0031-3998/89/2603-0250$02.00/0
PEDIATRIC RESEARCH

Vol. 26, No. 3, 1989
Printed in U.S.A.

Iron Absorption from Infant Foods

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ABSTRACT. To determine the bioavailability of iron from iron-fortified infant foods, we have determined erythrocyte incorporation of the stable isotope, ^{58}Fe, after feeding the following foods extrinsically labeled with ^{58}Fe: *1)* rice cereal with apples and bananas ("cereal-fruit product"), *2)* Mead Johnson Enriched Baby Food (MJEBF), a vitamin, mineral, and protein-enriched rice cereal, *3)* vegetables and beef ("vegetable-beef product"), *4)* grapejuice, and *5)* MJEBF. Foods 1–4 were fortified with ferrous sulfate, and food 5 was fortified with ferrous fumarate. Blood was obtained at ages 140, 168, and 196 d of age, and the test meal was fed under standardized conditions at 154 d of age. Erythrocyte incorporation of the ^{58}Fe label was determined from the increase in the mass isotope ratio, $^{58}Fe/^{57}Fe$, from the baseline value (at 140 d of age) to the follow-up values. The mass isotope ratio was determined by inductively coupled mass spectrometry. Geometric mean total iron incorporation into erythrocytes from the test meal of MJEBF fortified with ferrous sulfate (food 2) was 0.05 mg, and from the vegetable-beef product test meal (food 3) was 0.08 mg. The low value for MJEBF is presumably explained by the low level of iron fortification. The low value for the vegetable-beef product may reflect the presence of inhibitors of iron absorption. Geometric mean erythrocyte incorporations of iron from the test meals with foods 1, 4 and 5 were 0.15, 0.14, and 0.18 mg, respectively. These erythrocyte incorporation values are 20 to 26% of the estimated 0.7 mg requirement for absorbed iron, and therefore seem nutritionally important. (*Pediatr Res* 26: 250–254, 1989)

Abbreviation

MJEBF, Mead Johnson Enriched Baby Food

Iron deficiency in infancy and early childhood results in large measure from failure to meet the needs for absorbed iron—estimated to be 0.7 to 0.8 mg/day (1, 2). Because knowledge of iron intake without knowledge of the bioavailability of the iron is of little value, development of a sound approach to establishing strategies for meeting the infant's needs for absorbed iron requires knowledge of the bioavailability of iron in various infant foods.

The iron of human milk, although believed to be remarkably well absorbed, is insufficient to meet the needs of the infant during the latter part of the first year of life (3–5). The infant therefore requires iron in the form of medicinal iron or foods that provide an adequate form and amount of iron. Both direct (4, 6) and indirect (7–9) evidence indicates that infants fed iron-fortified formulas maintain good iron nutritional status; however, many infants are no longer fed formulas during the latter part of the first year of life and would benefit from foods that provide an adequate form and amount of iron.

Infant foods currently fortified with iron include dry cereals, wet-pack cereal-fruit products, and grape juice. We determined iron absorption from products in these categories. Technical problems prevent fortification of dry cereals with ferrous sulfate or most other iron salts known to be of good bioavailability (10). Currently, these products are fortified with electrolytic iron powder of questionable bioavailability (11). Moreover, cereals contain inhibitors of iron absorption and might not be the most appropriate foods for fortification. To determine whether inhibitors of iron absorption present in infant cereals (fiber and phytate) are likely to be an overriding problem in use of cereals as a vehicle for iron administration, we studied dry cereal fortified with ferrous sulfate. Because absorption of ferrous sulfate iron was found to be quite good, we studied absorption of ferrous fumarate—an iron salt that might be feasible to use for cereal fortification (10).

We also studied absorption of iron from a wet-pack cereal-fruit product fortified with ferrous sulfate, and from a vegetable-meat product fortified with ferrous sulfate.

