shown in Table I. This diet contained 3.9 ppm iron and adequate quantities of all other nutrients. During the depletion period the rats were fed the iron-deficient diet and given distilled–deionized water ad libitum. The animals were housed individually in stainless steel cages with wire mesh bottoms.

Blood was drawn from the tail for hematocrit determinations after feeding the depletion diet for 5 weeks. After discarding any rats with hematocrits in excess of 16.0%, the remaining 180 animals were divided into 26 comparable groups. The group hematocrits ranged from 13.0–13.6% with a mean of 13.3%. Blood samples were taken for hemoglobin and hematocrit determinations after the repletion diets had been fed for 2 weeks. Hemoglobin levels were determined spectrophotometrically (Crosby et al., 1954) and hematocrits were determined by the common clinical procedure using heparinized microhematocrit capillary tubes. Dietary iron sources were analyzed for iron content by atomic absorption spectrophotometry. Protein contents were calculated by analyzing for nitrogen by the Kjeldahl method and multiplying the percent N by 6.25.

The beef used was boneless chuck roast trimmed of all excess fat. These roasts were cooked in ovens at 350 °F for 3 h. The excess fat and liquid were poured off and the beef was then chopped, using a Hobart food chopper. The chopped beef was freeze-dried and reground in a Waring blender. The soybean analogue was processed from a soybean protein isolate (Promine F) and the same soybean protein isolate was used as received from the supplier. The ferrous sulfate monohydrate was Mallinckrodt dried USP (powder) and contained 32.0% iron.

Table I. Composition of Basal Diet

	g/100 g of diet
sucrose	32.73
corn starch	32.73
casein	20.00
corn oil	10.00
vitamin mix[a]	0.50
mineral mix[b]	0.75
choline chloride	0.20
$CaCO_3$	1.25
KH_2PO_4	1.75
L-methionine	0.10

[a] Vitamin mix, g/100 g of vitamin mix: 0.32, vitamin A palmitate; 0.08, vitamin D_2; 0.125, thiamin hydrochloride; 0.25, riboflavin; 1.5, niacin; 0.8, calcium D-pantothenate; 0.7, pyridoxine hydrochloride; 1.0, folic acid (1% in sucrose); 0.005, menadione; 0.02, *d*-biotin; 0.5, vitamin B_{12} (0.1% in mannitol); 7.68, vitamin E acetate (500 IU/g); 87.02, sucrose. [b] Mineral mix, g/100 g of mineral mix: 25.4, NaCl; 39.6, $MgSO_4$; 0.393, $CuSO_4 \cdot 5H_2O$; 3.08, $MnSO_4 \cdot H_2O$; 0.75, $ZnCl_2$; 0.0039, KI; 0.000736, $(NH_4)_6MO_7O_{24} \cdot 4H_2O$; 0.0146, Na_2SeO_3; 0.0205, $CrCl_3 \cdot 6H_2O$; 30.7372, sucrose.

Since the cooked beef, the soybean protein isolate, and the processed soybean protein isolate were all high protein sources, they replaced an equal weight of casein in the repletion diets. These foods contained the following concentrations of iron and protein: freeze-dried cooked beef, 73 ppm iron and 66.9% protein; soybean protein isolate, 150 ppm iron and 90.6% protein; and processed soybean protein isolate, 120 ppm iron and 58.1% protein. The freeze-dried cooked beef contained 23% lipid. All of the above iron sources contained less than 5% moisture. These foods were added to the diet to supply 4, 8, and 12 ppm added iron. Iron levels were confirmed by analysis. The diets containing ferrous sulfate were prepared by adding a dry premix containing ferrous sulfate at the expense of sucrose. Analysis of the final diets containing ferrous sulfate disclosed that they contained 5.7, 11.4, and 17.1 ppm iron rather than the 4, 8, and 12 ppm iron as originally intended.

The animals were fed in two ways. After depletion 13 groups of the rats were fed 8 g rat^{-1} day^{-1} of the respective repletion diets. The remaining 13 groups of depeleted rats were fed the same diets ad libitum. Food consumption for the ad libitum groups were measured three times weekly and body weight one per week.

