Nutritional Bioavailability of Iron

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FOREWORD

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PREFACE

IF NUTRIENTS FOUND IN FOOD were digested, absorbed, and made available to the human or animal body at the 100% level, the science and practice of nutrition would be indeed simplified. That nutrients vary in their bioavailability has been well established. The chemical nature of the specific form of the nutrient involved, the chemical and physical characteristics of the foods in which nutrients are contained, other constituents of the diet, the nature of the digestive and absorptive processes for the specific nutrients, and the physiological condition of the person consuming the food all may affect bioavailability. However, knowledge of specific individual and interacting factors affecting bioavailability and utilization of nutrients has not yet been fully elucidated and constitutes one of the most active areas of current nutrition research.

Iron deficiency anemia is commonly found in both affluent and economically deprived populations. In prevention of this nutritional deficiency disease, both increase in dietary iron and increase in the availability of this dietary iron for population groups at risk should be concurrently addressed. This is a problem for which the solution lies primarily not with the medical community but rather with the providers of food in agriculture and food industry.

The chapters were selected to give a broad overview of the topic of bioavailability of iron with special emphasis on topics of concern to food producers.

The editor expresses appreciation to all the contributors and to Donna Hahn, who did much of the organizational work involved in preparation of this volume.

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Efficiency of Hemoglobin Regeneration as a Method of Assessing Iron Bioavailability in Food Products

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The bioavailability of iron from any source (e.g., iron supplement, food or meal composite) is considered to be that portion of the total iron which is metabolizable. Philosophically, this concept is important because the amount of iron utilized by avian and mammalian species is directly associated with iron need. When assaying iron bioavailability, it is therefore necessary to use an organism whose need will exceed the amount provided. In animal assays of iron bioavailability, iron need is assured by a growth phase and/or creation of iron deficiency through feeding an iron deficient diet and phlebotomy. Because healthy subjects are usually used in human assays of iron bioavailability (Cook et al., 1981; Cook and Monson, 1976; Radhakrishman and Sivaprasad, 1980), it is inappropriate to compare the data obtained from animal and human assays. In fact it is questionable if assays of iron bioavailability yield good information on the quantities of metabolizable iron available when healthy human subjects are used.

The Committee on Dietary Allowances, Food and Nutrition Board, National Academy of Sciences (RDA, 1980) has estimated the amount of metabolizable iron (as absorbable iron) from meals consumed by human beings as ranging from 3 to 23 percent depending on the nature of the meal. For adult women of childbearing age, the committee has assumed that 1.5mg iron is lost daily and that 18mg should be consumed to meet this need. They have therefore assumed that approximately 8.3 percent of the dietary iron will be metabolized. For adult men and women over the age of 51 years, they estimate that 1.0mg iron will be lost daily and recommend that 10mg should be consumed to meet this need to offset only approximately 10 percent of the dietary iron being metabolized by these people. It should be noted, however, that what is metabolized from a food under such conditions does not necessarily reflect what is potentially metabolizable. Indeed, the majority of women of childbearing age consume less than the recommended 18mg iron

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and yet are not iron deficient (DHEW, 1968-70). Thus, much information is needed on the metabolizability of food iron.

Two basic methods have been used in the assay of iron bioavailability (Bing, 1972; Thompson and Raven, 1959). In the absolute method, the change in total body iron relative to that consumed is used. This necessitates making an estimate of the amount of iron present in the animal body at the initiation of the experiment and then determining the amount present at the termination. Usually in applying this procedure, a representative group of animals is killed at the beginning of the experiment to obtain the estimate of their initial body iron. Thus, one can obtain an average value for body iron content relative to weight that can be multiplied with initial body weights to estimate initial amounts of body iron for each test animal. Various modifications of the hemoglobin regeneration procedure have been used(Bing, 1972). In the one described here, the amount of iron gained as hemoglobin is estimated and expressed relative to the amount of iron consumed. An efficiency of the conversion of food iron into hemoglobin can be computed for each test animal knowing initial and final body weights, initial and final hemoglobin concentrations, the amount of food consumed, and the iron content of the food. It is calculated as follows:

$$\text{mg Hb Fe} = \text{BW} \times .067 \text{ ml bl/g BW} \times \text{g Hb/100 ml} \times 3.35\text{mg Fe/g Hb}$$

$$\text{Efficiency} = \frac{(\text{Final mg Hb Fe} - \text{Initial mg Hb Fe})}{\text{mg Fe consumed}} \times 100$$