To avoid the administration of radioisotopes to normal infants, we have used the least abundant stable isotope of iron, ^{58}Fe (natural abundance 0.322 wt %), and have judged relative bioavailability from erythrocyte incorporation of the isotope 14 d after administration. Analyses of blood for ^{58}Fe were carried out by inductively coupled plasma mass spectrometry (12). We have demonstrated the feasibility of using ^{58}Fe for studies of iron absorption from meals (13).

MATERIALS AND METHODS

Normal infants, eight to 12 per feeding group, were given a test meal under standardized conditions at 154 ± 4 d. All observations reported in the text were made within 4 d of the stated ages (140, 154, 168, or 196 d). The test meal contained a precisely weighed quantity of approximately 0.85 mg of ^{58}Fe. Relative bioavailaibility of iron from the test meals was evaluated by determining ^{58}Fe enrichment of erythrocytes 14 and 42 d after consumption of the test meal.

Subjects and feedings. The proposal for the study was reviewed and approved by the University of Iowa Committee on Research Involving Human Subjects. Infants who had been subjects for other studies in our unit were enrolled in the studies described here at 112 d of age. From soon after birth until 112 d of age, the infants had been fed milk-based or isolated soy protein-based infant formulas providing no less than 1.8 mg of iron/100 kcal. From 112 d of age until 4 d after the day on which the test meal

Received February 16, 1989; accepted April 26, 1989.
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Supported by USPHS HD 07578, AM 34964 and RR 59, DK 26678 and by grants from Mead Johnson Company, H.J. Heinz Company, and Beech-Nut Inc.

was fed, the infants were fed a milk-based formula (Enfamil, Mead Johnson Company) providing approximately 0.3 mg of iron/100 kcal. Thereafter, the infants were fed a milk-based formula (Enfamil with iron) that provided no less than 1.8 mg of iron/100 kcal.

Beikost (foods other than milk or formula) was not fed before 140 d of age. At 140 d of age, a food similar to that scheduled for use as the test meal was introduced and fed at least once daily. Then 4 d after consuming the test meal, the infants were permitted to receive other commercially prepared strained infant foods.

Test meals. The composition of the five test meals is presented in Table 1. The various foods are referred to as follows: food 1, cereal-fruit product; food 2 with ferrous sulfate, MJEBF fed with ferrous sulfate; food 3, the vegetable-beef product; food 4, grape juice; food 5 with ferrous fumarate, MJEBF fed with ferrous fumarate. At 154 d of age each infant visited the Lora N. Thomas Infant Metabolism Unit and was given the test meal midmorning, approximately 2 h after and 2 h before a formula feeding. Each test meal contained a precisely weighed amount (about 1 g) of a solution of ^{58}Fe-enriched ferrous sulfate, or, in the case of MJEBF fed with ferrous fumarate (food 5), a precisely weighed amount (about 14 mg) of a powder providing ^{58}Fe-enriched ferrous fumarate. Two solutions of the ^{58}Fe-enriched ferrous sulfate were used, one providing 0.998 and the other providing 1.016 mg of iron/mL. The iron was 85.03 wt% ^{58}Fe. The amount of iron added to the test meals from the solutions ranged from 0.934 to 1.116 mg. The amount of iron added with the ferrous fumarate powder ranged from 4.177 to 5.612 mg.

The cereal-fruit product (food 1) was similar to a commercially available wet-pack (*i.e.* marketed in jars) cereal with apples and bananas. As marketed, it provides (label claim) 5.3 mg of iron and 12 mg of ascorbic acid/100 g. It was specially produced for us with 3.6 mg of iron/100 g. The product was fed ad libitum from 140 to 154 d of age. At age 154 d, 1 g of a solution of ^{58}Fe-enriched ferrous sulfate was mixed with 50 g of the food immediately before it was fed. The ferrous sulfate solution provided 1 mg of iron and 0.85 mg of ^{58}Fe. The test meal provided 2.80 mg of iron (Table 1) and 6 mg (label claim) of ascorbic acid.

