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The Importance of Animal Products in Human Iron Nutrition

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I. INTRODUCTION

Long before iron deficiency was recognized as the major cause of hypochromic anemia, many clinicians believed that a high intake of meat would produce a hematologic response. However, increasing the intake of meat in the diet is not a satisfactory method of treating patients with iron deficiency anemia. Nevertheless, there is mounting evidence that the intake of animal foods is a major determinant of iron status. Recognition of the importance of animal protein in iron nutrition is based largely on the results of food iron absorption studies in human subjects. Because these measurements form the cornerstone of the present knowledge of iron balance, they are reviewed here before discussing the importance of animal foods in iron nutrition.

II. MEASUREMENTS OF IRON ABSORPTION IN MAN

Early studies of food iron absorption were based primarily on chemical balance measurements: absorption was calculated as the difference between iron intake and fecal iron loss. This approach proved to be unreliable because the small differences between iron intake and iron loss are often obscured by iron contamination. Most of the present knowledge of iron nutrition is based on isotopic measurements of iron absorption, which have been used extensively since the late 1940s.

A. Radioiron Absorption

There are several ways of determining the percentage absorption of orally administered radioiron: fecal radioiron balance, plasma radioiron tolerance curves, whole-body counting, and measurements based on incorporated red cell radioactivity. When assessing iron balance in patients with various clinical disorders, whole-body counting is the optimal method because the results are not affected by the distribution of absorbed radioactivity. However, when studying food iron absorption in normal individuals, measurements of red cell radioactivity are preferable because as many as four separate iron absorption tests can be performed in the same subject using dual isotopes of iron (^{55}Fe and ^{59}Fe). By calculating the ratio of absorption from different meals by the same subject, the effect of differences in iron status between subjects can be eliminated. Moreover, any errors in estimating blood volume from height and weight or assuming that all subjects incorporate the same proportion of absorbed radioactivity into circulating red cells are eliminated by calculating the ratio of iron absorption in a series of tests in the same subject.

One drawback in food iron absorption studies is that the percentage absorption

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of iron may vary markedly from study to study depending on the iron status of the subjects (Cook *et al.*, 1969). This problem can be circumvented by measuring absorption in all subjects from a standard reference dose consisting of 3 mg ferrous sulfate. By relating food iron absorption to reference dose absorption in each subject, the effect of differences in iron status of the subjects can be eliminated.

B. Biosynthetic Tagging

The most important aspect of food iron absorption is not the method for determining isotopic retention but rather the technique used for tagging dietary iron. Early studies were performed with single food items that had been biosynthetically labeled with radioiron (Moore, 1964). Vegetable foods were labeled by growing them in hydroponic media containing radioiron, and animal foods were tagged by injecting radioiron intravenously several months before sacrifice Studies with these foods provided the first evidence that iron bioavailability of animal foods is much higher than that of vegetable foods (Layrisse *et al.*, 1969; Martinez-Torres and Layrisse, 1974). Thus iron absorption from staple foods (e.g., maize, black beans, and wheat) averages 3-5% and absorption from animal foods (e.g., meat, liver, and fish) ranges from 15 to 30%.

A major limitation of studies with biosynthetically labeled foods became apparent when two foods tagged separately with ^{59}Fe and ^{55}Fe were administered in the same meal. It was found that iron absorption of one food item could be markedly affected by the presence of a second food. For example, when labeled meat was administered with biosynthetically tagged maize, absorption of meat iron was diminished but absorption of maize iron was greatly enhanced (Martinez-Torres and Layrisse, 1971). It was obvious from these mixing studies that data obtained with biosynthetically tagged foods are of limited importance because of the complexity of the average diet. Therefore, it became necessary to develop a technique to measure absorption from a complete meal.

C. Dietary Nonheme Iron

A major advance in studies of food iron absorption was the development in the early 1970s of extrinsic tagging to measure absorption of dietary heme and nonheme iron. This approach was based on the observation that if a small quantity of inorganic radioiron is simply added to a vegetal food at the time of its administration, absorption is nearly identical to that of a biosynthetic, or intrinsic, tag. Moreover, if a meal containing several vegetal foods is labeled extrinsically after thorough homogenization, absorption of the extrinsic tag is nearly identical to absorption of a food item in the meal that has been biosynthetically labeled (Cook *et al.*, 1972). These observations provide convincing evidence

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that a common pool of absorbable nonheme iron is formed with foods ingested in the same meal and that iron absorption from this pool can be measured by extrinsic tagging.

