blood 1969 33: 430-443

Food Iron Absorption: A Comparison of Vegetable and Animal Foods

M. LAYRISSE, J. D. COOK, C. MARTINEZ, M. ROCHE, I. N. KUHN, R. B. WALKER and C. A. FINCH

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published semimonthly by the American Society of Hematology, 1900 M St, NW, Suite 200, Washington DC 20036.

Food Iron Absorption: A Comparison of Vegetable and Animal Foods

By M. Layrisse, J. D. Cook, C. Martinez, M. Roche, I. N. Kuhn, R. B. Walker and C. A. Finch

MEANINGFUL ASSESSMENT of dietary iron requirements is not possible at present because of the limited information on absorbability of iron from various foods. A number of studies have attempted to evaluate absorption of iron from a single food; reference will be made only to those studies in which physiologic amounts of iron were administered (i.e., between 2 and 7 mg.) and in which 8 or more subjects were tested with a single food item. In these studies, radioiron was biologically incorporated into foods, and absorption of the tagged iron determined by feeding to normal and iron deficient subjects. Thus, Chodos et al.[1] found a mean absorption of 1.4 per cent (range 0.5–5) for iron of eggs, while Moore and Dubach[2] reported a mean of 4 per cent (range 1.1–8.4). Hussain et al.[3] found that wheat iron absorption in 21 normal subjects had a mean of 4.5 per cent (range 1.1–7.4), while in 21 iron deficient subjects the mean was 7.8 per cent (range 0.4-16.3). Using hemoglobin as the test substance, Turnbull et al.[4] found a mean absorption in 16 normal males of 9.1 per cent (range 3.7-19.1), while in the study of Callender et al.[5] the mean absorption of uncooked hemoglobin in 10 subjects of mixed sex was 19 per cent (range 1–21), and of cooked hemoglobin 7 per cent (range 0–16). They also reported that in 11 iron deficient subjects, uncooked hemoglobin had a mean absorption of 22 per cent (range 0–36) and cooked hemoglobin 12 per cent (range 6-21).

From other studies[6] performed on too few subjects to permit a quantitative evaluation of the biologic value of the food items studied, it appears that iron from animal sources is more available than iron from vegetable sources. When values of food iron absorption are compared to those of iron salts, it is evident

---

From the Instituto Venezolano de Investigaciones Cientificas (IVIC), Caracas, Venezuela; Department of Medicine, Loma Linda University, Loma Linda, California; and Departments of Medicine and Botony, University of Washington, Seattle, Washington.

This investigation was supported by U.S. Public Health Service Grant 5-RO1-HE-06242, Training Grant TI-AM-5130, AM-06983 (Uptake of Iron by Man), and N.I.H. Grant HE-06507.

First submitted August 1, 1968; accepted for publication October 21, 1968.

M. Layrisse,: *Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.* J. D. Cook.: *Instructor in Medicine, University of Washington School of Medicine, Seattle, Wash.* C. Martinez,: *Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.* M. Roche,: *Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.* I. N. Kuhn,: *Assistant Professor of Medicine and Head, Section of Hematology, Loma Linda University, Loma Linda, Calif.* R. B. Walker,: *Professor of Medicine and Chairman, Department of Botony, University of Washington, Seattle, Wash.* C. A. Finch,: *Professor of Medicine and Head, Division of Hematology, University of Washington, Seattle, Wash.*

that iron in food is less well absorbed. This difference is especially marked in iron deficient subjects. At the same time the wide range of absorption observed in normal subjects given either iron salts or tagged foods has made difficult any attempt to compare the nutritive value of these different sources of iron. The purpose of the present study was to characterize the absorbability of iron from different foods in both normal and iron deficient subjects using the absorption of inorganic iron as the reference with which to compare food iron absorption in each subject. In this way, individual variations in absorptive capacity were minimized. Food sources studied were corn, wheat, black beans, soybeans, lettuce, spinach, fish muscle, veal muscle and hemoglobin.

Materials and Methods

Iron absorption studies were performed on subjects from Seattle, Washington, and peasants from an agricultural area of Venezuela. Seventy-nine were male and 52 were female. Hemoglobin levels, packed red cell volume, serum iron, and iron binding capacity were determined in each individual. The lower limit of normal for hematocrit was taken as 42 in the male and 36 in the female[7] and the lower limits for plasma iron and iron saturation of transferrin were considered to be 50 μg. per cent and 17 per cent, respectively.[8]

^{55}Fe or ^{59}Fe tagged food was administered in the morning, after an overnight fast, and no food or drink was allowed for 3 hours after the test feeding. The following morning iron ascorbate tagged with the second isotope of iron was given by mouth. The dose was adjusted with nonradioactive iron to 2–5 mg. Blood was taken 15 days after the feeding and duplicate 10 ml. blood samples were prepared for counting by wet-ashing, iron precipitation and electroplating. The radioactivity of the material analyzed was counted either in Seattle according to the method of Peacock et al.,[9] or in Caracas by the method of Dern and Hart.[10] The per cent absorption was calculated by determining the radioactivity per milliliter of blood and multiplying by an assumed blood volume of 65 ml./Kg. Per cent absorption was calculated as the ratio of the amount of radioactivity in the blood to that injected.

Radioactive foods were prepared in the following manner. In the handling of the tagged food items, iron-free water and aluminum utensils were used. During and after cooking, when the preparation of food permitted, a dish-shaped aluminum foil covered by a thin layer of margarine was used to facilitate cooking, feeding and discarding of the contaminated material.

Wheat. The preparation of this food was described in detail elsewhere.[3]

Corn. Seeds of Idaho hybrid corn No. 216 obtained from the Charles E. Lilly Company were germinated in a flat box containing silica sand watered with tap water. Ten days later, plants were transferred to 20 liter tanks, 3 plants per tank. Each plant was supported in the solution with a styrofoam stopper and cotton. For two months the plants were grown on a "normal phosphate" solution including iron and other micronutrients* aerated con-

*Culture solutions used for growing plants in tanks.

