Factors affecting the absorption of iron from Fe(III)EDTA

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1. The modification of iron absorption from Fe(III)EDTA by agents known to promote or inhibit absorption was examined in 101 volunteer multiparous Indian women. Fe absorption from Fe(III)EDTA was compared with absorption of intrinsic food Fe in a further twenty-eight subjects. Finally the urinary excretion of radio-Fe after oral administration of ^{59}Fe(III)EDTA was studied in twenty-four subjects and evidence of intraluminal exchange of Fe was examined.

2. Fe absorption from maize porridge fortified with Fe(III)EDTA was more than twice that from porridge fortified with $FeSO_4 . 7H_2O$.

3. Although bran decreased Fe absorption from $FeSO_4 . 7H_2O$ approximately 11-fold, it had no significant effect on Fe absorption from Fe(III)EDTA. Nevertheless tea, which is a more potent inhibitor of Fe absorption, decreased absorption from Fe(III)EDTA 7-fold.

4. Fe absorption from Fe(III)EDTA given in water was only increased 40% by addition of 3 mol ascorbic acid/mol Fe but by 7-fold when the relative proportions were increased to 6:1. This enhancing effect was blunted when the Fe(III)EDTA was given with maize porridge. In these circumstances, an ascorbate:iron value of 3:1 (which doubles absorption from $FeSO_4 . 7H_2O$) produced no significant increase in Fe absorption, while a value of 6:1 produced only a 2·5-fold increase.

5. Fe absorption from Fe(III)EDTA was not altered by addition of maize porridge unless ascorbic acid was present.

6. Less than 1% of ^{59}Fe administered as ^{59}Fe(III)EDTA was excreted in the urine and there was an inverse relationship between Fe absorption and the amounts excreted (r 0·58, $P < 0·05$).

7. Isotope exchange between ^{55}Fe(III)EDTA and $^{59}FeSO_4 . 7H_2O$ was demonstrated by finding a similar relative value for the two isotopes in urine and erythrocytes when the two labelled compounds were given together orally. This finding was confirmed by in vitro studies, which showed enhanced ^{59}Fe solubilization from $^{59}FeSO_4 . 7H_2O$ in maize porridge when unlabelled Fe(III)EDTA was added.

8. Although Fe absorption from Fe(III)EDTA was marginally higher it appeared to form a common pool with intrinsic food iron in most studies. It is postulated that the mechanism whereby Fe(III)EDTA forms a common pool with intrinsic food Fe differs from that occurring with simple Fe salts. When Fe is present in the chelated form it remains in solution and is relatively well absorbed because it is protected from inhibitory ligands. Simple Fe salts, however, are not similarly protected and are absorbed as poorly as the intrinsic food Fe.

9. It is concluded that Fe(III)EDTA may be a useful compound for food fortification of cereals because the Fe is well absorbed and utilized for haemoglobin synthesis. The substances in cereals which inhibit absorption of simple Fe salts do not appear to inhibit absorption of Fe from Fe(III)EDTA.

The high incidence of iron deficiency in populations subsisting on cereal-based diets is largely due to the poor absorption of Fe from these foodstuffs. For example, the results of previous studies indicated that the geometric mean absorption of Fe from rice and maize in Fe deficient subjects was less than 5% (Sayers *et al.* 1973; Sayers, Lynch, Charlton & Bothwell, 1974; Sayers, Lynch, Charlton, Bothwell *et al.* 1974; Derman *et al.* 1977). These results underline the need for Fe-fortification programmes in populations subsisting on mainly

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cereal-based diets. Unfortunately, such programmes have not been successful in the past, since the Fe salts used for fortification are as poorly absorbed as the intrinsic Fe present in various cereals (Layrisse *et al.* 1973). This can be ascribed to the fact that the ligands inhibiting the absorption of the food Fe, have the same effect on the added Fe. One way of overcoming this problem has been to add ligands, such as ascorbic acid, which enhance Fe absorption (Sayers *et al.* 1973; Sayers, Lynch, Charlton & Bothwell, 1974; Sayers, Lynch, Charlton, Bothwell *et al.* 1974; Disler, Lynch, Charlton, Bothwell *et al.* 1975). While ascorbic acid has been shown to be effective, it has been difficult to find a suitable vehicle to which to add it, since it is a highly-reactive compound, which is easily oxidized and which sometimes causes unacceptable colour changes in food (Sayers, Lynch, Charlton, Bothwell *et al.* 1974). The results of recent work suggest that an alternative approach may be promising. It has been shown that the Fe present in the Fe(III)EDTA compound is better absorbed in the presence of a meal than is the Fe in other salts, possibly due to the fact that it is less susceptible to inhibitory ligands which form insoluble complexes with Fe (Layrisse & Martinez-Torres, 1977; Martinez-Torres *et al.* 1979).

In the present study further information was obtained on the absorption of Fe from Fe(III)EDTA and on the effects of various foods and of inhibitory and promoting ligands. In addition, information was obtained on the intraluminal mixing of Fe and on the excretion of Fe(III)EDTA in the urine.