Although most studies have shown that extrinsic labeling is a valid method of measuring nonheme iron absorption, there have been a few exceptions When biosynthetically tagged whole-grain rice was tagged extrinsically with a second form of radioiron, absorption of the two labeled foods differed markedly, whereas this difference was not seen when rice was administered as flour; apparently, the extrinsic tag does not permeate the whole rice kernel (Björn-Rasmussen *et al.*, 1973). Some forms of fortification iron, such as ferric orthophosphate and sodium iron pyrophosphate, do not exchange completely with dietary iron because of their high degree of insolubility (Cook *et al.*, 1973), and it is likely that iron contamination of food by dirt and/or food handling processes also results in an insoluble form of dietary iron that does not exchange with native food iron. Iron in ferritin and hemosiderin have been shown to exchange incompletely with an extrinsic tag, presumably because the protein coat of these storage iron proteins inhibits complete isotopic exchange (Layrisse *et al.*, 1975; Martinez-Torres *et al.*, 1976). Nevertheless, with these few exceptions, extrinsic tagging has proved to be a valid method of measuring dietary nonheme iron absorption.

D. Dietary Heme Iron

It is well known that iron enters the mucosal cell from the intestinal lumen not only as ionic iron but also as an intact porphyrin complex. This heme iron is rapidly degraded within the mucosal cell by heme oxygenase, and the liberated iron enters the same pathway as absorbed ionic iron. Therefore, it is either transported to the serosal surface where it enters the circulation or incorporated into ferritin as storage iron within the mucosal cell. It is important to distinguish between heme and nonheme iron because they are affected quite differently by the composition of the meal. Absorption of nonheme iron is markedly affected by substances that enhance iron absorption, such as ascorbic acid, and by inhibitors that bind nonheme iron and render it unavailable to the mucosal cell, such as tea or fiber. By contrast, the absorption of heme iron is little affected by the composition of the meal because this iron remains sequestered within the porphyrin complex until it enters the mucosal cell.

The same principle of extrinsic tagging for measurement of nonheme iron absorption can also be used to measure heme iron absorption; heme iron is labeled by adding a small quantity of radioactive hemoglobin to the test meal. Because there are two separate isotopes of iron available, ^{55}Fe and ^{59}Fe, it is possible to separately tag heme and nonheme iron in the same meal and thereby

provide a measure of total iron absorption from a complete meal. This two-pool model is particularly relevant in studies of animal foods because animal tissue contains both forms of iron.

The validity of the two-pool model for studying iron absorption has been evaluated by comparing iron absorption measurements with predicted values of absorption based on measurements of body iron loss. In one study, a meal composed of aliquots of all foods contained in a typical 6-week diet was tagged with heme and nonheme radioiron and administered to 32 normal male subjects (Björn-Rasmussen *et al.*, 1974). Dietary iron intake based on this composite meal was estimated at 17.4 mg iron daily of which 1 mg was in the form of heme iron. Absorption of nonheme iron averaged 5.3% or 0.8 mg/day while absorption of heme iron averaged 37% or 0.37 mg/day. The combined absorption of 1.2 mg iron daily is in close agreement with estimates of iron loss in normal males. Similar absorption data have been obtained by Layrisse and Martinez-Torres (1972) who studied a meal of meat, black beans, maize, and rice containing a total of 4.5 mg iron of which 1.5 mg was in the form of heme iron. In normal subjects, absorption of heme iron averaged 27% or 0.34 mg as compared with a value for nonheme iron of 6% or 0.12 mg; total absorption was 0.46 mg iron. In iron-deficient subjects, absorption of heme and nonheme iron increased to 37% (0.52 mg) and 14% (0.43 mg), respectively, to give a total absorption of 0.95 mg. These two studies not only validate double extrinsic tagging, but also underscore the importance of heme as a source of dietary iron.

III. BIOAVAILABILITY OF IRON IN ANIMAL FOODS

A. Meat

The use of biosynthetically tagged animal products in human iron absorption studies provided the first clear evidence of the beneficial effect of these foods on iron balance. Early studies were performed with chicken (Moore, 1964) and rabbit muscle (Heinrich *et al.*, 1969), but the most extensive observations have been made using veal (Layrisse *et al.*, 1969). Radioactive iron was injected into the jugular veins of 3-month-old calves; 2 to 4 months later, the animals were sacrificed, and the biosynthetically labeled muscle and liver were removed and kept frozen until administered in test meals to human subjects.

Veal muscle was cooked and served as hamburger to 107 fasting adult subjects, many of whom were iron deficient (Martinez-Torres and Layrisse, 1971). Geometric mean absorption in the composite group was 21.5%. In 58 of these subjects, absorption was also measured from a reference dose of ferrous sulfate; absorption averaged 23.8% giving a mean absorption ratio of veal:reference of

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1.12. The high bioavailability of veal muscle in this study was attributable, at least in part, to the high content of heme iron. In the supernatant of thoroughly homogenized meat 88–93% of the radioactivity was recovered, and 93–97% of this radioactivity was present as hemoglobin or myoglobin. Further evidence of the high heme iron content of veal was that substances that are known to alter the absorption of nonheme iron had little effect on the absorption of iron from veal muscle. For example, when 500 mg of ascorbic acid was added to veal muscle, a slight increase (16%) was observed, but this rise was not statistically significant. Similarly, the addition of 500 mg of desferrioxamine to the labeled food produced a relatively modest decrease (27%) in absorption. These results are in striking contrast to the profound effect that ascorbic acid and desferrioxamine have on the absorption of nonheme iron.