Compound	mM per liter: Normal Phosphate	mM per liter: Low Phosphate
$NH_4H_2PO_4$	1.0	0.2
$K NO_3$	6.0	6.0
$Ca (NO_3)_2$	4.0	4.0
$Mg SO_4$	2.0	2.0
NaCl	0.2	0.2
NH_4Cl		0.8
Micronutrients[1]		

[1]Micronutrients (mg./L.): Fe (as NaFe EDTA) 5.0; Mn 0.5, B 0.5; Cu 0.02; 2n 0.05; Mo 0.05

tinuously with compressed air through two capillary glass tubes. The solution was changed every 3 weeks. On the sixty-first day the solution was changed to a low phosphate composition adjusted to pH 4 with dilute hydrochloric acid. Two μc. of ^{59}Fe were added with 50 ml. of stable iron as NaFe EDTA in 10 ml. volumes to each tank. The corn plant silks had developed to a length of 7.5 cm. at that time and the tassels were beginning to shed pollen. On the one hundred twentieth day about 1.5 Kg. of corn kernels were harvested from 4 tanks (12 plants) by hand and ground in a porcelain mortar to a fine meal. Stone ground cornmeal used as carrier was obtained from Burr Mills, Seattle, Washington. Corn patties containing 35 Gm. of the radioactive cornmeal and 155 Gm. of carrier cornmeal were cooked before eating.

Radioactive corn was also prepared by injecting radioiron into the plant stem. This was done about 10–12 weeks after germination when the ear of the corn had reached about one-third of its full size. Three hundred μc. in the form of ferrous citrate were injected into the internode just below the first ear of corn. In 20 plants injected in this way, about 18 per cent of radioactivity administered was recovered in the grain at harvest time. The total iron content in the "hot" corn was 21 μg./Gm. Labeled corn, together with the carrier corn, was boiled in an aluminum pot containing 4–5 times the weight of corn in water. After the grain became swollen and soft it was ground and pancakes (arepas) of about 200 Gm. were prepared and grilled before being fed. Each subject received 4 mg. of corn iron. (In a separate study the absorbability of iron introduced by stem injection was found to be identical to iron introduced via the roots.)

Black beans (*Phaseolus vulgaris*). Seeds were germinated in moist filter paper. Five days after germination, each plant was transferred to a jar containing 400 ml. of nutrient solution[11] and covered with aluminum foil. The solution was changed weekly. About 6–8 weeks after germination 20 μc. of ^{59}Fe or ^{55}Fe were added to the medium once a week in the following 3 weeks. The beans were harvested 12–13 weeks after germination. About 13 per cent of the ^{59}Fe or ^{55}Fe added to the medium was recovered in the seeds. The iron content in the seeds varied from 85–90 μg./Gm. The labeled black beans, together with the carrier beans were boiled in an aluminum pot containing about 5–6 times the weight of the beans in water. Once the beans became swollen and soft they were mashed and mixed with the residual water to produce a homogenous paste. This paste was then heated again to evaporate more water, and an amount containing 3 or 4 mg. of food iron was given to each subject.

Lettuce. Seeds were germinated in moist silica sand. Three weeks after germination, each plant was transferred to modified Hoagland Arnon's solution in a jar covered with a coat of black paint and a layer of aluminum foil or paint. The lettuce leaves were harvested about 12–14 weeks after sprouting and 3–5 weeks after adding ^{59}Fe. About 15 per cent of the ^{59}Fe added to the solution was recovered in the green parts of the plants. Iron content varied from 9–26 μg./Gm. The labeled lettuce was given with tomato juice so that the subjects ingested 1.9–1.7 mg. of iron, from which only 0.4–0.6 mg. of iron came from lettuce.

Spinach. Nobel Giant seed from G.S. Seedhouse was germinated in moist sand. Nineteen days after germination, plants were transferred to Hoagland Arnon's solution. Spinach leaves were harvested 87 days after the seeds had been germinated and 20 days after the addition of ^{59}Fe. About 12 per cent of the ^{59}Fe added to the spinach plants was recovered in the leaves at the time of harvest. The analysis of iron content showed 11.5 μg./Gm. of the greenhouse fresh spinach leaves. The labeled spinach, with sufficient carrier spinach to bring the total dose to about 2 mg. of iron per subject was placed in aluminum baking pans and cooked in a preheated oven at 350 F. for 15 minutes.

Soybeans. Hawkeye soybean seeds (Olds Seed Co., Madison, Wisconsin) were started in silica sand watered with tap water. When 3 weeks old, 28 plants per tank were placed as previously described in 20 l. of "normal phosphate" solution.* Solutions were continuously aerated and changed about every 10 days. With the development of pods on the fifty-third day from seeding, the solution was changed to a "low phosphate" solution* with pH adjusted

*Refer to footnote on page 431.

to 4.2 with dilute hydrochloric acid. During the preceding 2 weeks, daylight was shortened to 9 hours by covering the plants with black cloths. This was stopped after the change to "low phosphate" solution. Flowers appeared on the plant one week later. Four days later 2 mc. of ^{59}Fe mixed with 10 ml. sodium Fe EDTA containing 50 mg. of iron were added to each tank. At this time, some plants appeared chlorotic. Harvesting was carried out 1 month later and about 1.15 Kg. of soybeans were obtained from the 5 tanks. The mature pods were shelled by rolling between rubber-covered wooden blocks and a ridged rubber mat in a flat porcelain pan. They were ground to a fine bean flour in an electrically driven mortar made of earthenware. Dried soybeans to serve as carrier were purchased in a local market and processed to a flour in the manner described above. The test meal was composed of 25–40 Gm. of radioactive bean meal and 10–25 Gm. of carrier bean meal. Labeled and carrier material were boiled separately for about 15 minutes in an aluminum pan containing 4–5 times the weight of powder in water. Boiling transformed the beans and water into a thick mush. The material was then mixed in proper proportions and aliquots were placed in an aluminum tray covered with margarine and heated for about 1 hour in an oven at 300 F. Four mg. of soybean iron containing about 5 μc. of ^{59}Fe were given to each subject.