The MJEBF (food 2 with ferrous sulfate) was a vitamin, mineral, and protein-fortified rice cereal in dry form, supplied to us in 2.5 oz containers. It required only the addition of water before feeding. As marketed, it provided (label claim) 28 mg of ascorbic acid and 27 mg of iron (91% electrolytic iron powder and 9% ferrous sulfate)/100 g. It was produced for use in our test meals without added iron, and, after dilution with water, provided less than 0.128 mg of iron/100 g. Beginning at 140 d of age, approximately 30 cm^3 (one scoop) of the commercial product was mixed with an equal volume of water and offered to the infant. Most of the infants consumed less than 50 g of the product as fed. At age 154 d, 8 g of the product prepared without the addition of iron was diluted with 32 g of water. To this mixture, we added 1 g of a solution of ^{58}Fe-enriched ferrous sulfate, which provided 1 mg of iron, including 0.85 mg of ^{58}Fe. The 40 g test meal provided 1.07 mg of iron and 5 mg (label claim) of ascorbic acid (Table 1).

Table 1. *Test meals*

Food	Description*	Test meal: Amount (g)	Test meal: Iron† (mg)	Test meal: Ascorbic acid‡ (mg)
1	Rice cereal with apples and bananas (cereal-fruit product)§	50	2.8	6
2	Rice cereal with formula (MJEBF with ferrous sulfate)	40	1.1	5
3	Vegetables and beef (vegetable-beef product)	50	3.2	
4	Grapejuice (grapejuice)	86	2.8	27
5	Rice cereal with formula (MJEBF with ferrous fumarate)	30	4.6	4

* Foods 1 and 3 were supplied by the H. J. Heinz Co., Pittsburgh, PA; foods 2 and 5 were supplied by Mead Johnson Company, Evansville, IN, and food 4 was supplied by Beech-Nut, Inc., Canajoharie, NY.

† The iron in the test meal included intrinsic iron, fortification iron, if any, and a precisely weighed quantity of ^{58}Fe-enriched ferrous sulfate (foods 1–4) or ferrous fumarate (food 5); mean values are labeled.

‡ Values for ascorbic acid are label claims.

§ Designations in parentheses are used in text.

The vegetable-beef product (food 3) was a wet-pack strained vegetable and beef product similar to the commercially available product except that it was fortified with ferrous sulfate to provide 4.0 mg of iron/100 g. The product was not fortified with ascorbic acid. It will be referred to as the "vegetable-beef product." It was fed *ad libitum* from 140 to 154 d of age. At age 154 d, 1 g of a solution of ^{58}Fe-enriched ferrous sulfate was mixed with 50 g of the product immediately before it was fed. The ferrous sulfate solution provided 1 mg of iron, including 0.85 mg of ^{58}Fe. The test meal provided 3.22 mg of iron (Table 1).

The grape juice (food 4) was a commercially available product that supplies 2.1 mg of iron (from ferrous sulfate) and 31 mg of ascorbic acid/100 mL. The juice was fed *ad libitum* beginning at 140 d of age. At age 154 d, 1 g of a solution of ^{58}Fe-enriched ferrous sulfate providing 1 mg of iron, including 0.85 mg of ^{58}Fe, was added to 30 mL of grape juice in a feeding bottle and fed to the infant. An additional 30 mL of the juice was added to the bottle, shaken well, and fed to the infant, and a final 26 mL of juice was added to the bottle, shaken, and fed. The entire test meal (86 mL of juice with the added ferrous sulfate) provided 2.86 mg of iron and 27 mg (label claim) of ascorbic acid (Table 1).

MJEBF (food 5 with ferrous fumarate) was the same as food 2 except for the different iron salt fed with the test meal. The commercial product was fed *ad libitum* beginning at 140 d of age. At age 154 d, about 14.0 mg of ^{58}Fe-enriched ferrous fumarate powder providing 0.3263 mg of iron/mg, including 0.05961 mg of ^{58}Fe, was added to 6 g of the dry cereal product prepared for our study and then mixed with 24 g of water. The test meal provided 4.56 mg of iron, including 0.826 mg of ^{58}Fe, and 4 mg (label claim) of ascorbic acid (Table 1).