B. Liver

Similar studies were performed with labeled veal liver (Martinez-Torres *et al.*, 1974). When cooked veal liver was fed to 74 adult subjects, absorption averaged 19.8% with a typically broad range of 2.8–55.4%. In 27 of these subjects, reference dose absorption was measured simultaneously and averaged 16.8%; the mean absorption ratio of liver:reference was 1.00. Analysis of the labeled veal showed that 78% of the radioactivity was in the form of hemoglobin while the remaining 22% was contained in either hemosiderin or ferritin. As with veal muscle, the addition of compounds such as ascorbic acid or desferrioxamine, which markedly influence nonheme iron absorption, had little or no effect on the assimilation of labeled liver. When 500 mg ascorbic acid was added to the labeled liver, iron absorption in 10 subjects actually decreased from a mean of 15.3 to 13.8%. When 500 mg desferrioxamine was added to the same food, mean absorption decreased from 20.6 to 8.6%. Although this difference was statistically significant, the effect was far less striking than the effect of this chelate on nonheme iron absorption.

C. Other Foods

Apart from veal muscle and liver, fish is the only other food that has been studied extensively by biosynthetic labeling (Martinez-Torres and Layrisse, 1970). Radiolabeled fish muscle was fed to 19 adult subjects who absorbed a mean of 18.3%; reference dose absorption averaged 44.8%. The iron in fish muscle is apparently less available than the iron in either veal muscle or liver. Iron absorption from eggs has also been studied by biosynthetic labeling (Moore, 1964; Chodos *et al.*, 1957; Callender *et al.*, 1970). Mean absorption from iron in tagged eggs was consistently less than 5%; this low iron availability is in striking contrast to results obtained with animal tissues.

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IV. EFFECTS OF ANIMAL PROTEIN ON FOOD IRON ABSORPTION

A. Effect on Dietary Nonheme Iron Absorption

Animal foods are an important constituent of the American diet, not only because of high intrinsic iron availability but also because of their ability to increase the absorption of all nonheme iron ingested simultaneously. This enhancing effect was first demonstrated in a series of studies by Layrisse *et al.* (1968). Vegetable and animal foods biosynthetically tagged with different radioiron labels were fed both separately and simultaneously to volunteer subjects to determine the extent to which the absorption of one food was affected by the other. Because both foods were tagged with separate radioiron labels, it was possible to detect any increase in iron absorption from the staple food as well as any decrease from the animal food.

Representative mixing studies with biosynthetically tagged foods are listed in Table I. Maize or black beans were used as the staple food and veal muscle, veal liver, or fish as the animal food. In the first study iron absorption from a meal containing only maize (4 mg iron) averaged 4.1%, but, when maize (1 mg iron) was fed together with veal muscle (3 mg iron), absorption of maize iron increased to 7.6%. The absorption ratio of with:without animal protein averaged 1.87, an effect that was highly significant. Indeed, in all six studies shown in Table I, the increase in iron absorption from staple foods when taken with veal muscle (3 mg iron), veal liver (2 mg iron), or fish (1 mg iron) was highly significant and veal liver was at least as effective as veal muscle.

Although these studies identify the enhancing effect of animal tissue on nonheme iron absorption, it is difficult to estimate their quantitative effect on iron absorption from a normal diet. In order to obtain a constant total iron content in all meals in the studies listed in Table I, a smaller quantity of the staple food was given with animal tissue than when it was given alone. Because the quantity of food in a meal may have a more important effect on absorption than its iron content, it may be preferable to make substitutions on the basis of protein or caloric content rather than on iron content.

A final point in these mixing studies is the relatively minor effect of maize and black beans on the absorption of iron from veal muscle, veal liver, or fish. For example, absorption of veal muscle fell by only 14 and 17% when consumed with maize and black beans, respectively. Absorption of veal liver was reduced by 25 and 44% when consumed with 1 and 2 mg of maize iron, respectively. The enhancing effect of animal tissue is much greater than the inhibitory effect of vegetal foods.

