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IRON ABSORPTION. IV. THE ABSORPTION OF HEMOGLOBIN IRON *

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The accepted mechanism of absorption of iron by the gastrointestinal tract involves solution and reduction to the ferrous form before its uptake by the mucosal cell (1). Food iron, much of which is bound in organic complex, has been assumed to follow the same pathway with the assistance of the digestive enzymes. Studies supporting this hypothesis have been conducted mainly with iron salts, and the extent of its applicability to absorption of iron in food remains uncertain. Measurements of absorption of radioiron biosynthetically incorporated into various foods (2–6) have shown enhancement of absorption by ascorbic acid and other reducing agents, but have not further defined the mechanism by which complex iron is absorbed. That there may be more than one pathway is suggested by the differences in level of absorption of iron in different foods, and of food iron and iron salt in comparable dosage. Hemoglobin is a form of food iron that can be prepared in standard form and that is absorbed sufficiently well for measurement of both increased and decreased absorption (7). Comparison of absorption of hemoglobin iron and iron salt reported here indicates a difference in the manner of absorption of these two forms of iron.

METHODS

Observations were made in healthy volunteers, most of them men between the ages of 20 and 30, and in subjects with iron-deficiency anemia. Five volunteers, W. M., R. B., E. B., C. J., and W. H., had each given more than 3 pints of blood for transfusion purposes over the last 5 years. These five, and one other male volunteer, G. F., whose plasma iron was 46 μg per 100 ml with transferrin saturation of 15 per cent, were considered likely to have suboptimal iron stores. The other volunteers with normal plasma iron and transferrin values were assumed to have normal stores.

Preparation of labeled materials. Doses of labeled ferrous salt were prepared by the mixture of a suitable amount of radioiron as ferric chloride with a weighed quantity of either ferric chloride or ferrous sulfate as noted in the text and tables and the addition of 5 moles of ascorbic acid to each mole of iron immediately preceding its administration. Since it is still uncertain whether ascorbic acid has any effect on iron absorption apart from its action as a reducing agent, a similar amount was added to all doses of the other labeled compounds administered except where noted in the text.

Hemoglobin labeled with either Fe^{59} or Fe^{55} was prepared in New Zealand white rabbits weighing 2 to 3 kg that had been bled until their hemoglobin was about 7 g per 100 ml. The isotope was given as ferric citrate (500 to 750 μc Fe^{59} or 1,500 to 2,000 μc Fe^{55}) by intravenous injection in divided dosage over 24 hours, and the rabbits were exsanguinated by cardiac puncture about 6 days after the last injection. The radioactive red cells were washed free of plasma with 0.9 per cent saline and hemolyzed by freezing and thawing. The solution so obtained, containing hemoglobin and red cell stroma, was administered as described below.

Radioactive hemin was prepared from labeled hemoglobin by the method of Labbe and Nishida (8), in which the solution of hemoglobin is treated with 12 volumes of a mixture of acetone and 2 per cent strontium chloride in glacial acetic acid. Hemin that crystallized from this mixture after heating to 102° C was washed once with distilled water and dissolved in 0.1 N sodium hydroxide before administration.

Ferritin labeled with radioiron was prepared in rabbits that had been given repeated intravenous injections of iron dextran complex [1] over a 10-day period to a total dose of approximately 60 mg iron per kg. Under barbiturate anesthesia, 100 μg radioiron as ferric citrate (150 μc Fe^{59} or 1,000 μc Fe^{55}) was injected into the portal vein, and 16 hours later the rabbits were exsanguinated

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[1] Imferon, kindly supplied by Lakeside Laboratories, Inc., Milwaukee, Wis.

by cardiac puncture. Ferritin was prepared by the method of Granick (9). The livers were homogenized with 5 volumes of saline and the suspension heated to 80° C. After filtration the ferritin was precipitated with 30 per cent ammonium sulfate. The precipitate of crude ferritin was dissolved in distilled water and dialyzed against water for 24 hours at 4° C before administration.

Administration of labeled materials. Preliminary studies were made to evaluate the variability in absorption from day to day and with time of day. For this purpose radioactive ferrous salt was given in 100 ml water. In all subsequent studies the radioactive materials were administered in 100 ml canned tomato juice, to disguise their appearance, with or without the addition of food or other substances as described under Results. The healthy subjects took the test doses between 4 and 5 p.m. after fasting for a least 4 hours except as otherwise specified. The patients with iron-deficiency anemia were given their doses at 9 a.m. after fasting overnight. No subjects were allowed food or drink for 2 hours after the test substance was administered.

In most studies the subjects received a dose labeled with one radioisotope on the first day and a second dose labeled with the other isotope on the following day. The iron content of the radioactive material to be ingested was calculated on the basis of either a hemoglobin determination or an iron analysis, and an appropriate amount of nonradioactive "carrier" material was added to make up the desired dose. The amount of radioactivity administered in each dose was that which was expected to lead to the absorption of about 0.5 to 1 μc Fe^{59} or 5 to 10 μc Fe^{55}. Suitable amounts of the radioactive materials were set aside as standards.

