Bioavailability of Iron to Rats from Nitrite and Erythorbate Cured Processed Meats

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The bioavailability of iron from bologna-type sausages cured with 550 μg/g erythorbate and 156 μg/g nitrite was determined in a heme repletion assay with iron-deficient rats. Rats were limit fed for 2 weeks six diets that contained as their sole protein sources uncured meat, meat cured only with erythorbate (+E), meat cured only with nitrite (+N), meat cured with nitrite and erythorbate (+E+N, a typical commercial formulation), and lactalbumin with (L+Fe) and without (L) supplemental iron. Curing with nitrite and/or erythorbate had no significant effect on iron absorption or iron incorporation into tissues. Inorganic iron from $FeSO_4$ was better utilized than the total iron in meat as measured by blood parameters and by iron levels in tissues. In this regard the rat model may not reflect what has been observed for humans.

The bioavailability of iron from meat to man is good and this has been documented extensively (Layrisse et al., 1969; Forth and Rummel, 1973; Hallberg et al., 1979). It is also well established that porphyrin-bound iron, as in hemoglobin or myoglobin, has a consistently high bioavailability in contrast to nonheme iron. This is likely due to different absorption pathways for heme and nonheme iron (Raffin et al., 1974). However, there is evidence that nitrite curing of beef (Mahoney et al., 1979) and nitrosylation of purified heme compounds (Park et al., 1983) may decrease iron bioavailability to rats. This may be of practical significance, as about one-third of all the meat consumed in the United States is nitrite cured (Goyan and Forman, 1980).

Commercially available nitrite-cured meats are a mixture of components that may increase or decrease iron bioavailability. Factors that increase iron bioavailability include an unidentified "meat factor", the presence of highly bioavailable heme iron, and the curing additives ascorbate or perhaps erythorbate. Factors that decrease iron bioavailability may include nitrite (Mahoney et al., 1979) and the presence of nitrosated heme (Park et al., 1983). The bioavailability of iron from this mixture of inhibitors and enhancers has not been studied. This work was designed to examine the independent and cumulative effects of the two curing additives, nitrite and erythorbate, at commercially used levels on the bioavailability of iron to rats.

EXPERIMENTAL PROCEDURES

Sausage Preparation. Bologna-type meat emulsions were formulated according to a standard formula (Komarik et al., 1974) using lean beef, pork trimmings, crushed ice, spices, sodium chloride, and sodium nitrite and/or sodium erythorbate (*d*-isoascorbate). Added nitrite levels were 0 to 156 μg/g, and added erythorbate levels were 0 and 550 μg/g of meat. This resulted in four sausage treatments: without curing additives (uncured), cured only with erythorbate (+E), cured only with nitrite (+N), and cured with both nitrite and erythorbate (+E+N). Lean beef, spices, and curing salts were mixed with half of the ice and chopped at high speed in a stainless steel Kramer and Grebe (Kutter Supplies, Inc., Randolph, MA) meat chopper for 2 min. Pork trimmings and the remaining ice were added to the beef emulsions, and the mixture was chopped 2 min. The batter was not allowed to exceed 16 °C. The emulsions were stuffed into 3 in. diameter presoaked cellulose casings, smoked overnight at 12 °C to allow for complete cure, and cooked at 71 °C to an internal temperature of 67 °C. After being racked overnight at 12 °C, the sausages were sliced, vacuum sealed in Curlon 550 (nylon/saran/surlyn laminated film) bags, frozen cryogenically, and stored at −40 °C until use. The bologna was lyophilized and blended into the diets.

Experimental Design. Forty-eight male weanling Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were used. Six rats were killed 1 day after arrival (initial) and tissues collected to provide base-line data. The remaining 42 rats were depleted of iron by feeding them ad libitum a basal diet with low (12 μg/g) iron for 4 weeks. Their mean hematocrit after the depletion period was 31%. Following 4 weeks of depletion, six rats were killed and their tissues collected to establish the effects of iron depletion. The remaining 36 rats were assigned on the basis of weight and hematocrit to one of six treatments in a randomized complete block design. The average weights and hematocrits of rats in each treatment group were statistically the same.