In another study, several animal foods were evaluated with regard to their effect on nonheme iron absorption by substituting them in two types of test meals

TABLE I

Effect of Animal Tissue on the Absorption of Nonheme Iron from a Staple Food

Animal food	Staple food	Number of subjects	Iron content of staple food (mg)		Iron absorption from staple food (% of dose)		Mean absorption ratio (with:without animal food)
			Fed alone	Fed with animal food	Fed alone	Fed with animal food	
Veal muscle[a]	Maize	15	4	1	4.1	7.6	1.87
(3 mg iron)	Black beans	11	4	1	4.8	10.7	2.21
Veal liver[b]	Maize	12	2	2	4.9	10.9	2.22
(2 mg iron)	Maize	14	3	1	2.2	7.0	3.17
Fish[c]	Maize	6	3	2	4.2	7.5	1.78
(1 mg iron)	Black beans	8	3	2	1.0	1.7	1.70

[a] From Martinez-Torres and Layrisse (1971).
[b] From Martinez-Torres *et al.* (1974).
[c] From Layrisse *et al.* (1968).

(Cook and Monsen, 1975). The first, considered to be a standard meal, was a meal containing beef, potatoes, corn, bread, margarine, ice milk, and peaches. This meal contained 696 calories and 4.1 mg total iron; beef provided most of the protein. The second was a semisynthetic meal containing semipurified ingredients in which ovalbumin was the only source of protein. This meal was designed to match exactly the composition of the standard meal in respect to food energy, fat, protein, carbohydrate, calcium, phosphorus, and iron. Several animal foods were substituted in both meals including pork, lamb, beef, chicken, fish, whole milk, cheese, and powdered egg. These foods were substituted for beef in the standard meal and for ovalbumin in the semisynthetic meal in amounts that provided the same quantity of protein. When the animal foods were substituted for beef in the standard meal, no adjustments were made for differences in nutrient content. However, with the semisynthetic meal the other constituents were varied so that the substituted meal had the same total nutrient content as the

TABLE II

Effect on Nonheme Iron Absorption of Substituting Various Animal Products in Meals Containing Either Albumin (Semisynthetic) or Beef (Standard) as the Source of Protein[a]

		Semisynthetic meal			Standard meal		
		Mean iron absorption (% of dose)		Absorption ratio	Mean iron absorption (% of dose)		Absorption ratio
Substitute		Alb	Sub	(Sub:Alb)	Beef	Sub	(Sub:Beef)
Beef	(7)[b]	1.7	5.1***	2.96(a)[d]	—	—	—
Pork	(7)	1.6	5.2***	3.21(a)	9.7	9.3	0.95(a)
Lamb	(7)	1.6	5.2***	3.23(a)	7.6	7.8	1.03(a)
Liver	(8)	1.4	5.4***	3.77(a)	3.8	3.9	1.01(a)
Chicken	(7)	1.4	3.4**	2.43(ac)	11.5	10.4	0.90(a)
Fish	(8)	1.9	3.9*	2.11(ac)	14.8	12.2*	0.82(a)
Milk	(8)	0.7	0.9	1.22(bc)	5.5	1.6**	0.29(b)
Cheese	(8)	2.0	2.4	1.20(bc)	9.4	3.6**	0.38(b)
Egg	(10)	0.8	0.7	0.88(b)	8.2	1.8**	0.22(b)
Albumin	(7)	—	—	—	10.0	3.9**	0.39(b)

[a] From Cook and Monsen (1976).

[b] Number of subjects.

*, Significant difference, $p < 0.05$; **, significant difference, $p < 0.01$; ***, significant difference, $p < 0.001$.

[d] Mean ratios with differing lower case letters (a,b,c) are significantly different by Duncan's multiple range test.

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original meals. Four iron absorption test meals were administered to each volunteer subject: standard, semisynthetic, substituted-standard, and substituted-semisynthetic meals. The results of the study are summarized in Table II.

When pork, lamb, liver, chicken, and fish were substituted for beef in the standard meal, absorption ratios (substitute:beef) ranged from 0.82 to 1.03; in no case did the ratio reflect a significant difference. However, with dairy products, which contain no animal tissue, absorption ratios ranged from 0.22 to 0.39, all of which were statistically significant. Comparable results were obtained with the semisynthetic meal. Therefore, when animal tissue was substituted for ovalbumin, the increase in absorption ranged from 2.11 to 3.77. Some enhancement was also observed with chicken and fish (2.43 and 2.11, respectively), although the increase was less pronounced than with other animal tissues. In contrast, milk, cheese, and eggs were all similar to ovalbumin in their effect on the absorption of nonheme iron; ratios ranged from 0.88 to 1.22. These results indicate that dairy products have little or no enhancing effect on the absorption of dietary iron, and should not be considered as animal products with respect to food iron availability.