The ^{58}Fe-ferrous fumarate was prepared by one of us (D.G.G.) as follows: to an 0.5M ^{58}Fe-ferrous sulfate (80 wt % ^{58}Fe) solution in 2 N sulfuric acid was added an amount of unenriched ferrous sulfate sufficient to achieve approximately a 5 to 1 ratio of ^{56}Fe to ^{58}Fe. The resulting solution was adjusted to pH 2.2 and heated. A hot 1.1 M disodium fumarate solution was added to achieve a 1.5 to 1 molar ratio of fumarate to iron. The solution was mixed at 94°C, pH 3.5–4.5, until ferrous fumarate precipitated as a dark orange "rust." Mixing was continued for 20 h at room temperature. The precipitate was washed three times with distilled water and dried in vacuum to constant weight. All of the sulfate ion was recovered in the pooled supernatant and washings. The final product was 94.2% ferrous fumarate.

Although we realized that interpretation of the results would be aided by providing closely similar amounts of iron in each of the test meals, several practical considerations led us to vary the intakes. In the case of MJEBF when fed with ferrous sulfate (food 2), our major question was whether inhibitors of iron absorption in infant cereals are so effective in preventing iron absorption that cereal should not be pursued as a vehicle for iron fortification. Because we were uncertain about the extent of ^{58}Fe enrichment that might be achieved in the erythrocytes, and because percentage absorption of iron decreases with increasing intakes (14), we elected to study the food fortified only with the enriched ^{58}Fe-ferrous sulfate. Thus, iron intake from the food 2