The six dietary treatments contained uncured meat, erythorbate-cured meat (+E), nitrite-cured meat (+N), erythorbate and nitrite cured meat (+E+N), lactalbumin with no supplemental iron (L), and lactalbumin supplemented with $FeSO_4$ (L+Fe). Rats within each block were pair fed so food consumption between treatments did not differ. Feces were collected during the last 7 days. Following 2 weeks of repletion, all 36 rats were killed.

Throughout the study, animals were in individual stainless steel cages and demineralized water was provided ad libitum. Animals were weighed twice weekly.

Diet Composition. The basal diet fed to all rats during the iron deplection period contained 15% lactalbumin (Teklad Test Diets, Madison, WI), 35.2% sucrose (Kohl's), 35.2% cornstarch (Argo), 5.0% corn oil (Mazola), 5% nonnutritive fiber (Teklad Test Diets), 3.5% AIN-76 mineral mixture (Teklad Test Diets) without ferric citrate, zinc carbonate, nor cupric carbonate (American Institute of Nutrition, 1977), 1.0% AIN-76 vitamin mixture (Teklad Test Diets), and 0.2% choline dihydrogen citrate (Teklad Test Diets). Zinc and copper sulfates were added so that the depletion diet contained 29 μg of Zn/g, 9 μg of Cu/g, and 12 μg of Fe/g.

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Table I. Formulation of Meat and Lactalbumin (Control) Repletion Diets, g/kg of Diet

	meat diets				lactalbumin diets	
	no additives (uncured)	erythorbate only (+E)	nitrite only (+N)	nitrite and erythorbate (+E+N)	$FeSO_4$ supplemented (L+Fe)	iron deficient (L)
sausage[a]	487.6	487.6	487.6	487.6		
lard					186.1	186.1
lactalbumin[b]					103.2	102.2
sucrose	208.7	208.7	208.7	208.7	294.5	294.5
cornstarch	208.7	208.7	208.7	208.7	294.5	294.5
α-cellulose	50.0	50.0	50.0	50.0	50.0	50.0
mineral mixture (AIN-76)[c]	35.0	35.0	35.0	35.0	35.0	35.0
vitamin mixture (AIN-76)[d]	10.0	10.0	10.0	10.0	10.0	10.0
NaCl					26.7	26.7
choline citrate	2.0	2.0	2.0	2.0	2.0	2.0

[a] Freeze-dried prior to incorporation into diets. [b] Teklad Test Diets, Madison, WI. [c] Modified (iron, zinc, and copper free) AIN-76 mineral mixture containing (per kg) 500 g of calcium phosphate, dibasic, 74 g of NaCl, 200 g of potassium citrate monohydrate, 52 g of potassium sulfate, 24 g of magnesium oxide, 3.5 g of manganeous carbonate, 0.01 g of potassium iodate, 0.01 g of sodium selenite, 0.55 g of chromium potassium sulfate, and finely powdered sucrose to make 1000 g. [d] AIN-76 vitamin mixture containing (per kg) 600 mg of thiamin hydrochloride, 600 mg of riboflavin, 700 mg of pyridoxine hydrochloride, 3 g of nicotinic acid, 1.6 g of D-calcium pantothenate, 200 mg of folic acid, 20 mg of D-biotin, 1 mg of cyanocobalamine, 400 000 IU of vitamin A activity as *dl*-α-tocopheryl acetate, 2.5 mg of cholecalciferol, 5.0 mg of menaquinone, and finely powdered sucrose to make 1000 g.