B. Effect on Ferritin and Hemosiderin

Nonheme iron in animal tissue is composed of ferritin and hemosiderin, the two major forms of storage iron in tissue. Nonheme iron in animal foods averages between 20 and 60% depending in part on the body organ; muscle, for example, has a much higher nonheme iron content than liver. Results from early studies in which purified ^{59}Fe-labeled ferritin alone was administered to human subjects indicated an absorption of about 7% in normal subjects, a value distinctly lower than absorption from other animal foods (Hussain *et al.*, 1965). More extensive studies with radiolabeled ferritin and hemosiderin were reported subsequently by Layrisse *et al.* (1975) and Martinez-Torres *et al.* (1976). Iron absorption of purified ferritin by 108 subjects ranged from 0.1 to 13.5% with a geometric mean of 1.9%, a much lower absorption than was observed in earlier studies. When purified ferritin was administered with biosynthetically tagged maize, iron absorption from maize was actually higher than from ferritin (2.0 and 0.9%, respectively); similar results were obtained with wheat and soybeans. However, when purified labeled ferritin was fed with animal tissue, a striking increase in absorption occurred. Absorption increased from 1.3 to 12% when 2 mg of ferritin iron was administered with veal muscle containing the same quantity of iron, and in a second study using veal liver, absorption increased from 1.7 to 6.6%. These absorption levels were comparable to those observed in meals containing both vegetable and animal foods. Because low absorption of ferritin iron may be an artifact related to purification of the protein, it seems unnecessary to regard ferritin and hemosiderin as separate pools of dietary iron.

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C. Effect on Dietary Heme Iron Absorption

Animal tissue consists predominantly of heme iron and therefore is little affected by factors such as ascorbic acid or desferrioxamine which have a profound effect on nonheme absorption. It is unlikely that other foods have any effect on the absorption of purified hemoglobin. Surprisingly, however, iron absorption from labeled hemoglobin is much less when the hemoglobin is administered alone than when taken with meat. Martinez-Torres and Layrisse (1971) observed an increase in absorption from 12 to 22% when labeled hemoglobin was mixed with meat prior to administration, and this finding was recently confirmed by Hallberg *et al.* (1979). The enhancing effect of meat on hemoglobin iron absorption is more important from a physiologic than from a nutritional standpoint because, with the exception of heme iron fortification, dietary heme iron is consumed as a component of animal tissue.

D. Mechanism of Meat Effect

One of the vexing questions in iron nutrition is what mechanism accounts for the enhancing effect of meat and liver on nonheme iron absorption. Some inferences can be drawn from studies of the enhancing effect of ascorbic acid, the only other dietary substance that is known to be as effective as meat in increasing iron absorption. There is evidence that the enhancing effects of meat and ascorbic acid are not additive. When 100 mg ascorbic acid was added to a semisynthetic meal (Cook and Monsen, 1977), the absorption ratio of with:without ascorbic acid was 3.19. When the same quantity of ascorbic acid was added to a standard meal, a lower ratio of 1.67 was observed. In another study, the addition of 100 mg of fish to a meal containing fresh papaya (70 mg of ascorbic acid) produced no further enhancement in iron absorption (Layrisse *et al.*, 1974).

A series of iron absorption studies designed to identify the effect of animal protein on iron absorption have been reported by Björn-Rasmussen and Hallberg (1979). The possibility that meat acts by stimulating gastric secretion was ruled out when it was shown that meat increased iron absorption of maize from 0.7 to 2.1% in patients with histamine-fast achlorhydria. Furthermore, when betazole, a drug that stimulates gastric secretion, was administered to normal subjects, absorption did not increase significantly. A modest increase in absorption was observed when a mixture of amino acids in amounts corresponding to that contained in beef muscle was added to maize, but contrary to previous observations (Martinez-Torres and Layrisse, 1970), no increase was observed when cystine alone was added to maize. When beef was fractionated into a water extract (broth) and an insoluble residue and fed with a meal of maize, the residue increased iron absorption from 1.4 to 2.4% whereas the broth had no enhancing effect; therefore, the meat factor does not appear to be water soluble.

The possibility that meat acts by stimulating mucosal uptake, or iron transport, would explain the similar enhancing effect on nonheme iron, ferritin iron, and heme iron. However, a mucosal cell effect should also exist with inorganic iron, and meat does not increase the absorption of ferrous chloride (Layrisse and Martinez-Torres, 1972). Meat might act by stimulating gastric secretion or altering gastrointestinal motility in favor of iron absorption, but it is more likely that animal tissue promotes the solubility of both heme and nonheme iron, presumably by separate biochemical mechanisms. Whether a "meat factor" is present in meat when administered or only formed during digestion of the food in the gastrointestinal tract is still a matter of speculation.

V. EFFECT OF INTAKE OF ANIMAL FOODS ON IRON STATUS

A. Estimates of Food Iron Availability

Little or no association has been found in various nutritional surveys between total iron intake and iron status. This may indicate that blood loss due to menstruation or pregnancy is a more important determinant of iron nutrition than is the diet. Another possible explanation is that the total intake of dietary iron is less important nutritionally than the type of diet consumed. A model has recently been developed for estimating food iron availability and predicting absorption from the composition of the meal (Monsen *et al.*, 1978). It is of interest to review this model because the content of animal food is an important parameter in estimating iron availability.