In applying this method, weanling male rats are given free access to a low-iron diet and bled to remove about one ml of blood two times 4 days apart. Three days later, the animals are again bled of about 100 microliters blood for determination of hemoglobin concentration and are allotted to treatments of ten rats each such that mean body weights and hemoglobin concentrations are similar. The mean hemoglobin concentrations should be between 4 and 6 gm/dl. They are fed the test diets for ten days in amounts that very few orts are obtained. Any spillage and orts are weighed and recorded to account for unconsumed dietary iron. The low-iron diet should contain less than 10 ppm Fe and the test diets should contain approximately 35 ppm. This amount of dietary iron has been shown not to exceed the ability of this animal preparation to utilize iron, since the regeneration of hemoblobin iron is linear at least to 68 ppm dietary iron (Mahoney and Hendricks, 1976). Miller (1977) reported that iron gained as hemoglobin was linear (r=0.94) through intakes of 5.5mg iron as ferrous sulfate in 11 days. Her rats were made anemic by feeding low-iron diet for 24 days in preparation for the hemoglobin regeneration experiment.

The following criteria for a good bioavailability assay are appropriate. (a) It must be dose responsive. For an

assay to be useful in a variety of situations, it should not be affected by variations in amounts of iron consumed. Therefore, the dose-response relationship should be linear. (b) It must discriminate with good sensitivity among sources of iron and among treatments such as cooking or processing. (c) Bioavailability values obtained should be unaffected by factors unrelated to the food or iron source. Thus, the bioavailability assay should be insensitive to variations in caloric density of the diet, appetite of the animal, and animal maturity. (d) The procedure should yield reproducible results for the same iron source among experiments and laboratories.

The efficiency of converting dietary ferrous sulfate iron into hemoglobin by anemic rats has been calculated from the data of many experiments and laboratories (Table 1). The 'uncorrected' efficiency values represent the values obtained for the total amounts of iron in the diets and the 'corrected' values represent a mathematical estimation of the hematinic response to only the ferrous sulfate iron present in the diet. This estimate was made assuming that the amount of iron present in the low-iron basal diets reflects the cumulative iron provided by the basal ingredients of the ferrous sulfate test diets (e. g., casein, oil, dextrose, fiber, vitamin mixture and mineral mixture). Thus, knowing the amounts of diet consumed by the test animals, one can estimate the contribution of the basal ingredients to the total dietary intake of iron of the test animals. This value subtracted from the total iron intake yields the estimated iron intake from ferrous sulfate. Similarly, the amount of iron gained as hemoglobin by the rats fed the low-iron basal diet can be calculated and subtracted from the total iron gained as hemoglobin by the rats fed the ferrous sulfate test diets, which yields an estimate of the ferrous sulfate contribution to the iron gained as hemoglobin. This value, relative to the estimated quantity of iron consumed as ferrous sulfate, was used to compute the 'corrected' efficiencies presented in table 1. The 'corrected' values were computed similarly for the iron sources presented in table 2. The validity of this correction is doubtful when foods are the source of experimental iron because the amounts of basal ingredients are decreased depending on the iron content of food tested, which affects the amount of food that must be formulated into the diet to provide the desired iron content.

For ferrous sulfate, the average efficiency of converting dietary iron into hemoglobin was 52 percent with a coefficient of variation of 19 percent (Table 1) . When corrected for the basal dietary ingredients, the average efficiency was 61 percent, with a coefficient of variation of 33 percent. Making the correction for the basal ingredients did not improve the analysis. In two cases, the 'corrected' efficiency of conversion was greater than 100 percent.

Table 1. Efficiency of Converting Iron in $FeSO_4$ into Hemoglobin by Anemic Rats

Dietary Fe (mg/kg)	Efficiency Uncorrected	Efficiency Corrected[a]	Reference
33.0	80	111	Farmer et al. (1977)
40.4	50	52	Allred (1976)
31.2	38	42	Mahoney et al. (1979)
27	71	45	Rahotra et al. (1973)
27	69	44	
--	54	56	Anderson et al. (1972)
27.8	51	70	Mahoney et al. (1974)
16.2	47	68	Blumberg & Arnold (1947)
20.5	48	60	
29.2	52	61	
45.0	46	49	
18.2	44	33	Mahoney & Hendricks (1976)
25.6	41	34	
41.8	46	41	
68.9	36	39	
11.8	57	53	Miller (1977)
18.9	62	60	
23.8	72	71	
23.6	54	42	Cardon et al. (1980)
35.2	60	57	
48.2	66	66	
	49	55	Theur et al. (1971)[b]
	53	59	
	57	62	
	49	50	
	52	85	Theur et al. (1973)[b]
	57	76	
	48	58	
13.8	53	148[c]	Fritz et al. (1974)[b]
19.8	57	89	
31.8	47	61	
12.2	33	39	Fritz et al. (1970)[b]
17.2	38	45	
22.2	43	50	
27.2	42	46	
14	41	58	Shah et al. (1979)
20	60	78	
32	53	60	
15	51	71	Shah and Belonje (1973a)
22	67	81	
32	57	64	
16.5	54	74	Shah and Belonje (1973b)
26.5	57	66	
46.5	43	47	
Mean ± Sd	52 ± 10	61 ± 20	T=2.67 (P .02)

Continued on next page.