Absorption was estimated from the radioactivity in the blood 14 days after the test materials were taken. Duplicate or triplicate samples of whole blood and appropriate dilutions of the standards were digested, precipitated, and electroplated. Differential counting was carried out by the method of Peacock and co-workers (10). Absorption was calculated on the assumption of a blood volume of 65 ml per kg, a body: venous hematocrit ratio of 0.92, and, in the healthy subjects with normal iron stores, 80 per cent utilization of absorbed radioiron. In the subjects with iron-deficiency anemia the utilization was assumed to be 95 per cent, and in the subjects with suboptimal iron stores 85 per cent.

In studies of the separation of transferrin iron from hemoglobin or heme iron in plasma, one volume of plasma was dialyzed at pH 5.6 against 1,000 volumes of 0.4 per cent EDTA in iron-free saline. The pH was adjusted with dilute sodium hydroxide, and the dialysis was run for 24 hours at 4° C with constant stirring.

Hemoglobin was estimated as oxyhemoglobin or cyanomethemoglobin in an Evelyn colorimeter at 540 mμ. Hematocrits were spun in Wintrobe tubes at 2,400 G for 30 minutes. Plasma iron and iron-binding capacity were measured by the methods of Bothwell and Mallett (11) and Ressler and Zak (12), respectively. The sulfosalicylic acid method of Lorber (13) was used to measure the iron content of the solutions of heme and ferritin, and of meals identical to those eaten by the subjects.

RESULTS

1. *Variation in absorption of ferrous salt by normal subjects.* Before the absorption of hemoglobin iron could be compared with that of iron salt, it was necessary to assess the day-to-day variation in absorption in a group of normal subjects. Five subjects were given 5 mg iron as ferrous sulfate on two successive days at 8 a.m. (Table I,A). In two subjects absorption of the second dose was twice that of the first. In the other three the variation was much less, average absorption of the two doses in the whole group being 9.3 and 11.0 per cent, respectively. Three more subjects took this same dose of ferrous salt at 8 a.m. on two occasions at an interval of 2 weeks (Table I,B). Average absorption was 6.6 per cent of the first dose and 9.9 per cent of the second.

Since it was more convenient for the volunteers to take the labeled hemoglobin in the late afternoon, two studies were made to compare the absorption of iron given at this time with that of iron given first thing in the morning. On two separate occasions three normal subjects were given 5 mg iron as ferrous sulfate at 8 a.m. after an overnight fast on one day, and the same dose at 4 p.m. after a four-hour fast on the following day (Table II,A). With the exception of one test in one subject, absorption of the afternoon dose was

TABLE I

Variation in absorption of two doses of ferrous sulfate (5 mg Fe) by a normal subject *

Subject	PI	TIBC	Tfn sat	% Absorption	
				First dose	Second dose
	μg/100 ml		*%*		
A. Doses on successive days					
D.W.	126	275	46	5.3	10.9
B.H.	122	276	44	11.7	13.4
D.M.	130	340	43	8.5	16.1
R.B.	79	299	26	13.5	9.5
E.W.	101	323	31	7.4	5.3
Average	112	299	38	9.3	11.0
B. Doses 2 weeks apart					
R.M.	132	310	43	8.7	15.2
S.J.	66	282	23	2.8	2.7
K.H.	93	294	32	8.3	11.7
Average	97	295	33	6.6	9.9

* PI = plasma iron, TIBC = total iron-binding capacity, and Tfn sat = transferrin saturation.

TABLE II

*Absorption of ferrous sulfate (5 mg Fe) by normal subjects at different times of day**

Subject	PI	TIBC	Tfn sat	% Absorption: Morning dose	% Absorption: Afternoon dose
	μg/100 ml		%		
A. At 8 a.m. after a 10-hour fast and at 4 p.m. after a 4-hour fast					
R.M.	132	310	43	8.7	1.8
				15.2	11.4
S.J.	66	282	23	2.8	9.4
				2.7	1.5
K.H.	93	294	32	8.3	4.5
				11.7	9.6
Average	97	295	33	8.2	6.4
B. At 8 a.m. after a 10-hour fast and at 4 p.m. after an 18-hour fast					
W.L.	146	292	50	12.4	8.2
G.H.	62	270	23	4.0	4.9
E.M.	77	273	28	24.4	24.5
D.S.	86	282	30	12.9	4.5
Average	93	279	33	13.4	10.5

* Abbreviations as in Table I.

rather less than that of the morning dose, the averages being 8.2 and 6.4 per cent, respectively. In a further study, four subjects took a first dose of 5 mg iron as ferrous sulfate at 8 a.m. after an overnight fast, and then were allowed no food until 2 hours after a second similar dose at 4 p.m. the same afternoon (Table II,B). Absorption of the morning dose averaged 13.4 per cent and that of the afternoon dose 10.5 per cent.