Table II. Analyzed Compositions of Repletion Diets

	meat diets				lactalbumin diets	
	uncured	+E	+N	+E+N	L+Fe	L
moisture, %	3.7	3.9	4.2	4.3	3.8	3.7
residual nitrite, μg/g	a	a	33.9	42.7	a	a
residual erythorbate, μg/g	a	410	a	320	a	a
malonaldehyde, μg/g	2.34	0.63	15.94	0.95	0.42	0.26
total iron, μg/g	24.1	26.3	22.0	23.7	20.4	9.4
soluble iron, μg/g	2.7	4.0	2.4	3.2	8.0	2.0
nonheme iron, μg/g	15.1	16.1	13.0	14.3	20.4	9.4
total zinc, μg/g	35.3	37.3	36.9	35.9	43.1	39.5
total copper, μg/g	5.8	5.8	4.6	5.4	5.4	5.4

[a] Not measured. [b] Determined by the method of Weinfield (1964).

For the test period, four meat-based and two lactalbumin-based diets were formulated as shown in Table I. A lactalbumin–lard emulsion, closely resembling cured meat emulsions, was lyophilized and blended into the control diets. The $FeSO_4$-supplemented diet was made by adding 0.075 g of $FeSO_4 \cdot 7H_2O$/kg of low-iron lactalbumin diet, resulting in a final iron content that was similar to that of the four meat diets. Diets were supplemented with $CuSO_4$, $MgSO_4$, $MnSO_4$, NaCl, KH_2PO_4, and $ZnSO_4 \cdot 7H_2O$ to give the same final level of each mineral (Table II). The diets on the average provided 5.5 mg of Ca/g, 640 μg of Mg/g, 5.4 mg of P/g, and 15 mg of Na/g.

Analytical Procedures. Hematocrits and hemoglobin concentrations were determined on blood obtained through the orbital sinus capillary bed with heparinized microcapillary tubes. Hematocrits were measured at weeks 2, 4, and 6. Hemoglobin concentrations were assayed at weeks 4 and 6 by using the cyanmethemoglobin method (Crosby et al., 1954). Lyophilized human methemoglobin (Sigma Chemical Co., St. Louis, MO) was the standard. Animals were killed by cardiac puncture and livers, kidneys, and tibias collected. Tissues were cleaned and weighed immediately and held on ice until frozen at −20 °C. Blood was centrifuged and plasma retained for mineral analysis.

Total iron in plasma, livers, kidneys, and tibias was determined by atomic absorption spectroscopy (AAS). Plasma samples were diluted with deionized water, and their iron content was determined directly by AAS. Diet, liver, kidney, tibia, and dried fecal samples were heated in a muffle oven at 450 °C for 18 h and solubilized in acid before AAS determinations. Samples were compared to standards that were prepared daily. As a further quality control measure, bovine liver standards, which were obtained from the National Bureau of Standards, were analyzed by the same procedure with each batch of samples. These liver samples were determined to contain 258 ± 8 SD (n = 21) μg of Fe/g; this was 96% of the certified value.

Diet samples were also analyzed for zinc and copper by AAS, for residual nitrite by the Griess reagent as modified by Woolford and Cassens (1977), and for erythorbate by titration with 2,6-dichloroindophenol (Koniecko, 1979). All water was glass distilled and deionized.

Liver nonheme iron was determined by using both the methods of Torrence and Bothwell (1968) and Weinfield (1964). Efficiency of converting food iron to hemoglobin iron was calculated as described by Mahoney et al. (1974). Net hemoglobin iron gain was calculated as described by Miller (1977). Percent apparent iron absorption was calculated as (intake–fecal)100/intake.

Statistical Analyses. Data from all eight blocks of animals (initial, 4, and 6 week kills) were analyzed using one-way analysis of variance. The least significant difference (LSD) procedure was used to compare means. A Minitab computer program (Penn State University, 1983, MACC version 83.1-UW1.3) was used for all statistics.