The first step in estimating iron absorption from a given meal is to calculate the total content of heme and nonheme iron. It is assumed for this purpose that pork, liver, fish, beef, lamb, and chicken all contain 40% heme iron, although, as noted earlier, this percentage may vary appreciably. Because heme iron absorption is not affected by the nature of the meal, iron absorption from heme is calculated by multiplying heme iron content by 25%, the estimated level of percentage absorption of heme iron in an individual with 500 mg of body iron stores.

Estimating nonheme iron absorption from a meal is more complicated because of food interactions. Two enhancing substances in the diet are considered to have overriding importance: ascorbic acid and animal foods including fish and poultry. One gram of animal food is assumed to be roughly equivalent in its enhancing effect to 1 mg ascorbic acid. Meals were divided on this basis into those of low-, medium-, and high-iron availability (Table III). Absorption of nonheme iron from these meals was estimated at 3, 5, and 8%, respectively, in a subject with 500 mg of iron stores.

This model for estimating absorption of dietary iron is undoubtedly oversimplified and is at best a preliminary step in determining the importance of the

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TABLE III

Dietary Iron Availability[a]

Meal iron availability	Meat, fish poultry (g)		Ascorbic acid (mg)	Nonheme iron absorption (%)
Low	<30	and	<25	3
Medium	30–90	or	25–75	5
High	>90	or	>75	8

[a] From Monsen *et al.* (1978).

diet in iron nutrition. Other dietary factors that are known to influence iron absorption, such as tea or EDTA, have not been included in the estimates because precise quantitative data on their effect are not available. It is also not clear what factor should be used when the meal contains both ascorbic acid and animal food. However, even with these limitations, this model comes much closer to determining the importance of diet in maintaining iron balance.

B. Epidemiological Studies

Many attempts have been made to establish a correlation between iron intake and iron status of a population, but the correlation has been poor when based on total iron intake without regard to food iron availability. However, a recent study of 7000 people in Finland showed that the intake of meat products does indeed correlate with iron status (Takkunen and Seppänen, 1975). The intake of animal products was found to be significantly lower in both male and female anemic subjects as compared with those having a normal hemoglobin level. A correlation was also observed in this study between meat consumption and iron status as measured by either the serum iron or total iron-binding capacity (Fig. 1). A

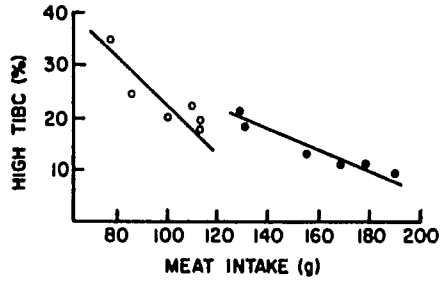

Fig. 1. Relationship between percentage of population with total iron-binding capacity (TIBC) greater than 400 μg/dl and daily intakes of meat in women (O) and men (●). (From Takkunen, 1976.)

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clearer picture of the importance of meat products should emerge when the model described in this section is used in future studies of dietary iron intake and its relation to iron status.

VI. IMPORTANCE OF ANIMAL FOODS IN IRON FORTIFICATION

A. Nonheme Iron Fortification

The only practical long-term approach for combating iron deficiency anemia in a population is to increase dietary iron intake by fortifying with iron a common item in the diet such as bread, sugar, or rice. However, recent studies suggest that the success of iron fortification depends largely on the intake of meat, fish, and poultry, and that without these animal foods, even heavy fortification is unlikely to be effective. For example, Layrisse *et al.* (1973) added increasing amounts of ferric chloride to meals containing either maize (1 mg iron), meat alone (3 mg iron), or a mixture the two foods in the ratio 2 mg meat iron to 1 mg maize iron. Ferric chloride was chosen as the iron salt because it undergoes complete exchange with dietary nonheme iron. Absorption values when 5, 20, and 60 mg iron were added are shown in Fig. 2. When 60 mg iron, a level far

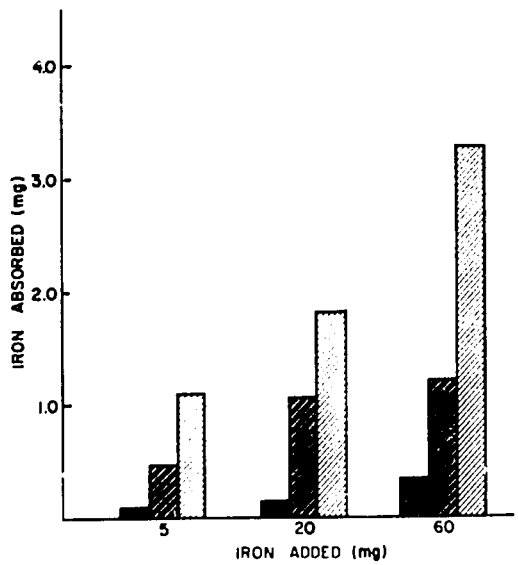

Fig. 2. Absorption of fortification iron (ferric chloride) added to meals containing (▩) maize alone (100g), (■) meat alone (150g), and ▨ maize plus meat (50 and 100 g, respectively). The enhancing effect of meat on nonheme iron absorption is apparent. (From Layrisse *et al.*, 1973.)