An important consideration in bioassays is selection of an appropriate statistical method to analyze the data. Either the slope-ratio or parallel-lines method can be valid for bioassays of this type (Hegsted et al., 1968; Finney, 1964). When we plotted our data according to both methods we found that the data more closely fit the criteria for the slope-ratio analyses. Therefore we employed the slope-ratio method as described by Finney (1964) to calculate the relative bioavailabilities of iron from the difference dietary sources of iron. *F* tests for linearity were conducted according to Finney (1964). In addition we conducted *F* tests for curvature within each individual treatment.

RESULTS

Effect of Iron Source on Weight Gain and Food

Table II. Weight Gain and Iron Consumption

dietary treatment	dietary iron concn, ppm	feeding regime: restricted wt gain, g	restricted added iron consumed, mg	ad libitum wt gain, g	ad libitum added iron consumed, mg
basal diet	0	9.1 ± 3.7[a]	0	38.1 ± 2.6[a]	0.0
ferrous sulfate	5.7	4.1 ± 3.0	0.64[b]	57.8 ± 6.6	1.19 ± 0.03[a]
	11.4	5.4 ± 2.8	1.28	56.3 ± 8.6	2.52 ± 0.28
	17.0	9.0 ± 2.3	1.90	70.4 ± 5.2	4.08 ± 0.18
cooked beef	4	16.3 ± 8.5	0.45	46.6 ± 11.3	0.86 ± 0.08
	8	13.7 ± 3.2	0.90	58.9 ± 5.7	1.56 ± 0.11
	12	−0.3 ± 5.1	1.34	61.0 ± 2.1	2.12 ± 0.07
soybean protein isolate	4	4.1 ± 4.2	0.45	48.2 ± 8.9	0.79 ± 0.02
	8	16.4 ± 6.6	0.90	60.3 ± 8.6	1.71 ± 0.08
	12	6.4 ± 4.1	1.34	52.9 ± 5.0	2.60 ± 0.15
processed soybean protein isolate	4	−2.1 ± 5.2	0.45	43.3 ± 4.1	0.88 ± 0.04
	8	14.0 ± 4.5	0.90	54.7 ± 7.1	1.78 ± 0.11
	12	8.0 ± 6.6	1.34	59.9 ± 5.6	2.95 ± 0.20

[a] Each value is the mean ± the standard error of the mean for six or seven animals. [b] There is no variation in these values since each animal received the same amount of diet.

Table III. Hematocrits, Hemoglobin Levels, and Total Hemoglobins

dietary treatment	iron dose, ppm	feeding regime: restricted hematocrit, % PCV	restricted hemoglobin, g/100 mL	restricted total hemoglobin,[a] mg/animal	ad libitum hematocrit, % PCV	ad libitum hemoglobin, g/100 mL	ad libitum total hemoglobin, mg/animal
basal diet	0	18.4 ± 0.9[b]	3.86 ± 0.2[b]	0.510 ± 0.033[b]	15.9 ± 0.9[b]	3.14 ± 0.19[b]	0.479 ± 0.023[b]
ferrous sulfate	5.7	25.0 ± 0.5	5.24 ± 0.13	0.695 ± 0.016	21.2 ± 0.7	4.40 ± 0.17	0.705 ± 0.044
	11.4	29.1 ± 0.3	5.86 ± 0.11	0.802 ± 0.019	29.4 ± 0.9	6.26 ± 0.24	0.999 ± 0.039
	17.0	32.3 ± 0.7	7.19 ± 0.16	0.985 ± 0.019	32.3 ± 0.6	8.07 ± 0.40	1.340 ± 0.064
cooked beef	4	19.7 ± 0.7	4.33 ± 0.2	0.573 ± 0.030	17.3 ± 0.7	3.53 ± 0.12	0.555 ± 0.028
	8	21.3 ± 1.3	4.56 ± 0.32	0.598 ± 0.037	18.9 ± 0.7	3.87 ± 0.20	0.636 ± 0.031
	12	24.9 ± 0.7	5.13 ± 0.2	0.648 ± 0.029	20.3 ± 1.1	3.93 ± 0.12	0.659 ± 0.020
soybean protein isolate	4	21.9 ± 0.5	4.67 ± 0.18	0.621 ± 0.026	18.3 ± 0.7	3.93 ± 0.13	0.623 ± 0.014
	8	25.3 ± 0.8	5.29 ± 0.24	0.701 ± 0.026	23.6 ± 1.2	4.76 ± 0.13	0.773 ± 0.025
	12	27.9 ± 0.5	6.09 ± 0.15	0.804 ± 0.019	28.4 ± 1.3	5.90 ± 0.28	0.989 ± 0.060
processed soybean protein	4	21.2 ± 0.6	4.35 ± 0.15	0.559 ± 0.028	18.6 ± 0.6	3.83 ± 0.24	0.605 ± 0.049
	8	24.3 ± 0.6	5.07 ± 0.25	0.689 ± 0.033	22.7 ± 1.2	4.46 ± 0.20	0.747 ± 0.045
	12	29.0 ± 0.7	6.40 ± 0.18	0.864 ± 0.065	29.3 ± 0.8	6.0 ± 0.16	0.994 ± 0.036