RESULTS

The four meat-based diets, containing uncured meat, erythorbate-cured meat (+E), nitrite-cured meat (+N), and erythorbate and nitrite cured meat (+E+N), were not significantly different in their iron repleting ability. There was no effect of nitrite or erythorbate curing as indicated by blood measures (final hematocrit, increase in hematocrit, final hemoglobin level, increase in hemoglobin level) shown in Table III. The concentrations of iron in tissues (i.e., plasma, kidney, tibia, and liver) of rats fed the four

Table III. Hematocrits and Hemoglobin Levels of Rats Fed Meat-Based and Lactalbumin-Based Diets for 2 Weeks after Being Depleted of Iron for 4 Weeks[a]

repletion diet	final hematocrit, week 6, %	increase in hematocrit, week 4 to week 6, %	final hemoglobin level, week 6, g/dL	increase in hemoglobin level, week 4 to week 6, g/dL
uncured	36.9[b]	4.3[a,b]	10.8[a,b]	1.2[a,b]
+E	38.8[b,c]	7.4[b,c]	11.8[b,c]	2.2[b,c]
+N	36.3[a,b]	5.1[a,b]	11.6[b,c]	2.1[a,b,c]
+E+N	36.4[a,b]	4.9[a,b]	11.0[a,b]	1.8[a,b]
L+Fe	41.8[c]	10.5[c]	12.4[c]	3.1[c]
L	33.7[a]	2.4[a]	10.4[a]	1.1[a]
pooled SD	1.9	2.1	0.6	0.6
F ratio	12.8	10.1	7.9	7.2

[a] Means ($N = 6$) in the same column with different superscripts are significantly ($p < 0.01$) different.

meat diets did not differ significantly (Table IV).

Although the total amounts of iron in the lactalbumin-based diet supplemented with iron (L+Fe) and the meat diets were similar, the inorganic iron in the L+Fe diet was more bioavailable to the rats than the inorganic plus heme-bound iron in the meat diets. The rats fed the L+Fe diet had significantly ($p < 0.01$) higher final hematocrits (Table III) and higher plasma, tibia, and liver iron levels (Table IV) than any of the meat-fed rats. Final hemoglobin levels and the increases in hemoglobin levels of rats fed the L+Fe diet were significantly higher than those of rats fed the uncured and +E+N diets. Increases in hematocrit levels among rats fed the L+Fe diet were significantly ($p < 0.01$) greater than the increases with the uncured, +N, and +E+N diets but not greater than with the +E diet. There were no significant differences in the concentration of iron in the kidneys.

The animals fed the lactalbumin diet with no iron supplementation (L) had similar iron levels in plasma, kidneys, and tibias as rats fed meat diets. However, rats fed the L diet had significantly ($p < 0.01$) lower hematocrits than the uncured and +E rats, lower hemoglobin levels than the +E and +N rats, and lower liver iron concentrations than the +N rats.

As expected, the iron level in tissues (tibia and livers) decreased during the 4-week depletion period (Table IV). The depletion diet and the L diet contained 12 and 9 μg of Fe/g of diet, respectively. Animals fed the L diet for 2 weeks had similar iron levels in plasma, kidneys, and tibias but significantly ($p < 0.01$) lower liver iron concentration than those killed after the 4-week depletion. The rats fed the L+Fe diet had significantly ($p < 0.01$) higher hematocrits, hemoglobin levels, and tissue (plasma, kidney, liver, and tibia) iron levels than animals fed the L diet and higher tibias and liver iron levels than the rats killed after the 4-week depletion.

The liver nonheme iron content was measured in two different ways. Analyses performed by the method of Weinfeld (1964) revealed 57–65% nonheme iron in the livers of rats fed the four meat diets and in rats killed after depletion (Table IV). There was 87% and 82% nonheme iron in the livers of rats fed the L+Fe and L diets, respectively. Analyses of liver nonheme iron by the method of Torrence and Bothwell (1968) detected nearly all liver iron in the nonheme form (not shown). Perhaps heme-bound iron was hydrolyzed during the prolonged hot acid conditions required in this procedure that were not required in the method by Weinfeld (1964). Rats fed the four meat diets as well as the L diet had significantly lower nonheme liver iron concentrations than the L+Fe fed rats (Table IV). The rats killed at the initiation of the study had even higher liver nonheme iron concentrations.

Although tissue iron levels differed among rats fed the six diets, there was no significant difference between treatments in apparent absorption of iron (Table V).