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above that which would be technically feasible on a national scale, was added to the maize meal, only 0.3 mg iron was absorbed. When this same amount of iron was added to a meal containing either meat alone or a mixture of meat and maize, an average of 3.27 mg and 1.22 mg, respectively, was absorbed.

Similar findings were observed in a later study by Hallberg *et al.* (1978). When 5 mg of fortification iron as ferrous sulfate was added to a rice-based meal containing only vegetables, absorption in an iron-deficient individual was estimated at 0.03 mg of iron. When 60 g of fish was added to the same meal, net absorption of fortification iron increased to 0.32 mg. Hallberg *et al.* concluded that iron fortification may be feasible with a rice-based diet, but that a certain minimal threshhold level of fortification iron would be required to achieve a reasonable level of absorption. The relatively high level of 5 mg of fortification iron per meal was considered realistic even with diets containing only negligible amounts of fish or animal foods.

B. Heme Iron Fortification

The low bioavailability of inorganic iron added to meals containing little or no animal products has prompted the suggestion that heme iron in animal blood recovered from the slaughterhouse could be employed for iron fortification (Reizenstein, 1975). Hemoglobin iron has the advantage that it is less inhibited by dietary substances that impair the absorption of nonheme iron. The dose-response curve also may be more favorable with hemoglobin iron than with inorganic iron, at least in patients with latent iron deficiency. Vehicles that have been suggested for heme iron fortification include blood sausage, pudding, meat products, and baking products.

The major limitation of fortification with animal blood is the risk of iron overload in iron-replete individuals. Because heme iron absorption is less affected by iron status than nonheme iron absorption, heme iron might be well absorbed in iron-replete individuals, which could conceivably create a risk of iron excess in susceptible segments of the population. In addition, the dark color of blood greatly limits the number of food items that could serve as vehicles for fortification. The costs of recovering hemoglobin and processing it to avoid bacterial contamination are undoubtedly high. Therefore, it is unlikely that slaughterhouse blood will be extensively used for iron fortification.

VII. SUMMARY

Studies employing double extrinsic tags of heme and nonheme iron to measure absorption of dietary iron have clearly established the importance of animal foods in iron nutrition. The major factor that accounts for high assimilation of iron from

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animal foods is their content of myoglobin and hemoglobin. These iron complexes enter the mucosal cell of the intestinal tract as intact molecules and therefore are little affected by dietary substances that impair the absorption of nonheme iron. Animal foods also play an essential role in iron nutrition because of their ability to enhance assimilation of all forms of dietary nonheme iron ingested simultaneously. It is unlikely that iron fortification programs would be effective in populations consuming only minimal quantities of meat, fish, or poultry.

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REFERENCES

Björn-Rasmussen, E., and Hallberg, L. (1979). Effect of animal proteins on the absorption of food iron in man. *Nutr. Metab.* **23**, 192-202.

Björn-Rasmussen, E., Hallberg, L., and Walker, R. B. (1973). Food iron absorption in man. II. Isotopic exchange of iron between labeled foods and between a food and an iron salt. *Am. J. Clin. Nutr.* **26**, 1311-1319.

Björn-Rasmussen, E., Hallberg, L., Isaksson, B., and Arvidsson, B. (1974). Food iron absorption in man. Applications of the two-pool extrinsic tag method to measure haem and nonhaem iron absorption from the whole diet. *J. Clin. Invest.* **53**, 247-255.

Callender, S. T., Marney, S. R., and Warner, J. T. (1970). Eggs and iron absorption. *Br. J. Haematol.* **19**, 657-665.

Chodos, R. B., Ross, J. R., Apt, L., Pollycove, M., and Hallkett, J. A. E. (1957). The absorption of radioiron labeled foods and iron salts in normal and iron-deficient subjects and in idiopathic hemochromatosis. *J. Clin. Invest.* **36**, 314-326.

Cook, J. D., and Monsen, E. R. (1975). Food iron absorption. I. Use of a semisynthetic diet to study absorption of nonheme iron. *Am. J. Clin. Nutr.* **28**, 1289-1295.

Cook, J. D., and Monsen, E. R. (1976). Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. *Am. J. Clin. Nutr.* **29**, 859-867.

Cook, J. D., and Monsen, E. (1977). Vitamin C, the common cold, and iron absorption. *Am. J. Clin. Nutr.* **30**, 235-241.