These results gave some indication of the variation to be expected in normal subjects. Clearly, only major differences in absorption of two doses of iron may be interpreted with confidence.

2. *Absorption of hemoglobin iron by normal subjects.* The comparative absorption of ferrous sulfate and of hemoglobin iron, both given in tomato juice, was evaluated in the following studies. Six subjects, who were all thought to have normal iron stores, absorbed an average of 4.1 per cent of a 5-mg dose of reduced iron (Table III,A). Thirteen subjects given from 4 to 6 mg hemoglobin iron (Table III,B) absorbed an average of 8.8 per cent; six given 2.5 or 3 mg absorbed an average of 12.7 per cent. Labeled hemoglobin was also given to six subjects who were considered likely to have suboptimal iron stores (Table III,C). Three subjects given 5 mg hemoglobin iron absorbed an average of 11.2 per cent; three others given 2.5 mg absorbed an average of 17.6 per cent.

In order to document further the effect of dosage on absorption of hemoglobin iron suggested by the observations shown in Table III, three subjects, T. D., C. P., and W. R., were given 3 mg hemoglobin iron on one day and 20 mg hemo-

TABLE III

*Absorption of forms of iron in tomato juice by healthy subjects believed to have normal or suboptimal iron stores**

Subject	PI	TIBC	Tfn sat	Dose of Fe	Absorption
	μg/100 ml		%	*mg*	%
A. Ferrous sulfate in subjects with normal iron stores					
M.R.	117	307	38	5	3.9
D.R.	89	298	30	5	3.1
B.B.	115	231	50	5	1.7
R.W.	75	340	22	5	5.1
J.E.	95	347	27	5	4.1
B.A.	79	312	25	5	6.9
Average	95	306	32	5	4.1
B. Hemoglobin iron in healthy subjects with normal iron stores					
F.W.	86	301	29	6	9.4
M.Sh.	59	289	20	6	10.1
J.M.	92	358	26	6	6.4
J.L.	79	274	29	5	4.8
J.D.	106	236	45	5	5.3
D.E.	96	253	38	5	8.5
W.E.	53	285	19	5	5.9
W.C.	126	271	46	5	3.7
S.H.	89	339	26	5	7.5
S.L.	106	311	34	5	6.7
D.S.	72	348	21	4	14.4
M.Sa.	72	264	27	4	12.1
E.T.	100	266	38	4	19.1
Average	87	292	31	5	8.8
T.D.	66	335	20	3	16.3
C.P.	95	282	34	3	9.5
W.R.	90	334	27	3	9.8
R.Ge.†	91	289	31	2.5	8.6
R.Go.†	65	289	22	2.5	15.9
R.K.	106	252	42	2.5	14.4
Average	86	297	29	2.75	12.7
C. Hemoglobin iron in healthy subjects with suboptimal iron stores (see text)					
W.H.	111	420	26	5	14.5
E.B.	96	330	27	5	6.4
C.J.†	78	275	28	5	12.6
Average	95	342	27	5	11.2
W.M.	112	305	37	2.5	22.4
R.B.	108	284	38	2.5	12.9
G.F.	44	302	15	2.5	17.6
Average	88	297	30	2.5	17.6

* Abbreviations as in Table I.
† Female.

TABLE IV

Absorption of hemoglobin iron (5 mg Fe) and of ferrous sulfate (5 mg Fe) by subjects with iron-deficiency anemia *

Subject	Diagnosis	Hct	PI	TIBC	Tfn sat	% Absorption	
						Hb Fe	Salt Fe
		%	μg/100 ml		%		
M.H.	Rectal ulcer, mild hypothyroidism	30	34	429	8	22.6	13.8
A.W.	G.I. bleeding, hiatal hernia	31	14	390	4	16.6	80.2
F.B.	Prepyloric ulcer	13	16	389	4	6.3	79.3
W.M.	Duodenal ulcer	25	10	335	3	7.7	13.1
V.M.	G.I. bleeding, peptic esophagitis	29	16	402	4	24.8	89.1
B.B.	Duodenal ulcer, malignant melanoma	31	21	358	6	9.6†	
K.A.	G.I. bleeding, cause unknown	29	14	359	4	9.3†	
	Average	27	18	380	5	15.6	55.1

* Abbreviations as in Table I; also, Hct = hematocrit and Hb = hemoglobin.
† Not included in average.

globin iron on the next. Absorptions of the 3-mg dose were 16.3, 9.5, and 9.8 per cent, averaging 11.9 per cent, and of the 20-mg dose, 3.5, 4.6, and 4.6 per cent, averaging 4.2 per cent.