Fish. Sweet fish (*Brycon whitei* and *Microptens salmonoides*) weighing about 200–400 Gm. were placed in appropriate tanks. About 15 days later, the fish were anesthetized with Tricain-methasulfonate and from 50–100 μc. of ^{55}Fe in the form of ferrous citrate were injected in the muscles, close to the caudal fin. After 3 months, the fish were sacrificed and muscles which were less than 4 cm. from the site of injection were discarded. The rest of the muscles (about 60 per cent of the total body weight) was washed with water in the same fashion used for culinary purposes, and then boiled and ground. The flesh was mixed with a small amount of flour to make an adherent paste. It was finally divided in about 50 Gm. pancakes and fried before it was given to the subjects. In the latter part of this study, snapper (*Lutjanus Sp.*) was used. Except for the area near the site of the ^{55}Fe injection, the muscles had a fairly uniform radioactivity with a variation of less than 20 per cent. The iron content of the fish muscles ranged from 4–6 μg./Gm. Only about 1–2 per cent of the radioactivity injected was recovered in the muscles. Due to the low iron content of the fish, a dose of only 1 mg. of iron was given to most individuals. In 4 instances, 2.5 mg. of fish iron was given.

Veal. Seven mc. of ^{55}Fe in the form of ferrous citrate was injected intravenously into the jugular vein of a 3 month old calf. Two months later, the calf was sacrificed and the flesh and liver kept frozen until used. The flesh was prepared as a hamburger after being ground. The iron content was 20–22 μg./Gm. A dose of 4 mg. of meat iron was given to each subject. The meal was cooked before administration.

Hemoglobin. A 3 Kg. rabbit was bled 40 ml., and after 2 days, 6 ml. of rabbit plasma containing 0.5 mc. of ^{59}Fe was injected into the ear vein. Three weeks later 60 ml. of whole blood were removed by 2 cardiac punctures 12 hours apart, using heparin as an anticoagulant. The blood was stored in a frozen state until its use. Subjects were given 4 ml. of "tagged" hemoglobin solution containing 5 μc. of ^{59}Fe and 4 mg. of iron.

Ferrous ascorbate. Two moles of ascorbic acid were added to each mole of iron immediately preceding its administration. The radioactive iron was in the form of ferric chloride, and carrier iron was added as ferrous sulphate to a total of 3–4.5 mg. iron.

Statistical Analysis

Since the absorption of food iron was directly proportional to iron ascorbate, the ratio was used to evaluate iron absorption from different foods. In order to satisfy prerequisites of standard statistical tests,[12,13] it was necessary to perform the analysis in the logarithms of the ratio of food to inorganic iron absorption in each subject. To recover the original units of per cent iron absorption, the results were retransformed as antilogarithms. (See Appendix)

Results

Data obtained on each of 131 subjects grouped according to the food administered are shown in Table 1. Attention will first be directed to the character-

Table 1.—Food Iron Absorption Studies

Name	Age	Sex	Packed Red Cell Vol. (%)	Serum Iron (μg./100 ml.)	Transferrin Sat. Index (%)	Food Iron Dose (mg.)	Food Iron Absorption (%)	Inorganic Iron Dose (mg.)	Inorganic Iron Absorption (%)
Wheat									
1) E.M.		M	47	90	31	3.4	2.5	3.4	3.1
2) J.M.		F	44	80	21	3.4	6.6	3.4	6.7
3) T.G.	42	M	44	125	36	3.8	5.2	4.0	7.2
4) H.M.	37	M	43	96	40	3.8	7.0	4.0	7.2
5) H.R.		M	48	114	34	3.4	5.8	3.4	7.3
6) R.V.	17	M	44	118	32	3.8	2.7	4.0	8.3
7) E.S.	12	M	43	108	31	3.8	3.6	4.0	9.2
8) H.F.		M	24	25	10	2.0	5.3	2.0	14.6
9) E.G.	17	F	39	69	19	4.0	14.3	4.0	24.7
10) M.G.	14	F	40	62	12	4.0	10.4	4.0	25.0
11) M.A.	17	M	26	82	18	2.4	8.8	2.5	38.0
12) J.M.	25	M	31	41	11	2.4	7.7	2.5	38.4
13) M.R.		F	27	27	11	2.0	0.4	2.0	42.4
14) F.R.G.	12	M	30	41	9	2.4	3.0	2.5	49.7
15) H.A.		F	26	37	9	2.0	3.6	2.0	58.1
16) P.V.	46	M	30	36	10	2.4	11.3	2.5	62.6
17) J.A.	15	M	41	64	13	4.0	22.5	4.0	65.1
18) G.Y.	17	M	44	116	25	4.0	20.8	4.0	71.6
Average			37	74	21	3.2	7.9	3.2	29.9
Corn									
1) J.E.G.	46	M	45	84	21	4.0	0.2	4.0	8.1
2) L.E.M.	30	M	41	84	24	4.0	4.6	4.0	9.9
3) C.H.	26	M	50	62	14	4.0	4.1	4.0	11.7
4) F.G.	54	M	43	71	24	4.0	2.4	4.0	11.9
5) Z.D.	30	F	38	58	14	4.0	2.2	4.0	12.6
6) L.P.	46	M	37	53	12	4.0	2.0	4.0	17.3
7) A.R.G.	18	M	42	88	24	4.0	1.5	4.0	22.7
8) C.H.	16	F	37	78	23	4.0	3.4	4.0	25.5
9) L.G.	14	F	43	50	11	4.0	4.0	4.0	30.8
10) I.P.	14	M	44	100	25	4.0	7.6	4.0	35.6
11) M.M.	53	F	36	64	17	4.0	7.7	4.0	41.2
12) M.C.	45	F	39	86	20	4.0	5.3	4.0	42.3
13) R.M.	33	M	45	134	33	4.0	3.5	4.0	46.6
14) M.C.	20	M	37	22	4	4.0	8.5	4.0	48.4
15) T.D.	43	M	35	76	18	4.0	9.0	4.0	57.7
16) P.G.	59	F	38	47	13	4.0	2.1	4.0	60.1
17) E.M.	24	F	37	46	13	4.0	4.2	4.0	63.5
18) V.P.	23	M	41	108	26	4.0	14.8	4.0	69.5
19) L.I.M.	12	M	39	52	13	4.0	12.9	4.0	72.5
20) M.V.	42	M	39	42	8	4.0	11.9	4.0	78.7
21) L.A.	23	M	34	43	8	4.0	10.1	4.0	80.5
Average			40	69	17	4.0	5.9	4.0	40.3
Black Beans									
1) L.Ch.	20	F	39	138	50	3.0	4.1	3.0	3.7
2) L.H.	40	M	42	84	22	3.0	0.7	3.0	3.8
3) M.Q.	24	M	45	108	32	3.0	2.1	3.0	5.1
4) R.V.	28	M	48	104	34	3.0	1.4	3.0	5.5
5) C.D.	34	M	38	112	25	4.0	1.1	3.0	7.3
6) C.H.	40	M	50	108	28	3.0	1.0	3.0	13.9
7) Z.L.	30	F	42	106	26	3.0	5.2	3.0	19.4