Net hemoglobin iron gain was shown by Miller (1977) to be a better measure of the relative biological value of iron in bread than final hemoglobin concentration or change in concentration. Net hemoglobin iron gain takes into account differences in initial hemoglobin levels (not different in this study) and differences in blood volume. The animals fed the L+Fe diet and the two meat diets that contained erythorbate (+E and +E+N) had significantly ($p < 0.01$) higher gains in net hemoglobin iron than the rats fed the L diet. Only the rats fed the +E diet had significantly ($p < 0.01$) higher gains in net hemoglobin iron than the rats fed the uncured and +N diets.

The food to hemoglobin conversion efficiency, as introduced by Mahoney et al. (1974), is a measure of the efficiency of conversion of total dietary iron into hemoglobin iron during the rat repletion period. It corrects for variation in food consumption an in blood volume of the animals. In this study, rats were pair fed so that the total weight of food consumed between treatments was similar. However, the animals fed the L diet consumed less than half the amount of iron consumed by the other animals during the repletion period because of the low iron content of this diet (Table II). Not surprisingly, the animals fed the L diet used dietary iron significantly ($p < 0.05$) more efficiently than rats fed the four meat-based diets. No other differences between the treatments were significant.

DISCUSSION

Effect of Curing Meat on Iron Bioavailability. Curing meat with nitrite had little effect on the bioa-

Table IV. Tissue Iron Levels of Rats Killed Initially, after 4-Weeks Depletion, and after 2-Weeks Repletion with Meat-Based and Lactalbumin-Based Diets[a]

	plasma, μg of Fe/mL	kidney, μg of Fe/g	tibia, μg of Fe/g	liver, μg of Fe/g	liver nonheme,[b] μg of Fe/g
initial		39.1	48.2	56.1	52.3
Fe depleted for 4 weeks	0.94	36.1	21.2	35.1	21.6
after 2-weeks repletion on					
uncured	0.71[a]	43.3[a,b,c]	22.8[a]	33.6[a,b]	21.7[a]
+E	0.62[a]	45.1[b,c]	25.4[a]	33.7[a,b]	19.1[a]
+N	0.69[a]	46.2[b,c]	22.6[a]	34.5[b]	21.3[a]
+E+N	0.60[a]	42.6[a,b,c]	22.9[a]	32.2[a,b]	18.4[a]
L+Fe	1.19[b]	48.3[c]	34.0[b]	45.7[c]	39.9[b]
L	0.54[a]	39.3[a,b]	23.1[a]	23.6[a]	19.5[a]
pooled SD	0.30	5.4	3.9	6.5	6.1
F ratio	4.0	3.5	34.5	13.9	25.0

[a] Means ($n = 6$) in the same column with different superscripts are significantly ($p < 0.01$) different. [b] By the method of Weinfield (1964).

Table V. Apparent Absorption, Net Hemoglobin Iron Gain, and Efficiency of Converting Dietary Iron to Hemoglobin Iron by Rats Fed Meat-Based or Lactalbumin-Based Diets for 2 Weeks after Being Depleted of Iron for 4 Weeks

repletion diet	apparent absorption of iron,[a] %	net hemoglobin iron gain,[b] %	efficiency of converting ingested iron to hemoglobin iron,[c] %
uncured	30	1.47[a,b]	36[a]
+E	32	2.20[c]	50[a,b]
+N	32	1.77[b]	48[a,b]
+E+N	23	1.79[b,c]	45[a]
L+Fe	28	1.85[b,c]	55[a,b]
L	27	1.13[a]	71[b]
pooled SD	10	0.44	10
F ratio	1.4	4.0	2.9

[a] Means (n = 6) in column are not significantly different. [b] Means (n = 6) in column with different superscripts are significantly ($p < 0.05$) different. [c] Means (n = 6) in column with different superscripts are significantly ($p < 0.01$) different.

