Absorption of iron from maize (*Zea mays* L.) and soya beans (*Glycine hispida* Max.) in Jamaican infants

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1. The absorption of iron from ^{59}Fe-labelled maize and soya-bean preparations was measured by whole-body counting in forty-two apparently healthy Jamaican infants and compared with the absorption of ferrous ascorbate.

2. The mean absorption of Fe from maize was 4·3% and from soya beans baked at 300°, 9·4%, compared with 28·5% for ferrous ascorbate. In a group of children given boiled soya beans the mean absorption of Fe was 2·8%, and of ferrous ascorbate 16·7%.

3. There was much variability between replicate tests made on the same child at intervals of 1–2 weeks.

4. The absorption of food Fe was not increased in children who were considered to be anaemic (haemoglobin less than 100 g/l) or Fe-deficient (serum Fe less than 500 μg/l and saturation of total Fe-binding capacity less than 15%).

5. The poor availability of Fe in maize meal, which is a staple food of children in Jamaica, is probably an important cause of the high prevalence of Fe-deficiency anaemia.

No systematic investigation of the prevalence of anaemia in Jamaican infants and young children has yet been reported, but the preliminary results of a longitudinal study of 300 babies born at the University Hospital, Kingston, show that by 12 months of age 24% had haemoglobin (Hb) concentrations less than 100 g/l, and by 18 months the proportion had reached 46% (S. A. Grantham-McGregor, personal communication). In rural Jamaica, Ashcroft, Milner & Wood (1969) found that in a sample of 100 infants aged from 3 to 11 months, 23% had Hb concentrations less than 100 g/l. The general impression from clinical experience is that some degree of anaemia is quite common in infants and young children here, mainly as a result of an inadequate intake of iron, although folic-acid deficiency is seen in some infants (MacIver & Back 1960).

In Jamaica, maize meal is the staple food of the weaning diet and mixed feeding is usually not fully established before the age of 3 years (Fox, Campbell & Morris, 1968). As a result, the consumption of maize-meal porridge tends to be high for a considerable period of time, extending throughout early childhood. Studies have shown that in adults only about 5% of the Fe in maize is absorbed (Layrisse, Martinez-Torres & Roche, 1968; Layrisse, Cook, Martinez, Roche, Kuhn, Walker & Finch 1969).

Much interest is at present being focused on the use of soya beans in the formulation of high-protein vegetable mixtures for the supplementary feeding of infants and children in developing countries. Besides being rich in protein, soya beans have a high Fe content (about 110 mg/kg compared with only 25 mg/kg in maize). In adults the availability of soya-bean Fe was found to be better than that from any other vegetable source (Layrisse *et al.* 1969).

In the absorption studies reported here we selected maize meal and soya-bean preparations as the test foods to determine (*a*) whether the Fe in maize is poorly absorbed by infants, as has been demonstrated in adults, and (*b*) whether soya-bean flour would be a useful source of Fe in the feeding of young children.

METHODS

Subjects

Forty-two Kingston children were studied, of whom thirty-nine were supposedly healthy children from two orphanages for abandoned children and three were children who had recovered from malnutrition in the Tropical Metabolism Research Unit. We have no information about the food intakes of individual children in the orphanages. Their ages ranged from 5 months to 2 years although precise ages were not known for abandoned children. Results for three children who had diarrhoea and fever at the time of the tests have been omitted.

Three series of tests were made at different times, with maize meal, boiled soya beans and baked soya beans. For each series a group of children was selected by the matrons of the orphanages. The criteria of selection were that the children should be apparently healthy, and that they should be small enough to be studied in the whole-body counter.

Hb concentration, packed cell volume (PCV), mean corpuscular Hb concentration (MCHC), serum Fe, total Fe-binding capacity (TIBC), unsaturated Fe-binding capacity (UIBC) and the percentage saturation of TIBC were measured for each child by methods previously described (Wood, Milner & Pathak, 1968). Hb electrophoresis was performed to exclude sickle-cell anaemia. These measurements were usually made at the time of the first absorption test. They were not repeated with subsequent tests.