Cook, J. D., Layrisse, M., and Finch, C. A. (1969). The measurement of iron absorption. *Blood* **33**, 421-429.

Cook, J. D., Layrisse, M., Martinez-Torres, C., Walker, R., Monsen, E., and Finch, C. A. (1972). Food iron absorption measured by an extrinsic tag. *J. Clin. Invest.* **51**, 805-815.

Cook, J. D., Minnich, V., Moore, C. V., Rasmussen, A., Bradley, W. B., and Finch, C. A. (1973). Absorption of fortification iron in bread. *Am. J. Clin. Nutr.* **26**, 861-872.

Hallberg, L., Björn-Rasmussen, E., Garby, L., Pleehachinda, R., and Suwanik, R. (1978). Iron absorption from South-East Asian diets and the effect of iron fortification. *Am. J. Clin. Nutr.* **31**, 1403-1408.

Hallberg, L., Björn-Rasmussen, E., Howard, L., and Rossander, L. (1979). Dietary heme iron absorption. A discussion of possible mechanisms for the absorption-promoting effect of meat and for the regulation of iron absorption. *Scand. J. Gastroenterol.* **14**, 769-779.

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Heinrich, H. C., Bartels, H., Gabbe, E. E., Meineke, B., Nass, W. P., and Whang, D. H. (1969). Die intestinale Resorption des Nahrungs-Eisens aus dem Hemoglobin, der Leber und Muskulatur bei Menschen mit normalen Eisenreserven und Personen mit prälatenen/latenen Eisenmangel. *Klin. Wochenschr.* **47**, 309-317.

Hussain, R., Walker, R. B., Layrisse, M., Clark, P., and Finch, C. A. (1965). Nutritive value of food iron. *Am. J. Clin. Nutr.* **16**, 464-471.

Layrisse, M., and Martinez-Torres, C. (1972). Model for measuring dietary absorption of heme iron: Test with a complete meal. *Am. J. Clin. Nutr.* **25**, 401-411.

Layrisse, M., Martinez-Torres, C., and Roche, M. (1968). The effect of interaction of various foods on iron absorption. *Am. J. Clin. Nutr.* **21**, 1175-1183.

Layrisse, M., Cook, J. D., Martinez, C., Roche, M., Kuhn, I. N., Walker, R. B., and Finch, C. A. (1969). Food iron absorption: A comparison of vegetable and animal foods. *Blood* **33**, 430-443.

Layrisse, M., Martinez-Torres, C., Cook, J. D., Walker, R., and Finch, C. A. (1973). Iron fortification of food: Its measurement by the extrinsic tag method. *Blood* **41**, 333-352.

Layrisse, M., Martinez-Torres, C., and Gonzalez, M. (1974). Measurement of the total daily dietary iron absorption by the extrinsic tag method. *Am. J. Clin. Nutr.* **27**, 152-162.

Layrisse, M., Martinez-Torres, C., Renzi, M., and Leets, I. (1975). Ferritin iron absorption in man. *Blood* **45**, 688-698.

Martinez-Torres, C., and Layrisse, M. (1970). Effect of amino acids on iron absorption from a staple vegetable food. *Blood* **35**, 669-682.

Martinez-Torres, C., and Layrisse, M. (1971). Iron absorption from veal muscle. *Am. J. Clin. Nutr.* **24**, 531-540.

Martinez-Torres, C., and Layrisse, M. (1974). Interest for study of dietary absorption and iron fortification. *World Rev. Nutr. Diet.* **19**, 51-70.

Martinez-Torres, C., Leets, I., Renzi, M., and Layrisse, M. (1974). Iron absorption by humans from veal liver. *J. Nutr.* **104**, 983-993.

Martinez-Torres, C., Renzi, M., and Layrisse, M. (1976). Iron absorption by humans from hemosiderin and ferritin, further studies. *J. Nutr.* **106**, 128-135.

Monsen, E. R., Hallberg, L., Layrisse, M., Hegsted, D. M., Cook, J. D., Mertz, W., and Finch, C. A. (1978). Estimation of available dietary iron. *Am. J. Clin. Nutr.* **31**, 134-141.

Moore, C. V. (1964). Iron nutrition. *In* "Iron Metabolism" (F. Gross, ed.), pp. 241-255. Springer-Verlag, Berlin and New York.

Reizenstein, P. (1975). Cattle haemoglobin—A possible dietary iron supplement. *Br. J. Haematol.* **31**, 265-268.

Takkunen, H. (1976). Iron deficiency in the Finnish adult population. *Scand. J. Haemat., Suppl.* **25**, 7-85.

Takkunen, H., and Seppänen, R. (1976). Iron deficiency and dietary factors in Finland. *Am. J. Clin. Nutr.* **28**, 1141-1147.