These results indicated that in normal subjects, hemoglobin iron given with fruit juice is absorbed as well as or better than ferrous salt and that absorption of hemoglobin iron is related both to the amount ingested and to the iron requirement of the subject.

3. *Absorption of hemoglobin iron by subjects with iron-deficiency anemia.* Absorption of hemoglobin iron was compared to absorption of iron salt in seven subjects with iron-deficiency anemia. In five of them a dose of labeled ferrous sulfate was administered on the following day, so that a direct comparison could be made of absorption of the two types of iron. Both doses were given in tomato juice with 80 mg ascorbic acid added. The average absorptions in these subjects (Table IV) were 15.6 per cent of the 5 mg of hemoglobin iron and 55.1 per cent of the 5-mg dose of ferrous salt. Although enhanced absorption of both iron salts and hemoglobin iron was observed in these iron-deficient subjects, in keeping with other reports (7, 14, 15), the increase was clearly less marked for hemoglobin iron than for ferrous salt.

4. *The effect of food on absorption of hemoglobin iron.* Food has been shown to reduce absorption of small amounts of iron salt by approximately one-half (2, 16, 17). This effect was confirmed in three normal subjects who were given on successive days 5 mg iron as ferrous sulfate and 80 mg ascorbic acid with and without a moderately large meal containing 4.4 mg of iron. Absorption averaged 0.9 per cent with and 2.9 per cent without the meal (Table V,A). The influence of food on absorption of hemoglobin iron was then measured in six healthy subjects. Three were given on successive days 3.8 mg hemoglobin iron without food, and 3.3 mg hemoglobin iron with a light meal containing 1.4 mg of iron. No ascorbic acid was given with these doses. Three other subjects, all suspected of having suboptimal iron stores, took 5.1 mg hemoglobin iron without food on the first day, and 5.1 mg with the larger

TABLE V

Effect of food on absorption of iron salt and of hemoglobin iron by healthy subjects *

Subject	% Absorption	
A.	Ferrous sulfate (5 mg Fe) without food	Ferrous sulfate (5 mg Fe) with food (4.4 mg Fe)
M.R.	3.9	0.7
D.R.	3.1	1.5
B.B.	1.7	0.6
Average	2.9	0.9
B.	Hb (3.8 mg Fe) without food	Hb (3.3 mg Fe) with food (1.4 mg Fe)
D.S.	14.4	17.1
M.Sa.	12.1	14.3
E.T.	19.9	16.1
Average	15.2	15.8
C.	Hb (5.1 mg Fe) without food	Hb (5.1 mg Fe) with food (4.4 mg Fe)
E.B.	6.4	10.8
W.H.	14.5	25.8
C.J.	12.6	25.2
Average	11.2	20.6

* Abbreviations as in Table IV.

meal on the second day. Eighty mg of ascorbic acid was added to both doses given to these subjects. The results in these six subjects are shown in Table V,B and C. Average absorptions were 13.2 per cent of the hemoglobin iron given without food and 18.2 per cent of the hemoglobin given with food. Thus food, in contrast to its effect on absorption of iron salt, did not reduce absorption of hemoglobin iron and in the iron-depleted subjects appeared to augment it.

5. *The effect of phytate on absorption of ferrous salt and of hemoglobin iron.* Phytate is believed to reduce absorption of ionic iron by forming an insoluble complex (17, 18). This reduction was confirmed in six healthy subjects given ferrous salt without and with food (Table VI,A and B). In the absence of food, average absorption in three subjects was 5.4 per cent of a dose of ferrous ascorbate (5 mg iron) given alone and 2.5 per cent of the same dose given with 4 g sodium phytate on the following day. Three other subjects took this same dose of ferrous ascorbate with a light meal (1.4 mg iron) but without phytate on the first day, and the same dose with the same meal and 10 g sodium phytate on the following day. Average absorption was 9.4 per cent of the dose without phytate and 4.4 per cent of that given with phytate. (The greater average absorption of iron salt in this group in spite of the presence of food was thought to be due to the use of two subjects who had given blood for transfusion.)

TABLE VI

Effect of sodium phytate on absorption of iron salt and of hemoglobin iron with and without food in healthy subjects

Subject	% Absorption	
A.	Ferrous ascorbate (5 mg Fe)	Ferrous ascorbate (5 mg Fe) with sodium phytate (4 g)
R.W.	5.1	2.4
J.E.	4.1	0.9
B.A.	6.9	4.1
Average	5.4	2.5
B.	Ferrous ascorbate (5 mg Fe) with food (1.4 mg Fe)	Ferrous ascorbate (5 mg Fe) with food (1.4 mg Fe) and sodium phytate (10 g)
M.Sm.	6.5	4.5
S.H.	19.5	7.8
D.W.	2.3	1.0
Average	9.4	4.4
C.	Hb* (6 mg Fe)	Hb (6 mg Fe) with sodium phytate (4 g)
F.W.	9.4	13.8
M.Sh.	10.1	10.0
J.M.	6.4	9.3
Average	8.6	11.0
D.	Hb (3.8 mg Fe) with food (1.4 mg Fe)	Hb (3.8 mg Fe) with food (1.4 mg Fe) and sodium phytate (10 g)
B.O'H.	9.9	14.8
T.J.	13.0	24.1
J.K.	10.6	22.5
Average	11.2	20.5

* Hb = hemoglobin.