vailability of iron from meat to rats in this study. One group of investigators studied extensively the effects of nitrite on iron bioavailability to rats. Their results were not consistent. Mahoney et al. (1979) demonstrated that anemic rats converted meat iron to hemoglobin iron less efficiently when fed diets containing bologna prepared with 10–400 μg of nitrite/g of meat than when fed diets containing bologna without nitrite. However, this inhibition was not observed at levels common in commercial practice, from 50 to 150 μg of nitrite/g of meat. In the same paper these investigators observed that anemic rats fed casein- or bologna-based diets regenerated hemoglobin more efficiently when nitrite (12 μg/g of diet) was added to the diets. Part et al. (1983) found the iron from diets containing nitrosylated purified pork or beef hemoglobin was 25% and 10% less available to anemic rats for heme synthesis than the unnitrosylated heme compounds. When 3.7 μg/g nitrite (the same amount required to nitrosylate the heme compounds) was added to a ferrous sulfate supplemented casein-based diet, no effect on hemoglobin regeneration efficiency was observed. The reason for discrepancies in these data are not clear.

We found that the efficiency of converting dietary iron to hemoglobin iron or rats fed uncured meat and nitrite cured meat (+N) were 36% vs. 48% and 45%, respectively. Mahoney et al. (1979) found rats fed uncured meat and meat cured with 50 and 100 μg of nitrite/g had conversion efficiencies of 36%, 48%, and 43%, respectively. these authors attribute the relatively low conversion efficiency of 36% in their study to oxidation of the untreated meat. This was not likely in our study because the degree of oxidation of the uncured meat was very small, as indicated by a TBA value of 2.35 mg of malonaldehyde/kg of diet (Table I). A TBA of 19.5 mg/kg was reported for the uncured meat in the study of Mahoney et al. (1979). In addition, it was later shown that the degree of rancidity must be very high before a significant depression in hematinic response was noted, e.g., 14 days at 21 °C in air (Cardon et al., 1980).

Erythorbate had little effect on iron bioavailability from cured meat diets to rats. However, the rats fed the meat cured only with erythorbate (+E) had significantly greater net gains in hemoglobin iron than the rats fed the other meat-based diets.

There are no published reports of studies that examined the effect of erythorbate on iron bioavailability. Erythorbate is the erythro isomer of ascorbate, which is a potent enhancer of nonheme iron absorption to rats (Monsen, 1974) and humans (Cook and Monsen, 1977). From 59% to 63% of the iron in the meat diets fed in this study was in the nonheme form (Table II). Thus erythorbate might have been expected to improve the absorption of iron by rats in this study. There are several possible reasons why this did not occur. Ascorbate is thought to enhance iron absorption by maintaining iron in the ferrous state and/or by forming soluble easily absorbed complexes with iron (Conrad and Schade, 1968). However, erythorbate and ascorbate have different chemical and nutritional properties. Erythorbate has 1/20 of the anti-scorbutic activity of L-ascorbate (Goldman et al., 1981). The two compounds have different redox potentials as erythorbate oxidizes 25–100% more rapidly than ascorbate in the pH range of 3–7 (Borenstein, 1965). If erythorbate is rapidly oxidized, it would not maintain iron in the ferrous form. The configurations of erythorbate and ascorbate also differ. Thus erythorbate may not form an easily absorbed complex with iron as does ascorbate.

It also should be noted that meat and ascorbate may be complementary rather than additive in enhancing iron absorption. Ascorbate is known to promote nonheme iron absorption but has little effect (Hallberg, 1981) or perhaps a negative effect (Lynch et al., 1983) on human heme iron absorption. This in combination with the different properties of erythorbate vs. ascorbate may explain the lack of a measurable effect of curing meat with erythorbate on iron bioavailability.

The Rat Model as a Predictor of Bioavailability of Iron in Meats for Humans. In this study rats utilized total heme and nonheme iron in the meat-based diets (uncured, +E, +N, and +E+N) less efficiently than the nonheme iron in the control diet (L+Fe). This is consistent with other studies, which have shown the absorption of iron from ferrous sulfate by the anemic rat was much greater than the absorption of iron from meats (Cardon et al., 1980; Farmer et al., 1977; Mahoney et al., 1974, 1979; Rotruck and Luhrsen, 1979). This is in contrast to that observed for humans, who absorb the iron from meat better than from ferrous sulfate iron (Layrisse et al., 1973). Ferrous sulfate absorption by rats is generally high, 30–84% of the dose in the solution (Bannerman, 1965; Raffin et al., 1974; Weintraub et al., 1965; Wheby et al., 1970). Ferrous sulfate absorption by humans in the absence of ascorbate is low: 4% absorption from solution (Hallberg and Solvell, 1967), 9.4% when given with a meat-containing meal (Hallberg et al., 1979), and 3.7% and 9.0% to iron-deficient humans when consumed in a vegetarian and a meat meal, respectively (Layrisse et al., 1973). The rat's preference for ferrous sulfate iron has not been observed in humans, and there may be several reasons for this species difference.