[a] Total hemoglobins were calculated by multiplying the hemoglobin level times blood volume. Rat blood volume is equal to about 6.5% of body weight (Wong, 1959). [b] Each value is the mean ± the standard error of the mean for six or seven animals.

Consumption. Weight gains and food consumption data for all animals are shown in Table II. These data show that food intake restriction reduced the weight gain as expected, eliminating significant effects on weight gain due to either iron level or dietary treatment.

If the animals were fed ad libitum, weight gains and food consumptions were variable. Increasing the level of iron supplied as ferrous sulfate increased both food consumption and weight gain. Increasing the level of iron supplied as processed soybean protein also increased food consumption and weight gain. However the effect of dietary iron level when supplied as soybean protein isolate was irregular. There was a significant increase in weight gain and food consumption if soybean protein isolate supplied either 4 or 8 ppm iron, but there was no further increase in either food consumption or weight gain if soybean protein isolate supplied 12 ppm iron. To the contrary, food consumption decreased when beef supplied increasing levels of iron to the diet. The decrease in food consumption by rats fed the beef-supplemented diet was not accompanied by a decrease in weight gain but in fact resulted in an increase in weight gain. The decrease in food consumption with the beef-suplemented diet is probably due to a change in caloric density because of the increased lipid supplied with the beef.

Generally, the data show that the source of iron and type of diet can have variable effects on food intake and weight gain.

Analyses of the Data. Hematocrits, hemoglobin concentrations, and total hemoglobin values for all animals are shown in Table III. The data from the animals fed ad libitum were analyzed either as a function of added iron or as a function of added iron consumed. The data from animals fed restricted levels of diet were analyzed only as a function of added iron. Since these rats consumed all of their diet, analysis by the other method would yield the same relative bioavailabilities.

Table IV shows F tests of all the data which were conducted to test the validity of the slope-ratio method as a technique for calculating iron availabilities. A significant F test shows that the assumptions required for the slope-ratio analysis are not met in some cases. Significant curvature is evident in the hematocrit data from the ad libitum fed animals if it is analyzed as a function of added iron or as a function of added iron consumed. Analyses of the hemoglobin concentrations and total hemoglobin data from the ad libitum fed animals did not reveal any violations of the requirements for the slope-ratio analysis.