Production of radioactive material

Radioactive maize (*Zea mays* L.) and soya bean (*Glycine hispida* Max.) were grown at the University of Washington, Seattle, Washington, USA. The plants were grown in a nutrient solution to which radioactive Fe (^{59}Fe) was added as described in detail by Layrisse *et al.* (1969).

Preparation of the test meals

Maize. An amount of radioactive maize which contained 0·1 μCi ^{59}Fe was added to unlabelled maize to provide a total of 0·5 mg Fe per test meal. The mixture was boiled with distilled water in an aluminium pan for 15 min and then homogenized in an electric blender so that the consistency was suitable for passing through a nasogastric tube.

Soya beans. In the first series of tests (group 1) with boiled soya beans, the procedure was the same as for maize, but in a second series of tests (group 2) the tracer and carrier soya beans were boiled with a little water and then transferred to an aluminium tray, covered with a little margarine and baked at 300° for 1 h (Layrisse *et al.* 1969). The baked soya-bean porridge was then homogenized as above.

Ferrous ascorbate. Absorption of Fe from the food was compared with that from ferrous ascorbate. Radioactive ferrous ascorbate was prepared by adding a tracer dose of 0·1 μCi $^{59}FeCl_3$ to 5 ml ferrous ascorbate solution containing 0·1 mg Fe/ml (25 mg $FeSO_4.7H_2O$ and 31·5 mg ascorbic acid in 50 ml water). Fresh carrier ferrous ascorbate was made each fortnight and kept in a refrigerator: the tracer dose was added immediately before the absorption test.

Measurement of Fe absorbed

Any oral dosing with supplementary Fe was stopped at least 4 d before the study began. All absorption studies were carried out after a 5–6 h fast and the children were not fed for a further 3 h after the test was performed. The test doses were given by nasogastric tube and washed through with 10 ml distilled water.

The amount of radioactivity in the child was measured in a 4 pi liquid scintillation counter (Garrow, 1965). On the day when the test was done the counting procedure was as follows:

A, background—counter empty
B, standard—0·1 μCi ^{59}Fe solution
C, child before dosing
D, child approximately 30 min after dosing
E, background—to check that no vomiting had occurred in the counter.

The value C – A represents the counts produced by natural ^{40}K in the child, plus any ^{59}Fe radioactivity remaining if the child had had a previous test. This 'background' count on the child was subtracted from all subsequent counts on the child to give a corrected count.

Whenever a count was made on a child the standard was counted as well, to correct for radioactive decay. No correction was made for self-absorption; in theory a small systematic error is introduced by the fact that at the time of the initial count the radioactivity is concentrated in the stomach and intestinal tract, whereas at the time of the final count it is distributed throughout the body. Because of the high energy of the ^{59}Fe radiation, the error produced in this way is not large and was ignored; to correct for it accurately would be extremely difficult.

The percentage absorption (more strictly, retention) of Fe was calculated from the corrected count on the child 5–7 d after the test feed. In pilot studies, serial measurements were made day by day on children in the ward. It was found that the count fell to a constant level after 4–5 d. Counts were routinely made on children from the orphanages, who were not in-patients, 7 d after the test feed.

The background value for the child was approximately 5500 counts/min, and the net value produced by 0·1 μCi was approximately 50 000 counts/min. Counts were made on the children for two 5 min periods. With these counting conditions the

Table 1. *Percentage absorption of iron by Jamaican infants in duplicate tests performed 2 weeks apart on the same subject*

Source of Fe	Test 1	Test 2
Ferrous ascorbate	9·0	31·1
	46·8	57·2
	25·7	27·7
	9·4	4·0
	42·2	73·4
	59·7	26·1
	23·0	40·3
	52·2	15·0
	4·5	7·1
Maize meal (boiled)	0·5	1·3
	2·8	0·6
	1·5	6·4
	5·7	5·1
Soya beans (baked)	1·1	0·7
	11·5	10·8
	15·1	19·4
	4·9	25·1

radioactivity retained could be estimated with a counting error of ±2% even when it amounted to only 10% of the dose.