The effect of phytate on absorption of hemoglobin iron was studied in six healthy subjects (Table VI,C and D). Three subjects took 6 mg of labeled hemoglobin iron alone. One month later this dose was repeated with 4 g sodium phytate. Absorption averaged 8.6 per cent without and 11.0 per cent with phytate. Three other subjects took 3.8 mg hemoglobin iron with a light meal (1.4 mg iron) on one day and the next day took 3.3 mg hemoglobin iron with the same meal and 10 g sodium phytate. Absorption of the dose given with food but without phytate was 11.2 per cent, and that of the dose given with food and phytate was 20.5 per cent.

Thus, a large quantity of sodium phytate reduced absorption of ionic iron in both the absence and the presence of food, but did not reduce absorption of hemoglobin iron; indeed, in the presence of food, phytate appeared actually to increase it. These effects are similar to those observed with food in the previous studies.

6. *The effect of ascorbic acid on absorption of hemoglobin iron.* Ascorbic acid increases the absorption of iron given as ferric salts and of iron in many foods (3, 16, 19) presumably by increasing the amount of ferrous iron presented to the mucosa. Absorption of hemoglobin iron given without food has been found not to be increased by ascorbic acid (7), and this observation was confirmed in two subjects with iron-deficiency anemia. These subjects, K. A. and B. B., were given 5 mg hemoglobin iron with and without 80 mg ascorbic acid on successive days. Absorptions were 19.3 and 9.6 per cent with ascorbic acid and 14.7 and 9.9 per cent without it, respectively. The effect

of a large amount of ascorbic acid on the absorption of hemoglobin iron given with food was studied in three healthy subjects, F. G., D. W., and R. S., who took 2.5 mg hemoglobin iron with a meal, containing 1.8 g iron, with and without 500 mg ascorbic acid on successive days. Absorptions with ascorbic acid were 19.4, 14.3, and 5.6 per cent, averaging 13.1 per cent, and absorptions without it, 27.1, 10.9, and 6.9 per cent, averaging 15.0 per cent. Thus, unlike the absorption of other forms of food iron, that of hemoglobin iron does not appear to be increased by ascorbic acid.

7. *The effect of the addition of ferrous salt on the absorption of hemoglobin iron.* It seemed possible that the increased absorption of hemoglobin iron in the presence of food and phytate might be due to the binding of soluble iron in the meal by the latter, since the soluble food iron might be expected to compete with the hemoglobin iron for absorption. Two studies were performed to assess the effect of soluble iron on the absorption of hemoglobin iron. In the first the quantity of soluble iron used was similar to that in the meals given in the previous studies. Three healthy subjects, D. E., J. D., and J. L., took 5 mg labeled hemoglobin iron on the first day, and the same with 5 mg carrier iron as ferrous sulfate on the following day; 80 mg ascorbic acid was added to both doses. Absorption of the hemoglobin iron of the first dose was 8.5, 5.3, and 4.8 per cent, averaging 6.2 per cent, and that of the dose with added iron salt was 11.4, 5.8, and 5.7 per cent, averaging 7.6 per cent. This result indicates that the soluble iron in the food given with labeled hemoglobin is unlikely to have affected its absorption.

The second study was designed to investigate the effect of a large amount of soluble iron on the absorption of a small amount of hemoglobin iron. Two healthy subjects, S. H. and J. L., were given 5.4 mg hemoglobin iron on one day and the same quantity with 100 mg carrier ferrous iron and 320 mg ascorbic acid on the following day. Two other subjects, W. E. and W. C., took the same doses on the same days but in reverse order. These doses were all given at 8 a.m. after an overnight fast. The results are shown in Table VII. Average absorption of labeled hemoglobin iron given alone was 5.9 per cent and of labeled hemoglobin iron given with 100 mg carrier iron 4.8 per cent. This difference is not greater than might be expected from normal variation, and it may be concluded that large amounts of iron do not greatly alter the absorption of hemoglobin iron.

TABLE VII

Effect of absorption of hemoglobin iron (5 mg Fe) of simultaneous administration of 100 mg salt iron as ferrous sulfate

Subject	% Absorption*	
	Hb. Fe alone	Hb Fe with iron salt
W.E.	5.9	6.5
W.C.	3.7	1.3
S.H.	7.5	5.6
J.L.	6.7	5.9
Average	5.9	4.8

* Hb = hemoglobin.