Table 2. *Erythrocyte incorporation of iron from test meals*

	140 days				168 days				196 days				Erythrocyte incorporation*	
Subject	Wt (g)	Hb (g/dL)	Ferritin (ng/mL)	Mass isotope ratio	Wt (g)	Hb (g/dL)	Ferritin (ng/mL)	Mass isotope ratio	Wt (g)	Hb (g/dL)	Ferritin (ng/mL)	Mass isotope ratio	% of Label	Total (μg)†
Food 1														
3688	6370	10.5	20	0.1473	6830	10.3		0.1547	7300	10.6		0.1533	2.7	77
3690	7010	10.7		0.1483	7455	12.0	30	0.1627	8050	12.0	26	0.1606	6.6	191
3692	6025	10.8		0.1500	6510	11.2	50	0.1571	7000	11.4	36	0.1585	3.2	92
3693	5675	11.8		0.1478	5755	12.2	130	0.1561					3.2	92
3718	5750	12.1		0.1455	6360	11.7	90	0.1544	6710	12.3	30	0.1552	3.9	113
3720	8550	11.7	94	0.1501	8910	12.2	84	0.1561	9875	10.5	55	0.1572	4.2	116
3740	8000	10.7	29	0.1495	8660	12.2	28	0.1720	8920	11.8		0.1686	12.2	344
3741	6565	9.9	31	0.1503	6800	10.6		0.1706	7500	11.8		0.1694	8.2	237
3744	7365	10.8	13	0.1493	7745			0.1659	8225	10.4	46	0.1731	11.4	321
3748	8000			0.1459	8110				8660	12.7		0.1706	15.4	436
3772	7150	11.6	61	0.1473	7565	12.1	80	0.1547	7945	11.9		0.1533	3.4	98
3775	5945	12.7	73	0.1490	6545	12.8	82	0.1533	6775	14.0	52	0.1574	3.3	92
Mean	6867	11.2	46	0.1484	7270	11.7	72	0.1598	7905	11.8	41	0.1616	5.4	153
SD	968	0.8	30	0.0016	968	0.8	34	0.0068	983	1.1	12	0.0074	2.9	83
													10.0	283
Food 2														
3700	6735	11.4	40	0.1471	7230	11.7	35	0.1532	7705	11.7	26	0.1564	3.7	41
3701	7000	11.9	83	0.1518	7445	11.8	100	0.1535	7715	10.3	113	0.1527	0.6	6
3702	6900	11.3	53	0.1472	7315	11.8	41	0.1676	7620				9.2	107
3725	8000	11.6	102	0.1479	8350	12.7		0.1580	8780	12.1	33	0.1646	7.9	86
3726	8075	11.8	138	0.1461	8425	12.0	138	0.1594	8975	11.4	190	0.1574	6.7	77
3727	7590	11.0	44	0.1486	8175	11.4	51	0.1554	8735	11.0	38	0.1522	2.6	29
3799	6355	11.3		0.1462	6775	11.6	16	0.1565	7275	11.2	18	0.1555	4.1	48
3862	7245	12.1	13	0.1502	8015	12.8	14	0.1810	8485	12.1	22	0.1884	19.0	211
3863	6390				6875	11.4	86	0.1509	7500	11.3	35	0.1525	3.0	34
Mean	7143	11.5	68	0.1481	7623	11.9	60	0.1595	8088	11.4	59	0.1600	4.4	50
SD	637	0.4	43	0.0020	631	0.5	44	0.0094	647	0.6	61	0.0122	1.6	19
													11.9	134
Food 3														
3523	7375	12.0	65	0.1460	7910	12.4	64	0.1505	8035	11.9	110	0.1510	2.5	82
3803	7160	9.8	62	0.1464	7750	10.7		0.1509	8060	11.2	50	0.1517	2.6	81
3804	6995	13.4	90	0.1468	7285	12.7	100	0.1456	7640	12.9	87	0.1494	0.4	13
3806	7775	12.7	41	0.1469	8195	12.8	45	0.1545	8615	12.9	50	0.1548	4.7	152
3807	6765	13.4		0.1473	7485	14.1	46	0.1533	7765				3.6	116
3865	6660	11.5		0.1462	6975	12.1	60	0.1532	7305	11.0	48	0.1542	3.5	112
3866	6775	11.3	70	0.1467	6880	11.0	57	0.1546	7575	11.2	80	0.1541	3.3	109
3867	7825	11.1	90	0.1466	8260	10.6	71	0.1541	8795				3.6	118
3868	7525	12.3	145	0.1470	8010	12.0	165	0.1481	8370	12.4		0.1495	1.0	33
3869	6630				7355	11.9	46	0.1551	7240	11.3		0.1545	4.2	135
Mean	7149	11.9	80	0.1467	7611	12.0	73	0.1520	7940	11.8	71	0.1524	2.5	80
SD	455	1.2	33	0.0004	490	1.1	39	0.0032	531	0.8	26	0.0023	1.2	37
													5.3	169
Food 4														
3697	6125	11.5	50	0.1491	6455	12.1	39	0.1569	6850	11.4	56	0.1585	3.9	109
3721	6095	11.1	60	0.1500	6725	11.8	62	0.1660	7180	11.1	62	0.1616	6.1	172
3723	6715	11.3	42	0.1510	7575	11.8		0.1551	7650	11.7		0.1522	1.2	35
3749	6945	12.3	56	0.1444	7555	12.3	98	0.1728	7810	11.4	110	0.1643	12.5	357
3750	7865	11.9	18	0.1467	8025	12.2	37	0.1523	8420	12.1	22	0.1492	2.3	65
3827	7345	12.0		0.1497	7700	12.3	34	0.1701	8295	11.1	44	0.1705	11.2	312
3831	7685	10.8	52	0.1456	8000	11.1	46	0.1656	8470	11.5	24	0.1593	8.7	249
3832	8675	12.3	140	0.1504	9505	11.9	28	0.1533	9615	12.4	56	0.1532	1.7	50
3856	7785	11.9	50	0.1502	8310	11.9		0.1708	8580	12.1		0.1714	11.0	320
3859	8155	12.6	250	0.1460	8945	12.6	46	0.1517	9455	12.5	50	0.1545	4.5	129
Mean	7339	11.8	80	0.1483	7880	12.0	49	0.1615	8233	11.7	53	0.1595	4.8	138
SD	857	0.6	72	0.0024	918	0.4	22	0.0084	892	0.5	27	0.0076	2.1	60
													11.1	316