Table IV. Analysis of Variance F Tests of All Data[a]

method of feeding	y-axis parameter	x-axis parameter	curvature	intersection	blanks	regression	individual tests of curvature			
							$FeSO_4$	meat	soybean protein	processed soybean protein
ad libitum	hematocrit	added iron	1.72	1.70	1.116	104.0*	8.50*	0.00	0.02	1.18
	hemoglobin level		0.93	1.33	1.10	126.30*	0.00	0.59	0.43	3.49
	total hemoglobin		0.57	1.00	1.02	107.5*	0.18	0.80	0.26	0.91
ad libitum	hematocrit	added iron	2.70*	3.02	0.24	72.08*	7.42*	0.01	1.00	0.99
	hemoglobin level	consumed	1.14	0.27	0.37	71.73*	0.76	0.08	2.05	1.44
	total hemoglobin		1.22	0.11	0.08	83.73*	1.45	0.39	0.61	1.33
restricted	hematocrit		0.67	3.99*	0.03	96.19*	0.50	0.77	0.32	1.06
	hemoglobin level		1.11	1.67	0.05	64.55*	5.04*	0.32	0.15	1.51
	total hemoglobin		0.54	2.42	0.07	73.01*	3.19	0.10	0.14	0.42

[a] Each number is the F value calculated using the slope-ratio analysis. Those values which are significant at $P < 0.05$ are followed by an asterisk.

Both the hematocrit and hemoglobin data yielded significant F tests, while the hematocrit data from the restricted fed animals showed a significant effect due to blanks. The hemoglobin level data show a significant curvature due to the ferrous sulfate treatment.

The total hemoglobin data consistently yielded the best results since in all cases these data met the requirements (i.e., linearity) for the slope-ratio analyses.

It appeared that the highest level of ferrous sulfate invalidated the slope-ratio analyses most probably because the highest level of ferrous sulfate supplied more iron than the highest level of the other iron sources tested. To test this possibility analyses of the hematocrit and hemoglobin level data were conducted after omitting the data from the animals fed the highest level of ferrous sulfate. These analyses are shown in Table V. Surprisingly, deletion of the data from animals fed the highest level of ferrous sulfate did not yield valid slope-ratio analyses. Analyses of the hematocrit data and hemoglobin level data from the ad libitum fed animals as a function of added iron consumed still shows significant curvature. Further investigation reveals that the curvature effect was due to the ferrous sulfate treatment.

Analyses of the hematocrit data and hemoglobin level data from the ad libitum fed animals as a function of added iron also did not reveal any violations of the requirements for the slope-ratio analysis. Likewise, the analyses of the hematocrit data and hemoglobin level data from the restricted fed animals were acceptable.

Slope Ratio. All of the slope ratios which meet the requirements of the analysis are shown in Table VI. Regardless of the method of analysis, the slope ratios reveal the same trends in iron availability: ferrous sulfate ≥ soybean protein isolate ≥ processed soybean protein > meat. The availability of iron in the soybean protein isolate ranged from 82% to 100% of ferrous sulfate. Except for the slope ratios calculated from the hemoglobin level data none of the slope-ratio values for the soybean protein isolate were significantly different from ferrous sulfate. The availability of iron from the processed soybean protein ranged from 71 to 102% of that of iron from ferrous sulfate. For the processed soybean protein four of the six statistically valid methods gave iron availabilities significantly less than ferrous sulfate. However, in no case was the iron availability of the processed soybean protein less than the iron availability of the soybean protein isolate. The availability of meat iron ranged from 26 to 55% of that of iron from ferrous sulfate, showing that in all cases the availability of meat iron was significantly less ($P < 0.05$) than the availability of any of the other sources of iron.

DISCUSSION

The major objective of this work was to compare the bioavailabilities of iron from meat and some soybean protein preparations. To do this we used two methods, with both methods employing the same basic depletion–repletion methodology recommended by Fritz et al. (1975). However, in addition, we employed either a restricted feeding regime or an ad libitum feeding regime during the repletion phase of the experiment.