8. *The effect of stimulation of gastric secretion on absorption of hemoglobin iron.* A possible explanation of the apparent immunity of hemoglobin iron to the expected blocking effect of food on absorption would be that this effect is masked by some other action of food on the digestive processes which tends to increase absorption of this form of food iron. Since one effect of food would be stimulation of gastric secretion, the effect of gastric secretogogues was studied in three of the subjects who had previously taken labeled hemoglobin with and without food and who had all shown better absorption with food. Two months after the first study, a third dose of 5 mg labeled hemoglobin iron with ascorbic acid was given 30 minutes after stimulation of gastric secretion, in two subjects, E. B. and W. H., by an injection of 1 mg of histolog,[2] and in the third, C. J., by an oral dose of 0.5 g of caffein sodium benzoate. As shown in Table VIII, absorption after gastric stimulation was the same as that of the first dose of labeled hemoglobin given alone.

9. *The absorption of iron in hemin.* During digestion of the hemoglobin molecule the globin part is presumably broken down by proteolytic enzymes with release of the heme moiety. Since globin, or an amino acid or polypeptide derived from it, might influence absorption of hemoglobin iron, the absorption of iron in hemin was studied in six healthy subjects. Three were given 2.5 mg

[2] 3-(β-aminoethyl) pyrazole.

TABLE VIII

Effect of stimulation of gastric secretion on absorption of hemoglobin iron (5.1 mg Fe)

Subject	% Absorption		
	Hb* alone	Hb + food (4.4 mg Fe)	Hb alone after gastric stimulant
E.B.	6.4	10.8	5.1
W.H.	14.5	25.8	14.0
C.J.	12.6	25.2	8.0
Average	11.2	20.6	9.0

* Hb = hemoglobin.

hemoglobin iron on one day and 2.5 mg hemin iron on the following day. The other three took the same doses as the first group, but with a meal containing 1.8 mg iron. The results of these studies are shown in Table IX. Average absorptions were 14.0 per cent of hemoglobin iron and 7.8 per cent of hemin iron when both were given without food, and 16.7 per cent of hemoglobin iron and 12.8 per cent of hemin iron when given with food. One month later a further dose of 2.5 mg hemin iron was given to five of these subjects either with or without food, so that the effect of food on the absorption of hemin iron could be assessed (Table X). Two subjects took the identical dose on each occasion so that variation due to sequential testing might be estimated. Average absorption of hemin iron without food was 10.2 per cent and with food 12.4 per cent.

TABLE IX

Absorption of iron in hemoglobin and in hemin without and with food by healthy subjects

Subject	% Absorption	
	Hb* (2.5 mg Fe) without food	Hemin (2.4 mg Fe) without food
G.F.	17.6	7.5
R.Ge.	8.6	6.9
R.Go.	15.9	8.9
Average	14.0	7.8
	Hb (2.5 mg Fe) with food (1.8 mg Fe)	Hemin (2.4 mg Fe) with food (1.8 mg Fe)
W.M.	22.4	14.1
R.K.	14.4	11.7
R.B.	12.9	12.5
Average	16.7	12.8

* Hb = hemoglobin.

TABLE X

Effect of food on absorption of hemin iron by healthy subjects

Subject	% Absorption	
	Hemin (2.4 mg Fe) without food	Hemin (2.4 mg Fe) with food (1.8 mg Fe)
R.K.	9.6	11.7
R.Go.	8.9	13.0
R.B.	12.1	12.5
R.Ge.	6.9* + 11.0*	
W.M.		14.1* + 12.9*
Average	10.2	12.4

* Not included in average.

In these studies, hemin iron was less well absorbed than hemoglobin iron, particularly in the absence of food, but its absorption, like that of hemoglobin iron, was not diminished by food. Thus, the apparent immunity of hemoglobin iron to the blocking effect of food cannot be due to the action of globin or a substance derived from it. The better absorption of hemoglobin iron than of hemin iron may be due to differences in solubility, since some precipitation of hemin was noted as soon as the dose was added to the tomato juice used to disguise its appearance. Further precipitation of hemin would be expected to occur in the stomach owing to the acidity of the gastric contents.

10. *The effect of cooking on the absorption of hemoglobin iron.* In an *in vitro* study, Kaldor (20) found that whereas incubation with hydrochloric acid and pepsin or gastric juice would free only traces of iron from native hemoglobin, the amount liberated was increased to about 12 per cent if the hemoglobin was first heated to 95° C for 30 minutes. The absorption of iron in cooked hemoglobin was therefore compared with that of native hemoglobin. Three subjects, R. N., M. S., and P. T., were given 2.5 mg hemoglobin iron with food containing 1.8 mg iron on one day and the same quantity of cooked hemoglobin, heated in a boiling water bath for 15 minutes, with a similar meal on the next day. Absorptions of the uncooked hemoglobin iron were 13.6, 21.5, and 12.0 per cent, averaging 15.7 per cent, and absorptions of the cooked were 19.1, 12.9, and 11.3 per cent, averaging 14.4 per cent. This study, in agreement with that of Callender, Mallett, and

Smith (7), indicated that cooking does not change the absorption of hemoglobin iron.