Table 2. *Continued*

Subject	140 days Wt (g)	Hb (g/dL)	Ferritin (ng/mL)	Mass isotope ratio	168 days Wt (g)	Hb (g/dL)	Ferritin (ng/mL)	Mass isotope ratio	196 days Wt (g)	Hb (g/dL)	Ferritin (ng/mL)	Mass isotope ratio	Erythrocyte incorporation* % of Label	Total (μg)†
Food 5														
3808	5760	11.1		0.1473	6135	11.7	39	0.1546	6490	12.2		0.1548	3.4	152
3810	6025	12.1		0.1470	6315			0.1522	6655	12.6	64	0.1524	2.9	124
3873	7805	11.8	50	0.1480	8290	11.7		0.1608	8725	12.6	29	0.1604	7.8	353
3874	8410	11.6	125	0.1474	8650	12.1	125	0.1522	9325	12.0	114	0.1525	2.6	146
3875	8270	13.5	111	0.1477	8950	12.4		0.1526	9110	12.4	84	0.1530	3.4	155
3877	6690	12.1	61	0.1478	7045	12.5	50	0.1595	7190	12.0	74	0.1594	6.4	276
3879	6185	11.8		0.1485	6560	11.0		0.1642	6885	11.4	70	0.1647	7.3	325
3883	7550	11.7		0.1480	7660	11.6		0.1515	8075	12.2	110	0.1517	2.1	92
3898	5910	12.4		0.1476	6405	12.2	74	0.1563	7345	12.0	31	0.1561	4.4	194
Mean	6956	12.0	87	0.1477	7334	11.9	72	0.1560	7756	12.2	72	0.1561	4.0	184
SD	1059	0.7	37	0.0004	1083	0.5	38	0.0045	1084	0.4	32	0.0045	2.5	116
													6.5	290

* Means for erythrocyte incorporation are geometric means with ± 1 SD.

† Calculated on the assumption that the percentage incorporation of total iron was identical to the percentage incorporation of ^{58}Fe label.

test meal was less than from the other test meals. Results of our studies with the cereal-fruit product (food 1) and MJEBF with ferrous sulfate (food 2) were available before we studied MJEBF with ferrous fumarate (food 5). However, the concentration of ferrous fumarate in food 5 was determined by the practical consideration that we wished to add at least 0.8 mg of ^{58}Fe to assure a sufficient enrichment of erythrocytes for measurement. This resulted in a larger quantity of iron in the test meal with food 5 than with the other foods.

Procedures. With the exceptions already noted, the infants were managed as described previously (15). Venous blood was obtained at ages 140, 168, and 196 d.

Laboratory analyses. Aliquots of the foods were placed in porcelain crucibles, dried at 100°C, and ashed for 12 h at 525°C. The ash was dissolved in nitric acid and the iron concentration was determined by atomic absorption spectrophometry using a Perkin-Elmer model 560 (Perkin-Elmer Corp., Eden Prairie, MN).

Hb concentration was determined on a Coulter Counter model M430 instrument (Coulter Electronics, Inc., Hialeah, FL). Serum ferritin concentration was determined by radioimmunoassay (catalog no. D-4401, Micromedic Systems, Inc., Horsham, PA). The ^{58}Fe/^{57}Fe mass isotope ratio ($MIR_{58/57}$) was measured by ICP/MS as described previously (12, 15).

Calculation of quantity of administered ^{58}Fe label incorporated into erythrocytes. The quantity of administered ^{58}Fe label incorporated into erythrocytes ($^{58}Fe_{inc}$) at a specified time t after administration of the dose of ^{58}Fe was calculated as follows:

$$^{58}Fe_{inc} = \frac{MIR^{t}_{58/57} - MIR^{o}_{58/57}}{MIR^{o}_{58/57}} \times Fe_{circ} \times 0.00322 \qquad 1)$$

where $^{58}Fe_{inc}$ is expressed in mg, $MIR^{t}_{58/57}$ is the determined $MIR_{58/57}$ at time t after dosing, $MIR^{o}_{58/57}$ is determined baseline ratio. Fe_{circ} is the quantity of total circulating iron (mg) at time t, and 0.00322 is the natural abundance (wt fraction) of ^{58}Fe.