We calculated that if the whole test dose had been retained, the total radiation dose averaged over a period of 13 weeks would be approximately 0·05 of the permissible whole-body burden for continuous exposure (International Commission for Radiation Protection, 1960).

Sequence and replication of tests

Since the liquid scintillation counter was not sensitive to ^{55}Fe, the double-isotope method (Brise & Hallberg, 1962) could not be used, and all tests were done with ^{59}Fe. The test food and ferrous ascorbate were compared at intervals of a week, the order being reversed in alternate subjects. In some subjects a third test was done with the same material as that used in the first test, either the labelled food or ferrous ascorbate. This provided two estimates of absorption of the same substance in the same subject; these paired estimates are referred to as duplicate tests, and the average of the two was used for calculating the mean absorption of the group.

RESULTS

Variation of absorption in duplicate tests with the same material

The variation between duplicate tests in the same subject was large (Table 1), and somewhat greater than the range found by Cook, Layrisse & Finch (1969) in the same subject tested on successive days.

Absorption of Fe from maize and soya beans

The haematological values and Fe status of the children tested with boiled maize meal are shown in Table 2, together with the values for Fe absorption. The mean absorption of maize Fe was 4·3% compared with 27·9% for ferrous ascorbate.

Table 2. *Blood picture, serum iron concentration, Fe-binding capacity and percentage absorption of Fe from maize meal and ferrous ascorbate in thirteen Jamaican infants*

(Mean values are given when duplicate tests were performed)

Child	Hb (g/l)	PCV (%)	MCHC (g/l)	Serum Fe (μg/l)	UIBC (mg/l)	TIBC (mg/l)	TIBC Saturation (%)	Absorption of ^{59}Fe(%) From maize	Absorption of ^{59}Fe(%) From ascorbate
DB	126	36	350	1300	2·40	3·70	35	5·6	13·9
MB	126	36	350	710	3·20	3·91	18	4·3	9·1
MR*	101	33	300	500	4·50	5·00	10	7·2	35·0
CW*	81	30	270	210	4·14	4·35	5	9·8	16·7
AR*	71	25	280	140	3·53	3·67	4	8·9	44·7
DC	79	29	270	500	2·68	3·18	15	1·7†	17·4
MS	92	28	320	700	2·44	3·14	22	4·0†	1·7
NS*	127	38	330	370	3·25	3·62	10	5·4†	60·6
ML*	104	32	320	280	2·60	2·88	9	2·5	20·0†
LL*	115	33	340	370	2·40	2·77	13	0·0	52·0†
TD*	107	32	330	280	2·68	2·96	9	0·0	26·7†
MC	111	34	320	540	2·00	2·54	21	2·5	6·7†
DS*	109	32	340	300	3·25	3·55	8	3·9	57·8†
Mean	103	32·1	317	480	3·00	3·48	13·8	4·3	27·9
SEM	5·1	1·0	7·9	84	0·20	0·19	2·4	0·8	5·3

Hb, haemoglobin; PCV, packed cell volume; MCHC, mean corpuscular Hb concentration; UIBC, unsaturated Fe-binding capacity; TIBC, total Fe-binding capacity.
* Fe-deficient.
† Duplicate tests.

The results for children tested with labelled soya-bean preparations are shown in Table 3. In group 1 (boiled soya beans), the mean Fe absorption was only 2·8%. This was considerably lower than the absorption from soya-bean flour reported by Layrisse *et al.* (1969) in adults. In group 2 (baked soya beans), the mean absorption of Fe was 9·4%.

There was also a difference between the two groups in the absorption of ascorbate Fe. In group 1 the mean was 16·9% and in group 2, 29·2%.