11. *The effect of food on the absorption of iron in ferritin.* Most of the iron in foods derived from animal sources is contained in porphyrin compounds, particularly hemoglobin, and in ferritin. Since the absorption of hemoglobin iron was not decreased by food, it appeared relevant to study the absorption of iron in ferritin. Three subjects, B. G., E. D., and D. H., were given 2 mg iron as labeled ferritin with food containing 1.8 mg iron and without it on successive days. Absorptions of ferritin iron with food were 0.3, 1.7, and 1.2 per cent, averaging 1.1 per cent, and those without were 1.7, 1.6, and 3.2 per cent, averaging 2.2 per cent. Thus, ferritin iron appeared to be less well absorbed when taken with food, but the very low absorption by these subjects makes it hazardous to draw any definite conclusion from this study.

12. *The nature of the absorbed iron in plasma after ingestion of hemoglobin.* The most likely explanation of the apparent immunity of hemoglobin iron to the blocking effect of food and of phytate, and to the facilitating effect of ascorbic acid, is that the hemoglobin molecule or an iron-containing portion such as hemin is absorbed intact. If this is so, the iron could either be carried into the blood stream in this form, or be split off in the intestinal wall. A study was therefore devised to determine whether after ingestion of labeled hemoglobin the radioiron in plasma was bound to transferrin or whether it was present in some other form. A preliminary experiment showed that when transferrin-bound radioiron in plasma was dialyzed against 0.4 per cent EDTA at pH 5.6 for 24 hours, more than 95 per cent of the iron was removed from the transport protein. In contrast, less than 6 per cent of the radioiron was dialyzable after this treatment when labeled hemoglobin or hemin was added to plasma in an amount insufficient to saturate haptoglobin or heme-binding proteins. This treatment was applied to samples of plasma from a normal subject at intervals after the ingestion of 5 mg hemoglobin iron labeled with Fe^{59} in an amount sufficient to lead to the absorption of 10 to 15 μc. As shown in Table XI, a maximum of 8 per cent of the Fe^{59} counts in the plasma was not dialyzed, a result which is consistent with transferrin binding of the absorbed iron.

TABLE XI

Radioactivity in plasma of Subject W.B. after ingestion of Fe^{59}-hemoglobin (5 mg Fe) before and after dialysis with acid and EDTA

Time	Radioactivity (Fe^{59} c/m/2ml)		
	Before dialysis	After dialysis	% Non-dialyzed
min			
30	61	1	1
47	135	3	2
60	179	3	2
75	216	2	1
92	294	25	8
105	312	23	7
120	314	21	7
150	307	4	1

In addition to the labeled hemoglobin, this subject was given at the same time a dose of ferrous sulfate containing 5 mg of iron labeled with Fe^{55}, so that the entry of the two types of iron into the plasma might be compared. The activity from the Fe^{55} isotope labeling the salt iron rose and fell about 20 minutes earlier than that from the Fe^{59} isotope in the labeled hemoglobin, a difference similar to that which has been observed by consecutive testing (7).

DISCUSSION

Any estimate of iron absorption must be considered in relation to the wide range of absorption seen in different individuals. This variation may be assumed to be related in part to the physiologic factors that influence absorption (14). Increased erythropoiesis and increased need for iron in pregnancy, growth, and iron depletion increase absorption, whereas decreased erythropoiesis and increased iron stores curtail it. Studies of apparently healthy subjects show a discouraging variation, one series reporting absorption of 5 mg ferrous iron from 11 to 68 per cent (7). Variation in body iron stores is probably the most important single intrinsic factor influencing absorption in these "normal" subjects, and it is of considerable importance to control this variable in an investigation of the kind reported here. An attempt was made to recognize the extreme variations by history of blood loss or blood donation and by determination of plasma iron and iron-binding capacity, although it is well known that these measures are inadequate to evaluate iron

stores. Accordingly, the investigation was based on comparative observation in the same individual with the two radioisotopes of iron, Fe^{55} and F^{59}. It was first necessary to determine the variation of results in the same subject. Although slightly lower values were obtained in studies done later in the day, it appeared that this was not a significant factor and that repetitive studies gave essentially similar results. Problems in methodology attending the measurement of iron absorption have been discussed in detail elsewhere (21). At the present time, measurement of red cell radioactivity is considered to be more accurate in normal and iron-deficient subjects than the determination of iron balance by fecal analysis, since the values so obtained agree with results of total body counting. Since the present studies are based on comparisons in the same individual, minor variations in the absolute figures that might be introduced by error in the calculation of blood volume are of no importance.