Using the slope-ratio method of analyzing the data we obtained the most acceptable statistical analysis (i.e., fewest deviations from linearity). It should be emphasized, however, that other experimental conditions may yield results which conform to other statistical analysis such as the parallel-lines methods. The appropriateness of a parallel-lines or slope-ratio analysis depends upon the range of doses investigated and probably the degree of iron

Table V. Analysis of Variance F Tests (Omitting Data from Animals Fed the Highest Level of $FeSO_4$)

treatment			curvature	intersection	blanks	regression	individual tests of curvature			
							$FeSO_4$	meat	soybean protein isolate	processed soybean protein isolate
ad libitum		hematocrit	0.29	0.91	2.67	71.62*	0.00	0.01	0.02	1.18
	added iron	hemoglobin level	1.18	1.49	0.95	78.60*	0.00	0.59	0.43	3.49
	added iron consumed	hematocrit	3.52*	1.61	0.00	51.35*	9.74*	0.01	0.99	0.99
		hemoglobin level	4.33*	1.27	0.83	45.55*	8.97*	0.08	2.05	1.44
restricted		hematocrit	0.59	2.02	0.16	65.58*	0.07	0.77	0.32	1.06
		hemoglobin level	0.52	2.31	0.02	36.75*	0.00	0.32	0.15	1.51

[a] Each number is the F value calculated using the slope-ratio analysis. Those values which are significant at $P < 0.05$ are followed by an asterisk. The total hemoglobin values were not reanalyzed, omitting data from animals fed the highest level of ferrous sulfate.

deficiency produced by the depletion diet. Thus one cannot a priori recommend one method of statistical analysis as Fritz et al. (1975) and Waddell (1973) have done.

In addition to the usual analysis of variance F tests, which test all treatments for curvature, we have tested the individual treatments for curvature. This is because the overall analysis may not reveal significant curvature, whereas the individual treatments do show significant curvature. Our data clearly show that this can happen (as shown in Table IV). We would recommend that other investigators conduct tests for curvature on individual treatments.

In our studies we analyzed the data as a function of hematocrit level, hemoglobin level, and total hemoglobin as a result of dietary treatment. We have found from all of these calculations that in some cases the analyses show significant deviations from the ideal slope-ratio analyses. These deviations were found entirely in the calculations based on the hemoglobin concentration and hematocrit data. In all cases the total hemoglobin data met all the requirements for the slope-ratio analysis, which suggests that the total hemoglobin method will consistently yield the best results.

Comparisons of all the valid slope ratios reveal the same basic trends regardless of the method employed to calculate iron bioavailability: ferrous sulfate ≥ soybean protein isolate (82 to 102% of ferrous sulfate) > processed soybean protein (71% to 102% of ferrous sulfate) > cooked beef (26 to 55% of ferrous sulfate). These data confirm the previous literature which indicates that soybean protein iron bioavailability is equal to or nearly equal to ferrous sulfate (Theuer et al., 1971; Fritz et al. 1970).

Our experiments do not allow us to recommend a specific experimental method for determining iron bioavailability. On the contrary, they suggest that either ad libitum feeding or restricted feeding will yield satisfactory results provided that the statistical method of analysis is carefully chosen and properly validated. Futhermore, the data suggest that basing the relative bioavailability on total hemoglobin results in a more satisfactory statistical treatment of the data (absence of nonlinearity). However, all of the hematological parameters yielded comparable slope-ratio values and thus comparable iron bioavailabilities, thus we have concluded that the bioavailability of iron from food sources can be satisfactorily determined by using a rat bioassay provided the assay is carefully executed and the results are correctly interpreted.

The studies of Mahoney et al. (1974) showed that iron bioavailability calculations based on total hemoglobin formation gave quite different results when compared to calculations based on hemoglobin level or hematocrit values, whereas the data reported in this paper show good agreement regardless of the hematological parameters used to calculate iron bioavailability. It is not clear why our data differ from that of Mahoney et al. (1974); however, it is possible that the difference between our studies and theirs could be a function of the time of depletion and repletion. We depleted rats for 5 weeks, resulting in a minimum hemoglobin and hematocrit values, and repleted the rats for 2 weeks. Mahoney et al. depleted their rats for 3 weeks and repleted for 3 weeks. The differences encountered might suggest that 3 weeks is insufficient to allow for maximum depletion of hemoglobin in the test rats. If this is the case, interpretation of Mahoney et al.'s data may be complicated by further "depletion of hemoglobin" during the repletion period. If the source of iron had a moderate or low bioavailability, and also dramatically stimulates