Correlation between Fe status and absorption of Fe

The results in Tables 2 and 3 suggest that in the series as a whole there was no evident relationship between the efficiency of Fe absorption and the Hb value, concentration of serum Fe, or percentage saturation of TIBC. When the children are divided into two groups, according to whether the Hb was above or below 100 g/l, absorption of ascorbate Fe was slightly lower in the 'anaemic' group (18·3 ± 4·4; nine children) than in the 'non-anaemic group' (27·1 ± 3·5; twenty-two children), but the difference was not significant. In Table 4 the children are again divided into two groups; 'Fe-deficient' and 'not Fe-deficient'. The criteria of Fe deficiency were that serum Fe was less than 500 μg/l, and the saturation of TIBC less than 15%. The absorption of ascorbate Fe was slightly higher in the Fe-deficient group, but the difference was again not significant.

Table 3. *Blood picture, serum iron concentration, Fe-binding capacity, and percentage absorption of Fe from boiled or baked soya beans and ferrous ascorbate in twenty-six Jamaican infants*

(Mean values are given when duplicate tests were performed)

Group and source of Fe	Child	Hb (g/l)	MCHC (g/l)	Serum Fe (μg/l)	UIBC (mg/l)	TIBC (mg/l)	TIBC Saturation (%)	Absorption of ^{59}Fe (%) From soya	Absorption of ^{59}Fe (%) From ascorbate
1, boiled soya beans	AL	113	300	600	3·71	4·31	13	1·6	18·5
	HD*	111	330	470	4·25	4·75	9	3·2	27·5
	CS*†	111	310	280	5·11	5·39	5	4·5	4·3
	W	109	290	1200	3·81	5·00	23	1·4	34·3
	PW*	102	310	420	3·98	4·40	9	1·5	25·4
	MC†	101	280	850	3·68	4·43	19	2·6	5·8
	AC*	92	280	310	4·75	5·06	6	3·1	12·4
	DM*	82	270	240	4·70	4·94	4	2·8	18·5
	SB*	89	290	200	3·82	4·02	5	4·5	8·0
	T*	73	290	220	4·57	4·79	4	2·6	12·1
	Mean	98	295	479	4·24	4·71	9·7	2·8	16·7
	SEM	4·4	5·6	102	0·16	0·13	2·1	0·3	2·9
2, baked soya beans	DA*	102	300	350	3·31	3·66	9	0·9‡	32·7
	CP	124	340	850	2·45	3·30	25	11·2‡	22·1
	EB	132	330	710	2·92	3·63	19	17·2‡	37·0
	SB	107	310	1050	2·31	3·66	30	15·0‡	27·5
	SW*	119	340	300	2·65	2·95	10	3·6	42·9‡
	CB*	107	320	450	3·35	3·80	11	8·4	31·7‡
	WD	95	310	640	3·11	3·75	17	10·5	33·6‡
	VH*	102	300	350	2·88	3·23	10	1·3	5·8‡
	CS*	114	330	450	3·50	3·95	11	16·7	—
	KH	120	340	620	3·19	3·81	16	4·8	—
	GB*	108	340	270	4·28	4·55	6	8·3	—
	AT*	116	330	420	2·70	3·12	13	3·0	—
	JJ†	115	320	540	4·00	4·54	12	16·2	—
	CB	107	330	810	2·44	3·25	25	1·9	—
	AA	111	330	560	3·69	4·25	13	19·1	—
	AN	125	340	670	3·18	3·85	17	12·0	—
	Mean	113	326	877	3·12	3·70	15·2	9·4	29·2
	SEM	2·4	3·5	335	0·14	0·12	1·7	1·5	3·7

Hb, haemoglobin; MCHC, mean corpuscular Hb concentration; UIBC, unsaturated Fe-binding capacity; TIBC, total Fe-binding capacity.
* Fe-deficient. † Sickle cell trait. ‡ Means of duplicate tests.