Table 1.—Food Iron Absorption Studies (Continued)

Name	Age	Sex	Packed Red Cell Vol. (%)	Serum Iron (μg./100 ml.)	Transferrin Sat. Index (%)	Food Iron Dose (mg.)	Food Iron Absorption (%)	Inorganic Iron Dose (mg.)	Inorganic Iron Absorption (%)
Black Beans (continued)									
8) E.A.	22	M	39	108	29	3.0	4.4	3.0	19.6
9) V.D.	39	F	39	55	16	4.0	1.7	3.0	20.9
10) M.D.	25	F	42	142	34	3.0	4.9	3.0	27.1
11) Z.M.	35	F	41	84	24	3.0	3.2	3.0	29.7
12) F.B.	28	M	44	90	21	3.0	4.5	3.0	31.1
13) M.V.	27	F	39	82	25	3.0	3.3	3.0	35.4
14) A.H.	12	M	34	53	17	4.0	3.9	3.0	39.4
15) M.A.	20	F	48	94	20	3.0	6.4	3.0	59.0
Average			42	98	27	3.2	3.2	3.0	21.4
Lettuce									
1) S.S.	22	M	49	125	40	1.7	1.3	5.0	1.3
2) Ph.B.	28	M	49	104	35	1.0	2.9	5.0	4.0
3) J.B.	24	M	49	167	65	1.5	1.1	5.0	4.4
4) M.F.	22	F	43	125	47	1.1	5.3	5.0	5.1
5) K.M.	23	F	39	56	16	1.2	3.9	5.0	9.2
6) M.H.	22	F	44	72	22	1.3	4.5	4.0	12.7
7) D.P.	21	M	49	160	48	1.2	2.2	5.0	13.3
8) S.L.	22	F	43	106	29	1.1	10.4	5.0	22.9
9) C.W.	21	F	43	67	23	1.0	7.0	5.0	23.9
10) P.B.	23	F	40	94	30	1.1	9.9	5.0	24.5
11) B.C.	23	F	40	126	42	1.0	6.9	5.0	34.0
12) R.J.	65	M	33	22	6	1.1	1.6	5.0	64.4
13) N.C.	22	F	42	122	37	1.2	18.0	4.0	95.0
Average			43	104	34	1.2	5.8	4.9	24.2
Spinach									
1) W.C.	23	M	44	96	30	1.9	1.0	5.0	1.0
2) F.B.	24	M	46	96	32	1.9	1.4	5.0	3.3
3) D.C.	24	F	41	129	59	2.0	1.1	5.0	9.8
4) O.H.	26	M	48	112	36	2.0	0.5	5.0	12.6
5) A.J.	33	F	38	95	36	2.0	2.0	5.0	12.9
6) R.H.	26	M	48	115	46	2.0	0.3	5.0	14.7
7) D.K.	36	F	35	41	12	2.1	2.6	5.0	19.3
8) J.M.	22	F	43	87	25	2.0	1.5	5.0	22.0
9) L.P.	23	F	36	84	24	2.1	4.8	5.0	52.9
Average			42	95	33	2.0	1.7	5.0	16.6
Soybean									
1) P.N.M.	50	M	45	120	37	4.0	1.7	4.0	5.1
2) F.J.T.	20	M	50	76	26	4.0	4.7	4.0	6.0
3) M.M.O.	32	F	37	88	27	4.0	7.5	4.0	6.3
4) R.M.	29	M	44	62	18	4.0	22.6	4.0	8.4
5) C.F.B.	51	F	39	57	18	4.0	6.9	4.0	11.9
6) M.M.A.	16	M	44	144	42	4.0	2.5	4.0	19.7
7) A.M.	23	F	37	56	18	4.0	14.8	4.0	20.1
8) R.F.	46	M	44	67	21	4.0	6.2	4.0	21.0
9) J.H.	32	F	42	78	23	4.0	23.4	4.0	25.1
10) M.B.	46	F	42	74	25	4.0	1.5	4.0	25.9
11) R.B.	25	M	45	128	34	4.0	33.7	4.0	37.7
12) C.E.C.	18	F	39	107	23	4.0	37.2	4.0	48.6
13) P.F.S.	36	M	47	94	24	4.0	22.4	4.0	48.9

Table 1.—Food Iron Absorption Studies (Continued)