The quantity of total circulating iron (Fe_{circ}, expressed in mg) was estimated as follows:

$$Fe_{cir} = BV \times Hb \times 3.47 \qquad 2)$$

where BV is blood volume in mL, assumed to be 65 mL/kg of body wt, Hb is Hb concentration in g/mL, and 3.47 is the concentration of iron in Hb (mg/g).

$^{58}Fe_{inc}$ was expressed as a percentage of the administered dose of ^{58}Fe. Total erythrocyte iron incorporation was calculated from the percentage of the administered ^{58}Fe. incorporated times the total amount of iron in the test meal.

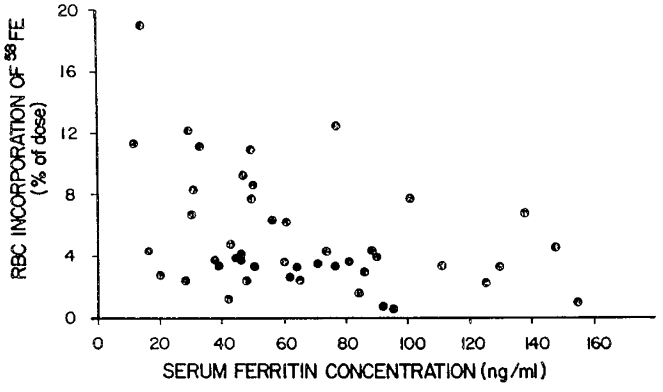

Fig. 1. Erythrocyte (RBC) incorporation of ^{58}Fe (percent of dose) in relation to serum concentration of ferritin (average value for ages 140 and 168 days—see text). Each symbol indicates the RBC incorporation by one infant.

RESULTS

Table 2 presents body wt concentrations of Hb and ferritin, and $MIR_{58/57}$ for each of the 50 infants at ages 140, 168, and 196 d. Also included are the calculated values for percentage of the administered label incorporated into erythrocytes and the calculated total quantity of iron from the meal incorporated into erythrocytes, assuming that percentage erythrocyte incorporation of the ^{58}Fe label is the same as that of the total iron of the meal.

The values for percentage of administered label incorporated into erythrocytes and for total iron incorporation into erythrocytes are the averages of the results of the calculations for 168 and 196 d. The geometric mean values for percent of administered label incorporated into erythrocytes ranged from 2.5% for the vegetable-beef product (food 3) to 5.4% for the cereal-fruit product (food 1).

The ^{58}Fe label contributed most of the iron in MJEBF fed with ferrous sulfate (food 2), and 20 to 35% of the iron in the other foods. However, in studies of absorption of fortification iron, the percentage of the added iron accounted for by the label does not influence the results.

As is evident from Figure 1, erythrocyte incorporation of iron

(as percent of dose) was inversely related to serum concentration of ferritin ($r = -0.337$, $p < 0.03$). In most instances, the values for ferritin in Figure 1 are the averages of the concentration at 140 and 168 d; when values were not available for both ages, the value at 140 or 168 d was used. Subjects for whom neither value was available are not represented in Figure 1. The difference in erythrocyte incorporation of iron from the various foods was not statistically significant (analysis of covariance with serum ferritin as covariate, $p = 0.3$).

COMMENT

A number of studies of iron absorption from infant foods have been carried out with the radioisotope, ^{59}Fe. Absorption of iron naturally present in human milk has been studied in adults (16–18) and infants (19) by use of ^{59}Fe as an extrinsic tag and subsequent whole body counting. Although the results have generally been interpreted (20–22) as indicating that about 50% of the iron is absorbed, validation of an extrinsic tag for human milk is lacking, and absorption may be much less than 50%.