DISCUSSION

In many tropical countries malaria is a very important cause of anaemia in infancy and early childhood: this has been clearly shown in studies from the Gambia (McGregor, Williams, Billewicz & Thomson, 1966) and from Tanganyika (Draper, 1960). Other important factors are the frequency of infectious disease and the extent of intestinal parasitism. In Jamaica, however, malaria was eradicated several years ago, intestinal helminths are rare in children under 2 years (Ashcroft *et al.* 1969) and infectious diseases such as measles and whooping cough are probably much less prevalent than in Africa. Yet anaemia in infants is common (Ashcroft *et al.* 1969) and

Table 4. *Absorption of inorganic iron and food Fe in Jamaican infants with a serum Fe value below 500 μg/l and a serum Fe saturation of less than 15% (Fe-deficient) compared with absorption by infants with higher Fe levels (not Fe-deficient)*

(Mean values with their standard errors: figures in parentheses denote number of children)

	'Not Fe-deficient'		'Fe-deficient'	
Source of Fe	Mean	SE	Mean	SE
Maize	3·6	0·6 (5)	4·7	1·2 (8)
Soya beans (baked)	12·0	1·8 (9)	6·0	2·0 (7)
Ferrous ascorbate	19·0	3·3 (12)	27·6	3·7 (20)

the present results show that the majority of apparently healthy children from two orphanages were Fe-deficient by the criteria commonly used.

There is very little information available in Jamaica on actual dietary intakes of Fe in young children. In a random sample survey undertaken more than 10 years ago in two districts, all food eaten during 1 week was weighed and the mean Fe intake of seventy-seven children aged 1–3 years was 5·5 mg/d (unpublished findings of E. K. Cruickshank and H. C. Fox).

From the most recent survey (1963–4) in which dietary intakes of 266 children aged 1–3 years were studied by 24 h recall for 6 d (Fox *et al.* 1968), we have estimated that Fe intakes were of the order of 5 mg/d and that approximately one-quarter of this was derived from maize meal and wheat flour. Even allowing an upper limit for Fe absorption of 15%, as suggested by the World Health Organization (1970) for diets in which 10–25% of the energy is from foods of animal origin, the net absorption of Fe would be only 0·75 mg/d, that is below the World Health Organization (1970) estimated daily requirement of 1 mg absorbed Fe.

The results of the present study indicate that anaemia in Jamaican infants is likely to arise from the very poor absorption of dietary Fe from maize, which is the staple food during early childhood. Of the modest amount of Fe present in maize only 4·3% was available for absorption in these children. This value is similar to the mean values of 5·2 and 5·9% found for adults by Layrisse *et al.* (1968, 1969).

Soya beans are not grown in Jamaica or consumed in any amount, but soya-bean flour is a component of some welfare foods and might be a source of extra Fe for children. In two studies on soya-bean flour, Layrisse *et al.* (1969) and Layrisse & Martinez-Torres (1971) found mean absorptions in adults of 17·9 and 6·9%. In the present work the mean absorption of Fe from baked soya-bean flour was 9·4%, which is in reasonable agreement with the finding of Layrisse and his co-workers.

Although there appeared to be some differences in the percentage absorption of Fe from the different foodstuffs, comparison of the mean values obtained in different groups of children is of doubtful validity because the groups were not exactly matched in all respects other than in the food tested. It is well recognized that there is great variability in the Fe absorption, both between subjects and in the same subject tested on more than one occasion (Brise & Hallberg, 1962; Cook *et al.* 1969). Because of this variability at least one test was done with Fe ascorbate in each child. The theory is that a subject who absorbs Fe ascorbate poorly will also absorb food Fe poorly, and vice versa. Thus the value for the absorption of Fe ascorbate can, it is held, be used as a correction factor for the absorption of food Fe, to make the results from different subjects more closely comparable. In the present study the children who received boiled soya beans, for reasons which are not known, absorbed less Fe ascorbate than those in the other two groups. Therefore the groups are not strictly comparable, and absorption from boiled soya beans may be underestimated. Cook *et al.* (1969) attempted to get round this difficulty by calculating for each subject a ratio, food Fe absorbed: ascorbate Fe absorbed. The ratios were expressed as logarithms for statistical analysis, because this was found to produce a more nearly Gaussian distribution. In the present study, however, this procedure is of no value because when the absorption of food Fe is plotted against that of ascorbate Fe, there is no evident relation between the two. (We are indebted to the Statistical Editor of the British Journal of Nutrition for this observation.) Therefore, the values obtained with Fe ascorbate cannot be used to correct the values obtained with food Fe. It is probable that this method of correction is more useful when the tests are done simultaneously by the double isotope method, as in the work of Cook *et al.* (1969), rather than at intervals of a week, as in our study.