Name	Age	Sex	Packed Red Cell Vol. (%)	Serum Iron (μg./100 ml.)	Transferrin Sat. Index (%)	Food Iron Iron Dose (mg.)	Food Iron Absorption (%)	Inorganic Iron Iron Dose (mg.)	Inorganic Iron Absorption (%)
Soybean (continued)									
14) B.R.	15	M	43	75	18	4.0	42.2	4.0	51.8
15) C.P.	17	M	31	56	14	4.0	4.5	4.0	59.9
16) P.C.R.	15	M	38	36	8	4.0	37.8	4.0	81.4
17) B.B.	18	M	35	28	6	4.0	34.0	4.0	105.7
Average			41	79	23	4.0	17.9	4.0	34.3
Fish									
1) A.V.	47	M	42	58	17	1.0	1.9	3.0	2.8
2) G.G.	46	M	40	114	28	1.0	7.4	3.0	10.9
3) M.V.	37	F	35	69	20	2.5	17.8	3.0	15.2
4) L.F.R.	42	M	43	88	37	1.0	8.9	3.0	15.8
5) P.A.	53	M	48	149	36	2.5	7.3	3.0	21.1
6) J.M.	44	M	51	106	25	1.0	7.7	3.0	21.8
7) A.A.	34	M	43	81	24	2.5	7.2	3.0	23.4
8) N.P.	13	M	43	46	11	1.0	16.0	3.0	27.0
9) R.P.	32	M	52	122	28	1.0	4.9	3.0	28.7
10) B.A.	34	M	39	72	29	1.0	37.2	3.0	41.6
11) A.M.	23	M	45	80	24	1.0	37.6	3.0	42.0
12) P.C.G.	55	M	40	70	22	1.0	6.3	3.0	60.6
13) R.J.F.	29	F	41	52	14	1.0	25.1	3.0	63.2
14) A.F.	67	M	16	32	7	1.0	20.6	3.0	65.9
15) L.F.	47	M	46	86	20	1.0	10.5	3.0	73.8
16) J.A.Z.	42	F	40	94	32	1.0	14.8	3.0	74.6
17) L.F.	25	M	49	144	31	1.0	32.2	3.0	82.0
18) C.L.T.	18	F	39	44	10	1.0	42.4	3.0	86.0
19) L.E.A.	20	M	42	86	24	2.5	41.7	3.0	94.0
Average			42	86	24	1.3	18.3	3.0	44.8
Veal Muscle									
1) P.A.	64	M	43	95	27	4.0	10.4	4.0	2.5
2) C.D.	38	M	47	112	25	4.0	12.7	4.0	7.3
3) L.E.M.	30	M	41	84	24	4.0	20.2	4.0	9.9
4) C.H.	26	M	50	62	14	4.0	23.2	4.0	11.7
5) F.G.	54	M	43	71	24	4.0	17.0	4.0	11.9
6) V.D.	39	M	39	55	16	4.0	15.2	4.0	20.9
7) C.H.	16	F	37	68	19	4.0	29.6	4.0	25.5
8) A.H.	12	M	34	53	17	4.0	15.8	4.0	39.4
9) M.C.	20	M	37	22	4	4.0	38.3	4.0	48.4
Average			41	69	19	4.0	20.3	4.0	19.7
Hemoglobin									
1) P.M.	20	F	44	104	31	5.0	11.4	4.0	2.6
2) A.C.	25	F	39	84	24	5.0	12.7	4.0	15.8
3) B.U.	21	F	39	88	24	5.0	6.7	4.0	16.6
4) K.G.	20	F	40	101	22	5.0	9.8	4.0	21.6
5) P.B.	27	F	37	88	27	5.0	12.5	4.0	26.2
6) K.H.	19	F	42	80	24	5.0	9.2	4.0	26.8
7) S.A.	21	F	39	91	22	5.0	22.4	4.0	42.5
8) L.J.	19	F	40	50	11	5.0	30.8	4.0	51.1
9) D.H.	21	F	42	39	10	5.0	20.1	4.0	63.6
10) K.A.	21	F	42	60	15	5.0	20.7	4.0	64.9
Average			40	79	21	5.0	15.6	4.0	33.2

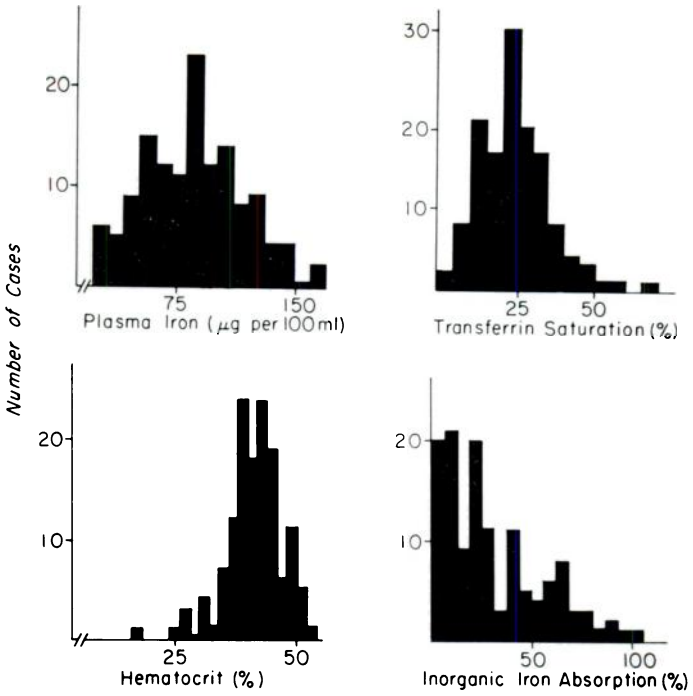

Fig. 1.—Frequency distribution of parameters reflecting the level of storage iron in the composite group of test subjects. The unimodal distribution observed with each parameter indicates a continuous spectrum in iron status ranging from normal to the iron deficient state.

istics of the subjects and the composition of the individual group. The frequency distribution of composite values for hematocrit, plasma iron, per cent transferrin saturation, and inorganic iron absorption are shown in Figure 1. According to the criteria employed, 25 per cent of the subjects were anemic, and of these, 80 per cent had iron depletion as evidenced by a reduced plasma iron or transferrin saturation or both. Of the total group, 16 per cent had a plasma iron of less than 50 μg./100 ml., 24 per cent had a transferrin saturation of less than 17 per cent, and 33 per cent had one or both abnormalities. Thus, by customary standards, 20 per cent of the group had iron deficiency anemia, and a further 13 per cent were iron depleted. The high proportion of iron deficient subjects is reflected in the composite means, which were 82.5 μg./100 ml. for the plasma iron, 23.6 per cent for the transferrin saturation, 40.7 per cent for the hematocrit, and 31.5 per cent for inorganic iron absorption. It is apparent from the distribution of these parameters (Fig. 1) that any arbitrary separation into iron deficient and normal subjects would be artificial, since in each case the distribution appeared to be a continuum.