Table VI. Slope Ratios

	feeding regime								
	ad libitum								
	data analyzed as a function of added iron			data analyzed as a function of added iron consumed			restricted		
iron source	hematocrit	Hb level	total Hb	hematocrit	Hb level	total Hb	hematocrit	Hb level	total Hb
$FeSO_4$	1.00[a a]	1.00[a a]	1.00[a a]	*b*	1.00[a a]	1.00[a a]	*b*	*b*	1.00[a a]
meat	0.448[b]	0.349[c]	0.422[c]	*b*	0.256[c]	0.408[c]	*b*	*b*	0.448[b]
soybean protein isolate	1.005[a]	0.827[b]	0.876[ab]	*b*	0.818[ab]	0.898[ab]	*b*	*b*	0.905[a]
processed soybean protein isolate	1.021[a]	0.804[b]	0.859[b]	*b*	0.705[b]	0.779[b]	*b*	*b*	0.968[a]
	Omitting Animals Fed Highest Level of $FeSO_4$								
$FeSO_4$	1.00[a a]	1.00[a a]	*c*	*b*	*b*	*c*	1.00[a a]	1.00[a a]	
meat	0.431[b]	0.349[c]	*c*	*b*	*b*	*c*	0.498[b]	0.548[b]	
soybean protein isolate	0.894[a]	0.849[ab]	*c*	*b*	*b*	*c*	0.833[a]	0.979[a]	
processed soybean protein isolate	0.908[a]	0.824[b]	*c*	*b*	*b*	*c*	0.847[a]	1.001[a]	

[a] Those slope ratios not followed by the same letter are significantly different at $P < 0.05$. [b] Slope ratios invalid because of nonlinearity. [c] Analyses were not conducted.

growth and therefore increases blood volume, it is possible that an increase in hemoglobin concentration may not occur.

In unpublished experiments we have actually observed decreases in hemoglobin level if cooked beef is used as the iron source for depleted rats. In this case, we observed a dramatic non-iron-dependent stimulation in growth when rats were fed cooked beef as the iron source. Mahoney et al. (1974) also noted a similar growth stimulation which was not iron dependent because iron supplied as ferrous sulfate yielded much less growth stimulation. We subsequently found that the diets employed in these unpublished experiments were marginally deficient in vitamin B_6 and pantothenate [this diet was reproduced from the literature, Fritz et al. (1975)]. We reasoned that the cooked beef was increasing the level of these B vitamins and thereby increasing growth. We could not find evidence of nutrient deficiencies in the diet of Mahoney et al. (1974). Thus, the data of Mahoney et al. (1974) cannot apparently be explained by a dietary deficiency of one or more nutrients. There were other differences between our experiment and that of Mahoney et al. (1974) (dietary iron level, initial body weight, and possibly differences in storage iron). However, it is unclear whether these differences can explain the results.

The next part of this discussion is devoted to a comparison of rat data to human data. Although use of the iron-deficient rat as a model for people has been criticized (Monsen, 1974), our data show reasonable agreement to human data. Monsen (1974) has pointed out that iron deficiency would tend to minimize differences in the absorption of iron sources. Although this is true, we feel the iron-deficient rat model more closely parallels the human population which is at risk, that is, the iron-deficient population. As people and rats become iron deficient, iron absorption is increased, thus there is a compensatory mechanism to increase iron absorption in times of need. In fact, iron absorption is increased before hemoglobin levels or hematocrits are decreased. Although differences in iron absorption between iron sources will not be as great among the iron-deficient population, this is obviously the most important population.

Others have argued that the rat does not use heme (the predominant form of iron in beef) iron as efficiently as people. This conclusion would appear to be largely based on studies conducted by Weintraub et al. (1965) and Conrad et al. (1966a), which showed poor absorption of heme iron by rats. To the contrary, other studies by Bannerman (1965) demonstrated as much as 50% absorption of heme iron.