Table 1-Continued

Note: Uncorrected efficiencies of 82, 77, 74, 65, 63, 84, 82, and 65 percent were calculated using data presented by Cowan et al. (1967). Because there were insufficient data published to calculate the corrected efficiencies, these data were not included in Table 1.

[a]The efficiency was corrected by estimating the contribution of iron in the basal diet to iron intake and hematinic response.

[b]Supplemental data necessary for computations supplied by authors.

[c]Values greater than 100 percent not included in the mean.

Table 2. Efficiency of Converting Iron From Various Sources Into Hemoglobin By Anemic Rats.

Source	Dietary Fe (mg/kg)	Efficiency Uncorrected	Efficiency Corrected[a]	Reference
$FePO_4$	18.8	26	4	Blumberg & Arnold (1947)
	29.2	18	4	
	54.9	21	18	
	118.0	14	12	
$FePO_4$	19.8	22	34	Fritz et al. (1974)[b]
	31.8	24	32	
	55.8	23	27	
$FePO_4$	24.2	21	26	Mahoney & Hendricks (1976)
	32.6	17	18	
	38.2	23	25	
	49.7	26	30	
Ground Beef	26.6	34	42[c]	Mahoney et al.(1974)
Beef Shank	31.0	63	87	Farmer et al.(1977)
Beef Plate	33.0	61	79	Farmer et al. (1977)
Bologna	29.0	46	62	Mahoney et al. (1979)[b]
Beef	26.0	49	37	Cardon et al. (1980)
Turkey	23.0	45	74	Mahoney et al. (1980)
Turkey	30.4	43	---	Cardon et al. (1980)
Enriched Flour	24.4	24	33	Mahoney et al. (1974)
White Bread	10.7	28	49	Miller (1977)
Whole Wheat Flour	28.0	43	54[c]	Mahoney et al.(1974)
Rice	28.0	30	31	Shah et al. (1979)
	48.0	43	41	
Dried Egg	21.2	43	41	Mahoney et al.(1974)

[a]The efficiency was corrected by estimating the contribution of iron in the basal diet to iron intake and hematinic response.

[b]Supplemental data necessary for computations supplied by authors.

[c]Due to calculation errors, the original value was reported as 45 for ground beef and 33 for whole wheat flour.

In ten cases, the 'corrected' values were less than the uncorrected ones. Because of this inconsistency and because correction does not reduce variability within nor among experiments, attempting to correct for the iron contribution of the basal ingredients to the hematinic response does not seem to improve this assay of iron bioavailability.

Dietary iron level does not seem to affect the efficiency with which dietary iron is converted into hemoglobin when ferrous sulfate (Table 1) or when ferric orthophosphate (Table 2) is the primary source of dietary iron. This is also true for white bread (Table 2); however, the source of the iron in the enriched flour used in the bread is unknown. That the efficiency of converting food iron into hemoglobin is not affected by dietary iron concentration is important to bioavailability testing because it is often difficult to formulate diets with precise amounts of iron, especially when foods are the sources of iron.

The effects of carbohydrate and fat on the efficiency with which dietary iron is converted into hemoglobin have been studied. Miller and Landes (1976) used starch, sucrose or glucose as the carbohydrate source and ferrous sulfate as the iron source. The respective efficiencies of converting dietary iron into hemoglobin were 72, 65, and 46 percent. Amine and Hegsted (1971) obtained similar carbohydrate effects studying iron absorption. Glucose is the most commonly used source of dietary carbohydrate in semipurified diets. Pennell et al. (1976) reported that beta-lactose in place of sucrose reduced the relative biological value of iron as sodium iron pyrophosphate when fed to rats. However, alpha-lactose or glucose in place of the sucrose did not affect the bioavailability of this iron source. Similarly, the source of fat can affect the bioavailability of dietary iron; but, the level of dietary fat has no effect (Mahoney et al., 1980). The casein concentration of diets fed rats does not affect iron absorption (Amine and Hegsted, 1971, Carmichael et al., 1975); however, effect of protein source was not studied by these authors. Thus, the sources of carbohydrate and fat can markedly affect the utilization of dietary iron and should be considered as important variables in bioavailability experiments. The amount of protein, however, does not seem as critical.