The relationship between iron ascorbate absorption and either the plasma iron or transferrin saturation was examined in the total group of subjects. While there was some degree of correlation as shown by the correlation coefficients of − 0.392 for plasma iron and − 0.501 for transferrin saturation (sig-

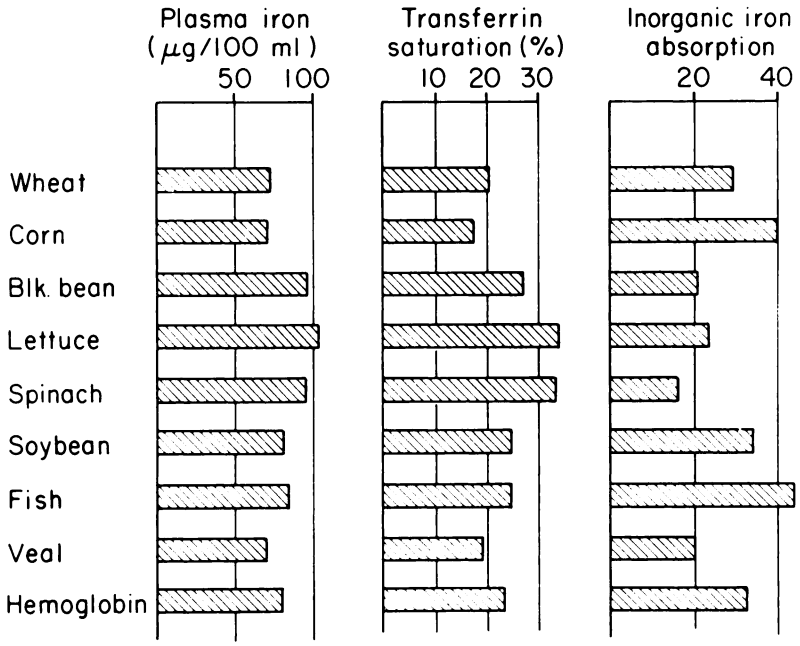

Fig. 2.—Mean values for plasma iron, transferrin saturation, and inorganic iron absorption in test groups studied with different types of dietary iron. The wide range of means reflect differences in the incidence of iron deficiency which varied from roughly 10 per cent in the group given spinach to about 60 per cent in subjects given tagged corn. A close reciprocal relationship between inorganic iron absorption and the plasma iron measurements is apparent.

nificant to $p < 0.001$), a given value for inorganic iron absorption was still consistent with a wide range of plasma iron or transferrin saturation values.

Appreciable differences existed in these three parameters among the 9 groups of individuals studied with a specific food (Fig. 2). Thus, the mean plasma iron of individual groups varied from 69–104, the mean transferrin saturation from 17–34, and the iron ascorbate absorption from 17–45 per cent. These differences reflect the large variation in body iron stores in the different groups, which is to be expected from the contrasting socio-economic conditions of subjects living in different countries.

The mean absorption varied from a low of 2 per cent for lettuce to a high of 20 per cent for veal. A relatively low absorption with mean values ranging from 1.7–7.9 was found for wheat, corn, black beans, lettuce* and spinach; higher mean values ranging from 15.6–20.3 were observed with soybeans, fish, veal and hemoglobin.

It seemed likely that the group differences described above in relation to plasma iron, transferrin and iron ascorbate absorption might affect the values obtained with the individual food items. A method was, therefore, sought for

*Absorption values for lettuce are not strictly comparable to other foods since it was given with tomato juice. This vehicle undoubtedly enhanced absorption through its ascorbic acid content.

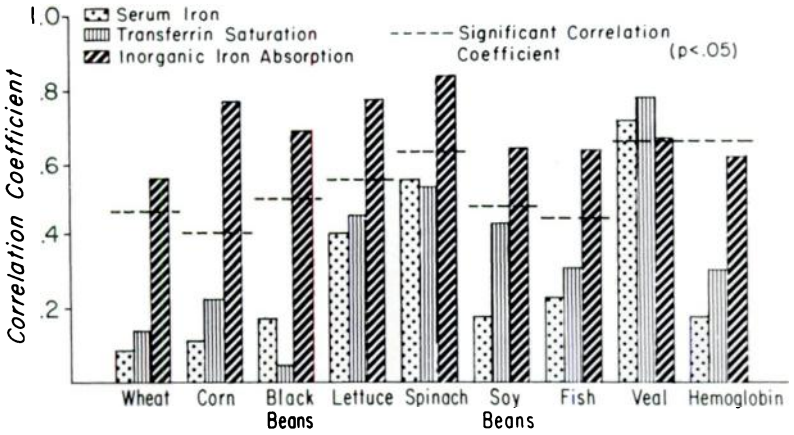

Fig. 3.—Correlation between iron absorption from various foods and the plasma iron transferrin saturation or absorption of inorganic iron. The interrupted horizontal lines represent the correlation coefficient at the 5 per cent level of significance. The absorption of iron ascorbate was the only parameter which consistently correlated with food iron absorption.

correcting food iron absorption values for subject differences. To determine the most reliable correction index, the correlation between food iron absorption and plasma iron, per cent transferrin saturation, and inorganic iron absorption were evaluated among members of each group. Despite the limited number of subjects, significant correlation coefficients were obtained between food iron and inorganic iron absorption in all but one group. Conversely, in only one instance was the correlation of food iron absorption with plasma iron or transferrin saturation significant (Fig. 3). Absorption of food iron was, therefore, expressed in relation to the absorption of ferrous ascorbate. The ratios for the individual foods calculated as described under Materials and Methods were as follows: wheat 0.29, corn 0.13, black beans 0.17, lettuce 0.31, spinach 0.11, soybean 0.46, fish 0.40, veal 1.31, and hemoglobin 0.57. The expected absorption curve of each food related to ferrous ascorbate is shown graphically in Figure 4.