Bannerman's (1965) studies were conducted in both iron-deficient and iron-supplemented rats with rats made iron deficient by feeding a low iron diet. The dose level of iron ranged from 1 to 500 μg of iron. The data from these studies showed that heme iron was absorbed approximately one-half as efficiently as ferrous sulfate at all dose levels in iron-supplemented animals, while in iron-deficient animals absorption of heme iron was one-third to one-half as efficient as ferrous sulfate. The iron-deficient animals dosed with 500 μg of heme iron show about one-fourth the iron absorption of those animals dosed with an equivalent dose of ferrous sulfate.

One difference between the two heme absorption studies was dose level; however, this does not completely explain the contrasting results. The major difference in the two studies could be in the method of preparing iron-labeled hemoglobin. Since hemoglobin is known to polymerize, precipitate, and become insoluble (Conrad et al., 1966b; Shack and Clark, 1947), slight differences in preparation could have yielded different amounts of insoluble and thus unabsorbable hemoglobin.

Bannerman's (1965) results are consistent with our studies showing that meat iron (which is predominantly heme iron) is utilized about one-third to one-half as efficiently as ferrous sulfate. As a result of Bannerman's (1965) and our own studies we conclude that the ability of the rat to utilize heme iron has been previosly underestimated.

Studies in iron-adequate people have shown that iron from heme is absorbed as well as if not better than iron from an inorganic source, such as ferrous sulfate (Turnbull et al., 1962; Hussain et al., 1965). The data substantiate this statement for iron-adequate people (Turnbull et al., 1962). However, in iron-deficient people, heme iron is absorbed only about one-third as efficiently as inorganic iron (ferrous sulfate or ferrous ascorbate) (Turnbull et al., 1962; Hussain et al., 1965). Thus, the absorption data comparing heme iron to inorganic iron in iron-deficient people are in reasonable agreement without animal data showing that meat iron (predominantly heme iron) is about

one-third to one-half as available as inorganic iron (such as ferrous sulfate or ferrous ascorbate). Thus, the rat data actually agree closely with the human data, suggesting that iron absorption patterns in the rat may more closely parallel those seen in humans than has been previosly supposed. Furthermore, the data suggest that meat or heme iron may not be as good a source of iron as previously supposed.

The excellent availability of iron from soybean protein in rats (Fritz et al., 1970; Theuer et al., 1971; and our study) and in man (Layrisse et al., 1969) would appear to be contradictory to the widely supposed inhibitory effect of phytate [present in soybean protein at 2.0 to 3.0% (Okubo et al., 1975)] on iron absorption. The exact origin of the postulate that phytate inhibits iron absorption is unclear, but it would appear to be largely based on the inhibitory effect of purified sodium phytate on iron absorption in rats (Davies and Nightingale, 1975) and in man (Sharpe et al., 1950; Hussain et al., 1959; Hallberg and Solvell, 1967; McCance and Widdowson, 1943).

This apparent discrepancy is readily explained by a careful examination of the experiments. All studies which show an effect of phytate on iron absorption or iron availability have employed purified sodium phytate (Sharpe et al., 1950; Hussain et al., 1959; Hallberg and Solvell, 1967). However, studies which have carefully examined the effect of naturally occurring phytate have failed to demonstrate an inhibitory effect of phytate on iron absorption or iron availability in either man or rats (Sharpe et al., 1950; Welch and Van Campen, 1975; Welch et al., 1974). The contrasting effects of sodium phytate and phytate occurring in foods can be readily explained. The sodium phytate is easily ionized and thus more easily forms poorly adsorbed iron complexes or precipitates, whereas naturally occurring phytate such as that in soybean protein probably occurs as a mixed metal salt of calcium and magnesium and phytate as such may be unable to react with the iron. Regardless, as Waddell (1973) has stated, it is quite obvious that sodium phytate and the phytates occurring in foods are quite different compounds as regards their effects on food iron. Morris and Ellis (1976) have isolated monoferric phytate from wheat and have shown it to be a highly available source of iron for the rat. This observation led Morris and Ellis (1974) to propose that phytate may be a carrier for iron absorption rather than an inhibitor of iron absorption. Thus naturally occurring phytate has much less of an effect on iron absorption than had been previously supposed.

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