Iron retention from iron-fortified milks and formulas is influenced by the size of the feeding, the quantity of iron in the feeding (with greater amounts fed, percentage absorption is less), the quantity of ascorbic acid in the feeding and the iron status of the infant. The studies that seem most relevant to usual feeding circumstances are those of Rios *et al.* (23) and Stekel *et al.* (24). In each case, the extent of iron absorption was judged by the radioactivity of the erythrocytes 14 d after consumption of the labeled test meal. With feedings that provided 1.4 to 2.6 mg of iron (from ferrous sulfate) and approximately 6 mg of ascorbic acid in 120 mL of formula, Rios *et al.* (23) reported geometric mean absorption by 4- to 7-mo-old normal infants to be 4.2% of the dose.

Stekel et al. (24) studied 5- to 18-mo-old infants, including many with iron deficiency, fed 1.5 to 2.4 mg of iron (from ferrous sulfate) in a test meal of approximately 120 mL. Geometric mean absorptions of iron from six milks or formulas without added ascorbic acid ranged from 2.9 to 5.1% of the dose. Geometric mean absorptions of iron from five ascorbic acid-containing formulas (approximately 4 to 12 mg of ascorbic acid per test meal) ranged from 5.9 to 11.3% of the dose. The greater absorptions reported by Stekel *et al.* (24) than by Rios *et al.* (23) probably reflect the difference in iron nutritional status of the study groups.

Human studies of iron absorption from beikost (foods other than milk or formula fed to infants) have generally been carried out with adult volunteers. Reports of such studies have been reviewed by Cook and Bothwell (20). Few studies of infants have been reported. Schulz and Smith (25), using radioiron balance, reported a mean value of 9.1% for absorption of iron from mixed cereal fortified with sodium iron pyrophosphate; however, this value was later questioned (23). Rios *et al.* (23) determined radioiron absorption from mixed-grain infant cereals by whole body counting of infants 10 d after administration of the last of five daily test meals labeled with ^{59}Fe. The subjects were "4 to 6 mo of age" (apparently more than 4 mo and not yet 7 mo of age). Geometric mean absorption was 0.7% for ferric orthophosphate (four infants), 1.0% for sodium iron pyrophosphate (nine infants), 2.7% for ferrous sulfate (25 infants), and 4.0% for electrolytic iron powder (12 infants). The electrolytic iron powder was of considerably smaller particle size than that used in commercial fortification of infant cereals (11, 23). Iron absorption from other infant foods has not been reported.

Geometric mean total iron incorporation into erythrocytes from the test meal of MJEBF fortified with ferrous sulfate (food 2 with ferrous sulfate) was 0.05 mg, and from the vegetable-beef product test meal (food 3) was 0.08 mg. The low value for MJEBF is presumably explained by the low level of iron fortification. The low value for the vegetable-beef product may reflect the presence of inhibitors of iron absorption. There was certainly no suggestion that the presence of beef enhanced iron absorption. However, the product provided only about 5% of solids from beef. We plan in future studies to determine erythrocyte incorporation of ferrous sulfate added to a meat and vegetable product that provides a greater amount of meat. Iron absorption from the iron-fortified grapejuice (food 4) may have been enhanced by the favorable ratio of ascorbic acid to iron.

Geometric mean erythrocyte incorporation of iron from three of the foods (the cereal-fruit product, grape juice, and MJEBF with ferrous fumarate—foods 1, 4, and 5, respectively) ranged from 0.14 to 0.18 mg. These foods and others that may promote as good or better retention of iron seem worthy of further study. Assuming a requirement for absorbed iron of 0.7 mg/day, erythrocyte incorporation of 0.14 mg amounts to 20% of the requirement for absorbed iron, and 0.18 mg/day amounts to 26% of the requirement for absorbed iron. These erythrocyte incorporations from meals of quite modest size thus appear to be nutritionally meaningful, particularly because erythrocyte incorporation is unlikely to account for all of the retained iron.

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