Discussion

Iron absorption measurements in normal man have been shown to have great variation from subject-to-subject, probably related to the iron status and requirements of the individual. Thus, the mean absorption of a group of men was 7 per cent as compared to the mean absorption of 18 per cent in a group of women, a difference almost exactly accounted for by the menstrual iron loss and the lower food iron intake of the female.[14] Unfortunately, there is no accurate method of directly assessing the amount of storage iron of the individual or his iron requirements. While plasma iron and transferrin values permit recognition of the iron deficient individual, they do not provide a means of evaluation within the wide variation of body iron stores short of depletion.

Evidence is presented elsewhere[13] and supported by data in this study (Fig.

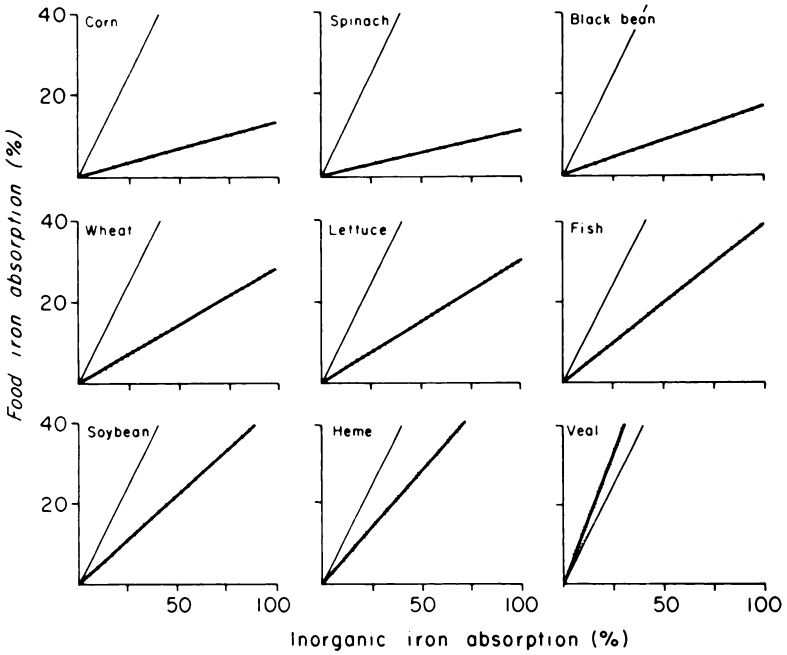

Fig. 4.—Estimates of the ratio of food to inorganic iron absorption with different forms of dietary iron. The shaded areas represent the ratio estimate ± 1 standard error of the mean. Estimates were obtained from statistical analysis of the logarithms of the ratio of food to inorganic iron absorption in each subject. The results were retransformed as antilogarithms to recover the original unit of percentage iron absorption. Because of the positively skewed distribution of iron absorption data, the confidence band lying above each ratio estimate is wider than the lower interval.

3) that ferrous ascorbate absorption may be the most useful way to characterize the iron balance of the individual and the "absorptive behavior" of his intestinal mucosa. The ratios of food iron to ferrous ascorbate absorption have been calculated as the difference in their logarithms. The mean difference for each group when converted by taking the antilogarithm represents the slope of food to inorganic iron absorption with a y-intercept of zero. From these slopes, absorption of iron from a particular food can be estimated at any given level of inorganic iron absorption ranging from normal to iron deficiency anemia. Furthermore, these estimates remove from consideration any group differences in iron status which might influence assimilation of the food iron being studied. The differences in the slopes, representing the ratio of food to inorganic iron absorption, were evaluated statistically by the multiple range test of Duncan.[15] The absorption of veal was significantly higher than all other foods, and appeared to exceed even the absorption of iron ascorbate (ratio 1.31). Two other subsets were found among the remaining foods. Spinach, corn and black beans were included in the first with absorption ratios of 0.11, 0.13, and 0.17, respectively. Wheat, lettuce, fish, soybean and hemoglobin formed the second with ratios of 0.29, 0.31, 0.40, 0.46, and 0.57, respectively. For three foods (black

beans, wheat and lettuce) it was not possible to state whether their mean absorption lay within a low or intermediate range.

These studies were designed so that iron absorption in iron depleted subjects could be compared with that of normal subjects. Indeed, the critical question is the extent to which iron may be absorbed in the iron deficient, since absorption measurements in people with adequate iron stores reflect the behavior of the mucosa rather than availability of food iron. The method of analysis used in this study permits a prediction of food iron absorption in individuals with variable requirements. In an average male with adequate iron stores and in whom iron ascorbate absorption is less than 7 per cent, absorption values predicted from Fig. 4 are: corn 0.9, spinach 0.8, black beans 12, wheat 2, lettuce 2.2, fish 2.8, soybeans 3.2, hemoglobin 4, and veal 9.2. In the individual with no iron stores and with iron deficiency anemia, absorption values at a ferrous ascorbate absorption of 60 per cent would be: corn 8, spinach 6.7, black beans 10.3, wheat 17.2, lettuce 18.6, fish 24.1, soybeans 27.8, hemoglobin 34, and veal 78.5.

In view of the well-established relationship between dose and per cent absorption of inorganic iron, it is desirable to employ a uniform dosage when comparing absorption of different types of iron. For six of the nine foods tested, a dose of 3–5 mg. was employed; by adjusting the quantity of inorganic iron to that of food iron and expressing the absorption of food iron as a ratio of iron ascorbate/absorption, differences within this range were largely eliminated. It was impractical to employ the same amount of iron in testing three foods (lettuce, spinach and fish) because of their low iron concentration. Here, doses of 1–2.5 mg. of food iron were given, but the dose of iron ascorbate was not comparably reduced. If corrections are made for the effect that dose* might have, the average absorption of lettuce is reduced by 18.6–12.8, spinach 6.7–5.2, and fish by 24.1–18.9 per cent. However, since dose response information with food iron is not available, the validity of this correction is conjectural.