Heme iron absorption by rats and humans may be different. Some investigations have suggested that rats are much less capable than humans in splitting heme in the intestine (Weintraub et al., 1965; Bannerman, 1965). The greater absorption of iron from the inorganic (L+Fe) iron source over the meat diets in this study may be due in part to a difference in heme iron absorption by rats. However, this difference can only be inferred since a lactalbumin diet with the same level of nonheme iron as the meat diets was not included here. Other researchers have observed that the ability of the rat to utilize heme iron has been underestimated (Rotruck and Luhrsen, 1979). Further, the absorption of hemoglobin iron increases during iron deficiency in rats and humans, and the amount of iron absorbed from hemoglobin by rats is about the same as the

amount of iron absorbed by humans [a mean of 23% absorption for an individual with 500 mg of iron stores (NAS, 1980)]. The practical significance of these observations may be obscured by the claim that the absorption of iron from purified heme compounds is not the same as from meat heme compounds (Hazell et al., 1978, 1980; Hazell, 1982).

Differences in reference iron salts, experimental protocol, iron status of subjects, and measures of response to dietary treatments make comparisons between rat and human iron bioavailability studies difficult. The reference salt used in most rat studies is ferrous sulfate, which is fed in a basal rat diet over a period of weeks, and then various parameters that indicate iron nutriture are measured in the rat. The reference salt used in most human bioavailability studies is ferrous ascorbate (a mixture of ferrous sulfate, ascorbic acid, and sometimes $^{59}FeCl_3$), which is often given in a single dose in solution. Percent absorption is then assessed by measuring circulating or whole body ^{59}Fe levels. Ferrous ascorbate and ferrous sulfate differ in bioavailability. Ferrous ascorbate has been shown to have a much higher bioavailability than the sulfate in both iron-replete and iron-deficient humans (Hallberg and Solvell, 1967; Hallberg et al., 1979; Layrisse et al., 1973). The absorption of ferrous ascorbate has not been directly tested in the anemic rat model. Conversely, the absorption of ferrous sulfate in the absence of ascorbate in a mixed diet has not been studied extensively in humans.

Although absorption of iron from ferrous sulfate is useful as an interlaboratory control for rat bioassays (Pla and Fritz, 1971), it may not be reasonable to compare the absorption of iron from different pools of iron, i.e., nonheme sources and heme sources (Chao and Gordon, 1983). The rat appears to be an acceptable model for the human when inorganic iron sources are compared or when the effects of dietary components (such as phosphorus, egg yolk, phytate, and fiber) on the absorption and utilization of inorganic iron were evaluated (Greger, 1982; Morris and Ellis, 1980; Reinhold, 1982). However, we are aware of no previous studies that evaluate iron bioavailability to both rats and humans from the same diets at the same time. This was done in this laboratory as reported in the following paper (Greger et al., 1984). The same meats prepared for this rat study were also fed to human subjects. Nitrite and erythorbate curing of meat did not affect the bioavailability of iron from meat for either human subjects or rats. On the basis of this observation, the rat may be a useful model for determining the relative bioavailability of iron to humans from similar meat sources. Further work needs to establish whether the rat is appropriate as a model for studies involving heme iron. This is important to resolve as the rat gains popularity as a cheaper and more convenient alternative to human subjects.

Registry No. Erythorbate, 89-65-6; nitrite, 14797-65-0; iron, 7439-89-6; iron sulfate, 7720-78-7.

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