Another difference between the groups is that rather more of the children who received boiled soya beans were Fe-deficient. However, the results summarized in Table 5 suggest that this would not necessarily reduce the absorption.

For these reasons we cannot say with confidence that the apparent differences in Fe absorption between baked soya beans, boiled soya beans and maize are real. Nevertheless, on general grounds it is likely that the amount of Fe absorbed from natural foodstuffs depends not only on the nature of the food, but also on the method of preparation. This needs further investigation. Heat treatment of soya beans has been shown to make the sulphur amino acids more available (Mickelsen & Yang, 1966). Cysteine has also been shown to improve the absorption of dietary Fe (Layrisse & Martinez-Torres, 1971). There is a possibility, therefore, that heat treatment may play a useful part in improving Fe absorption.

One important cause of variability in our subjects was probably infection and fever. Recent work by Beresford, Neale & Brooks (1971) has shown that even a transient fever reduces Fe absorption to very low levels. This effect may be seen even in children who are not actually febrile when tested. Although all children have been excluded from the present study who had fever or diarrhoea at the time of any test, we have no means of knowing whether any had a febrile episode shortly beforehand or afterwards. Conditions in one of the homes were far from ideal and it is possible that in some of the children Fe absorption may have been temporarily impaired as a result of subclinical infection. One child, not included in the present series, illustrates this problem. Fe absorption from boiled soya-bean and ferrous ascorbate was nil in three tests, although she was afebrile throughout. She was only 74% of her expected weight for height, and was therefore admitted to the ward for investigation. The erythrocyte sedimentation rate was raised to 80 mm/h, but no cause for this was found. A bone-marrow examination showed a general absence of Fe stores, but some reticulo-endothelial cells staining positively for Fe, suggesting a disturbance of Fe metabolism superimposed on a basically Fe-deficient marrow. The erythrocyte sedimentation rate gradually fell during the following 6 weeks to 24 mm/h, and at the same time the absorption of ascorbate Fe increased. The prevalence of minor subclinical infections in young children, particularly under institutional conditions, may explain much of the variability found in duplicate tests.

This example illustrates the difficulties which are encountered in comparing the absorption of Fe from different sources when the tests cannot be made simultaneously by the double isotope method.

Our results also suggest that these children face a further handicap. Adults, when Fe-deficient, usually compensate by absorbing more Fe than normal controls (Hussain, Walker, Layrisse, Clark & Finch, 1965; Cook *et al.* 1969; Heinrich, Bartels, Gabbe, Meineke, Nass & Whang, 1969) and this has also been found in children (Schulz & Smith, 1958*a*,*b*). Kimber & Weintraub (1968), however, found that absorption of haemoglobin Fe was impaired in eight children with Fe-deficiency anaemia of predominantly dietary origin, thus reversing the pattern found in a previous study with adults (Kimber, Patterson & Weintraub, 1967). In the present study, although infants considered to be Fe-deficient absorbed more inorganic Fe (Table 5) than normal infants, they did not always absorb more food Fe.

From the results of this work it is not surprising that there should be a high prevalence of Fe-deficiency anaemia in Jamaican children, since much of the food Fe is poorly available, and its absorption is further depressed by frequent minor infections.

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