Among experiments, the variability of the efficiency of converting iron from ferrous sulfate into hemoglobin (Table 1) was much greater than when ferric orthophosphate (Table 2) was the iron source. This variability is disturbing since ferrous sulfate is commonly used as a reference source of iron for bioavailability experiments, as well as an iron supplement clinically. Typically, this variability is dealt with by expressing the hematinic responses of the unknowns relative to ferrous sulfate (Shah et al., 1979; Coccodrilli et al., 1976; Amine et al., 1972).

Using the efficiency of converting dietary iron into hemoglobin, effects of food processing procedures on the bioavailability of iron in meat have been studied. Farmer et al. (1977) showed that the bioavailability of iron from mechanically deboned meat was less than that from hand deboned meat; but, more metabolizable iron was available in the mechanically deboned product because of its greater iron content. There was no difference, however, between the iron bioavailability from mechanically deboned and hand deboned turkey frame meat (Allred, 1976). The difference in iron bioavailability between the mechanically deboned turkey and the mechanically deboned beef might be attributed to differences in abrasiveness of the meat and bone mixture on the machinery, which would modify the amount and form of iron in the two products (Farmer et al., 1977). The bioavailability of meat iron is decreased due to curing. This decrease is dose dependent with nitrite added, until residual nitrite begins to accumulate (Mahoney et al., 1979). Residual nitrite was associated with an apparent increase in iron bioavailability, which was explained on the basis of some nitric oxide binding to hemoglobin, rendering a fraction of it unable to carry oxygen and thus stimulating hematopoiesis. Severe atmospheric oxidation of beef results in depressed iron bioavailability and growth in rats while similar oxidation of turkey meat did not (Cardon et al., 1980).

Based on the limited data available, the relative biological values of iron sources are similar whether determined by the slope-ratio assay or by efficiency of conversion of dietary iron into hemoglobin (Table 3). The most descrepancies are observed when the relative biological value is estimated by method "c" in Table 3. Much additional research is required to determine the utility of the simpler method of evaluating iron bioavailability by efficiency of converting dietary iron into hemoglobin. It does, however, take less time than the slope-ration method, apply to food stuffs of relatively low iron concentration (Ifon, 1981), provide for direct measurements of iron utilization, and apply to human subjects such as blood donors, anemic subjects (Norby and Solwell, 1977) and infants (Garry, et al., 1981). It, therefore, has many potential advantages as means of evaluating iron bioavailability.

Table 3. Comparison of Biological Values of Different Iron Sources Relative to Ferrous Sulfate

Iron Source	Rel. Biol. Value	Reference
$FePO_4$	51[a]	Mahoney & Hendricks (1976)
$FePO_4$	56[b]	Amine et al. (1972)
$FePO_4$	44.5 + 4.8[b]	Fritz et al. (1974)
$FePO_4$	23[a]	Blumberg & Arnold (1947a)
$FePO_4$	46[a]	Blumberg & Arnold (1947b)
$FePO_4$	14[c]	Fritz et al. (1970)
$FePO_4$	75[c]	Motzok et al. (1977)
$FePO_4$ in Breakfast Cereal	33[c]	Shah et al. (1979)
$FePO_4$ in Breakfast Cereal	38[c]	Coccodrilli et al. (1976)
Enriched Flour	55[a]	Mahoney et al. (1974)
Enriched Flour	32[c]	Fritz et al. (1970)
White Bread	53[b]	Miller (1977)
Turkey, raw	83[a]	Mahoney et al. (1980)
Turkey, raw	72[a]	Allred (1976)
Whole egg, dried	84[a]	Mahoney et al. (1974)
Egg yolk	33[c]	Fritz et al. (1970)
Beef, raw	80[a]	Cardon et al. (1980)
Beef, cooked	67[a]	Mahoney et al. (1974)

[a] Relative Biological Value calculated by dividing the efficiency of converting iron from the test diets into hemoglobin iron relative to that for diets containing ferrous sulfate.

[b] Relative Biological Value determined by slope-ratio assay.

[c] Relative Biological Value is calculated (Pla and Fritz, 1971):

$$RBV = \frac{\text{mg Fe/kg from test diet}}{\text{mg Fe/kg from } FeSO_4 \text{ diet}} \times 100$$

that give the same response in hemoglobin

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