Summary

Iron absorption measurements have been made in 131 individuals relating the absorption of nine different foods tagged biosynthetically with radioiron. Relatively low absorption, ranging from 1.7–7.9, was found with wheat, corn, black beans, lettuce and spinach. Higher values of from 15.6–20.3 were observed with soybeans, fish, veal and hemoglobin. When these values were related to the absorption of ferrous ascorbate determined simultaneously in each subject, food iron absorption could be predicted over the spectrum of normal to iron deficient states.

SUMMARIO IN INTERLINGUA

Mesurationes del absorption de ferro esseva effectuate in 131 subjectos, con le relation del absorption de novem differente alimentos etiquettate biosyntheticamente con radioferro. Relativemente lente absorptiones (inter 1,7 e 7,9) esseva trovate pro frumento, mais, fabas

*The correction was based on a dose/absorption relationship observed with inorganic iron.[16]

nigre, lactuca, e spinacia. Plus alte valores (inter 15,6 e 20,3) esseva observate con soja, pisce, vitello, e hemoglobina. Quando iste valores esseva ponite in relation con le absorption de ascorbato ferrose, determinate simultaneemente in omne subjecto, le absorption de ferro dietari poteva esser predicite a transverso del spectro ab normalitate usque a statos de carentia de ferro.

APPENDIX

When the availability of different iron compounds is assessed by relating their absorption to that of inorganic iron, some method is required for handling the atypical frequency distribution of absorption data. The development of the model employed in the test is described in this section. The relationship between percentage absorption of food in inorganic iron was initially assessed for each food by regression analysis. The 95 per cent competence interval for the y-intercept of a least-squares regression line (food plotted against inorganic iron absorption) encompassed the origin in each instance. Scanner diagrams indicate the relationship to be linear over the range of values observed. Subsequent development of the model was, therefore, based on the assumption that food iron absorption is best expressed as a ratio of the value for inorganic iron.

The average ratio of food to inorganic iron for a given substance can be calculated in three ways :(1) a least-squares estimate with the restriction that the line must pass through the origin, (2) mean of the ratios observed with each subject, and (3) the ratio of the mean food iron absorption to the mean inorganic iron absorption. These three estimates are equally correct if no further statistical evaluation of the data is required. However, if one wishes to evaluate the significance of differences observed with each food, it is necessary for the ratio estimate chosen to satisfy the prerequisites of the statistical technic employed. If classic methods are to be used for this purpose, it is necessary to establish that the degree of variation about the ratio estimate is similar for each of the foods. Validity of this assumption was tested for the three estimates listed by a modification of Kruskal-Wallis One-Way Analysis of Variance by Rank.[17] Because of the peculiar frequency distribution of iron absorption measurements, homogeneity of deviation could not be established for any of the models usually employed.

The only model in which proportionality between food and inorganic absorption is retained and in which criteria for common methods of statistical analysis could be satisfied was one in which the logarithm of the ratio of food to inorganic iron were employed. Statistical tests including calculation of means and standard deviations for each food are performed on the logarithmic scale. To express the absorption of a particular food as a per cent of the reference value for inorganic iron, the mean and confidence limits are retransformed by taking antilogarithms.

REFERENCES

1. Chodos, R. B., Ross, J. F., Apt, L., Pollycove, M., and Halkett, J. A. E.: 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, 1957.

2. Moore, C. V., and Dubach, R.: Observations on the absorption of iron from

foods tagged with radioiron. Trans. Ass. Amer. Physicians 64: 245–256, 1951.

3. Hussain, R., Walker, R. B., Layrisse, M., Clark, P., and Finch, C. A.: Nutritive value of food iron. Amer. J. Clin. Nutr. 16: 464–471, 1965.

4. Turnbull, A., Cleton, F., and Finch, C. A.: Iron absorption. IV. The absorption of hemoglobin iron. J. Clin. Invest. 41: 1897–1907, 1962.

5. Callender, S. T., Mallett, B. J., and Smith, M. D.: Absorption of hemoglobin iron. Brit. J. Haemat. 3: 186–192, 1957.

6. Moore, C. V.: Iron nutrition. In F. Gross (Ed.): Iron Metabolism, an International Symposium sponsored by CIBA, July 1963. Berlin, Springer-Verlag, 1964, pp. 241–255.

7. Iron Deficiency Anaemia, report of a study group. World Health Organization Tech. Report Series, Geneva, 182:3–15, 1959.

8. Bainton, D. F., and Finch, C. A.: The diagnosis of iron deficiency anemia. Amer. J. Med. 37:62–70, 1964.

9. Peacock, W. C., Evans, R. D., Irvine, J. W., Jr., Good, W. M., Kip, A. F., Weiss, S., and Gibson, J. G.: The use of two radioactive isotopes of iron in tracer studies of erythrocytes. J. Clin. Invest. 25: 605–615, 1946.

10. Dern, R. J., and Hart, W. L.: Studies with doubly labeled iron. I. Simultaneous liquid scintillation counting of isotopes Fe^{55} and Fe^{59} as ferrous perchlorate. J. Lab. Clin. Med. 57:322–330, 1961.

11. Villegas, L.: Incorporación y translocación del Fe-59 en *Phaseolus vulgaris*. 2do. Simposio Internacional sobre la aplicación de la energía nuclear para fines pacíficos. Buenos Aires, p. 169–172, 1960.

12. Snedecor, G. W., and Cochran, W. G.: Statistical Methods (ed. 6). Ames, Iowa, The Iowa State University Press, 1967.

13. Cook, J. D., Layrisse, M., and Finch, C. A.: The measurement of iron absorption. Blood 33:421, 1968.

14. Kuhn, I. N., Monsen, E. R., Cook, J. D., and Finch, C. A.: Iron absorption in man. J. Lab. Clin. Med. 71:715–721, 1968.

15. Duncan, D. B.: Multiple range and multiple F tests. Biometrics. 11:1–42, 1955.

16. Bothwell, T. H., and Finch, C. A.: Iron Metabolism. Boston, Little, Brown and Company, 1962, p. 98.

17. Kruskal, W. H., and Wallis, W. A.: Use of ranks in one-criterion variance analysis. J. Amer. Stat. Assoc. 260:584, 1952.