Since it is currently believed that dietary iron must be liberated from complexes and reduced before it can enter the mucosal cell, soluble ferrous iron must be considered the prototype to which other substances may be compared. In the present studies, average absorption of doses of 4 to 6 mg elemental iron by healthy subjects was 9.6 per cent for ferrous salt alone, 4.1 per cent for ferrous salt given with tomato juice, and 9.2 per cent for hemoglobin iron. The figure for ferrous salt alone is similar to the 10.8 per cent average absorption of the same dose reported by Brown, Dubach, and Moore (22); that for hemoglobin iron is similar to the 10 per cent average observed by Callender, Mallett, and Smith (7). Average absorption of this amount of ferrous salt in the subjects studied by Callender and co-workers, however, was 24.8 per cent, a difference probably related to differences in body iron stores, since iron-deficiency appears to facilitate absorption of iron salt more than that of hemoglobin iron.

In some respects there are similarities between the absorption of hemoglobin iron and that of iron salt. The limited data so far available indicate that the fraction absorbed is related to the quantity ingested; with larger doses, the percentage absorption decreases although the total amount absorbed increases. Absorption of hemoglobin iron is also enhanced by iron-deficiency. In other respects there are marked differences. Absorption of soluble ferrous iron is reduced by food and by phytate, and in the presence of food is increased by ascorbic acid. In contrast, the absorption of hemoglobin iron is not reduced and may even be increased by food and by phytate, but is unaffected by ascorbic acid. At the dosage studied it does not seem to be affected by added iron salt. These antitheses suggest that hemoglobin iron is absorbed in a manner different from soluble food iron.

The possibility was considered that the digestion process might be involved in the absorption of hemoglobin iron, for the increased absorption when food was present might reflect secretory or motility changes effected by food. Stimulation of gastric secretion, however, did not increase absorption in the absence of food. Although direct studies of duodenal or pancreatic secretions were not made, studies with phytate present indirect evidence of their limited importance. Nearly all the subjects given phytate complained of abdominal cramps and had marked diarrhea. In this case there was no reason to expect an outpouring of intestinal secretions, and despite rapid mobility absorption was enhanced. These studies suggest that food and phytate do not facilitate absorption by increasing digestive secretions; possibly they increase surface activity of the mucosal cells and thereby facilitate absorption of hemoglobin iron.

The apparent insusceptibility of hemoglobin iron to the action of blocking substances and reducing agents suggests that the iron is either absorbed within the hemoglobin molecule or bound in complex to some portion of it. Since absorption of hemin iron was likewise unaffected by food, it seems unlikely that globin plays an essential role as a vehicle. While it is theoretically possible that after absorption hemoglobin or heme (or a fragment of heme) might be bound directly to plasma proteins and be processed by the reticuloendothelial tissues at some distant site, this possibility was not supported by the finding that the absorbed iron in plasma behaved as transferrin-bound iron. If it had entered the plasma as hemoglobin, a detectable amount would have been found in nondialyzable form, since the half-time of plasma hemoglobin turnover is of the order of 20 minutes (23).

Whether this is indeed the mechanism whereby hemoglobin iron is absorbed, the differences noted between its absorption and that of iron salt demonstrate the inadequacy of the present model of iron absorption. It remains uncertain to what extent the various iron compounds in food behave like iron salts or hemoglobin. Clearly a more detailed study of various food iron compounds is indicated if the normal physiology of iron absorption is to be understood. In animal foods most nonheme iron is in ferritin and hemosiderin, and it seemed of great interest to study the effect of food on absorption of ferritin iron. Food appeared to diminish absorption in two of three subjects, but the level of absorption was so low that the study was inconclusive.

SUMMARY

The absorption of radioiron in rabbit hemoglobin, hemin, and ferritin has been compared to that of ferrous salt in healthy volunteers and in subjects with iron-deficiency anemia.

At a dosage level of 5 mg elemental iron, hemoglobin iron was as well or better absorbed than ferrous salts in the normal subject. Absorption of hemoglobin iron increased less, however, in the iron-depleted or iron-deficient subject. In contrast to the absorption of ferrous salts, that of hemoglobin iron was not decreased by food or by phytate nor increased by ascorbic acid. The absorption of hemin iron was also not decreased by food. Iron absorbed from hemoglobin appeared in the plasma later than that from ferrous salts, but was found to be similarly dialyzable at acid pH with EDTA.

These findings suggest that iron in heme complexes is absorbed as a porphyrin complex without conversion to the free ionized form. It is further apparent that there is less effective mucosal regulation of absorption of iron in this form. Finally, the present hypothesis of iron absorption based on the behavior of iron salts is not adequate for all types of food iron.

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