EXPERIMENTAL

Subjects

The subjects were 153 multiparous Indian housewives aged between 21 and 71 years (mean 36 years) living in a municipal housing complex at Chatsworth, near Durban. They belong to a low socio-economic group in which Fe deficiency is common (Mayet *et al.* 1972).

Preparation and administration of the meals

Sufficient maize meal to provide 50 g dry maize meal/subject was mixed with a small amount of water to make a paste. This was added to four times its weight of boiling water and cooked at 90° for 20–25 min to make a porridge. When intrinsically-labelled maize (Hussain *et al.* 1965; Layrisse *et al.* 1969) was used, sufficient to provide 3 μCi ^{55}Fe/subject was blended with dry carrier maize to provide 50 g dry maize/subject. In some experiments the meal was fortified with 3 or 5 mg Fe labelled with 3 μCi/subject by mixing the appropriate radio-Fe compound into the paste. In other experiments the porridge was cooked without added Fe and individual servings were subsequently fortified by adding 3 ml portions of solutions containing 3 or 5 mg of Fe either as Fe(III)EDTA in water or as $FeSO_4 . 7H_2O$ in 0·01 M-hydrochloric acid. In each instance the portion contained 3 μCi of the appropriate radio-Fe labelled compound. The final mass of porridge eaten by each subject was 250 g. Tea was made by adding 45 g leaves (Pot o' Gold; O.K. Bazaars Ltd, Johannesburg) to 1800 ml boiling water to provide 200 ml/subject. The tea was sweetened with cane sugar fortified with the appropriate radio-Fe compound. The traditional Indian dhal (Sayers, Lynch, Charlton & Bothwell, 1974) and the fortified sugar (Disler, Lynch, Charlton, Bothwell *et al.* 1975) were prepared as described previously.

In each study the meal was consumed after an overnight fast and no food or drink was allowed for 4 h after the test meal had been eaten. The same procedure was followed the next day, but the meal was labelled with the other Fe isotope. After a period of 2 weeks, blood samples were taken after an overnight fast for the determination of the concentrations of ^{59}Fe and ^{55}Fe, haemoglobin, serum Fe, unsaturated Fe-binding capacity and serum ferritin. Each subject then drank a 'reference Fe salt' consisting of 50 ml of a solution

containing 30 mg ascorbic acid and 3 mg Fe as $^{59}FeSO_4 . 7H_2O$ (3 μCi ^{59}Fe). No food or drink was allowed for the following 4 h period. Samples of blood were again obtained a further 2 weeks later and the ^{59}Fe concentrations determined. The percentage absorption of the Fe from the solution was calculated by difference and provided an index of each individual's absorbing capacity.

Isotopic and chemical methods

Labelled Fe(III)EDTA was prepared by mixing equimolar solutions of disodium EDTA and carrier ferric chloride with a tracer amount of radio-labelled $FeCl_3$. The pH of the solution was then adjusted to pH 5 with sodium bicarbonate. The Fe(III)EDTA complex formed was precipitated by ethanol, the supernatant fraction discarded and the precipitate redissolved in water. The complex was then precipitated again with ethanol and washed three times with ethanol (Sawyer & McKinnie, 1960).

Duplicate blood samples (10 ml) and appropriate standards were prepared for differential radioactivity determination by the method of Katz *et al.* (1964). The quantities of ^{55}Fe and ^{59}Fe in the processed samples were determined using a liquid-scintillation system (Insta-Gel; Tri-Carb AAA Spectrometer model No. 3375; Packard Instrument Co., Downers Grove, Illinois, USA). The ^{59}Fe activity in the 4 ml blood samples collected immediately before the 'reference Fe salt' was administered, and 2 weeks later, was assessed against suitable standards using a liquid-scintillation spectrometer (Auto-Gamma Tri-Carb Spectrometer model No. 3001; Packard Instrument Co.). All values for absorption (%) were calculated on the assumption that 100% of the absorbed radioactivity was present in the haemoglobin of circulating erythrocytes, and that the blood volume of each subject was 65 ml/kg. Although the red cell utilization of radioiron varies according to iron status, it is normally above 80% and in an iron deficient group such as the one investigated in the present study, most values would be expected to be close to 100%. Furthermore, any errors introduced by the assumption would not invalidate the findings, since the design of the absorption experiments was such that each subject served as her own control, with comparisons being made on the basis of paired data and not between different individuals.

When large volumes of urine were prepared for the determination of differential radioactivity using the same method as for blood, a black compound was obtained which was unsuitable for mixing in insta-gel. To overcome this problem, half the volume of urine passed was evaporated to 10–15 ml by gentle boiling and prepared according to the method used for blood samples. The resulting black compound was dissolved in 1 M-HCl and all the Fe was converted to the ferric state by the addition of potassium permanganate solution. The ferric-Fe was complexed with potassium thiocyanate and immediately extracted with diethyl ether. The water phase was found to be free of radioactivity after four to five diethyl ether extractions. The diethyl ether phase was evaporated to dryness and then redigested and prepared for radioactivity determination as described previously.

Serum Fe concentrations were measured by the International Committee for Standardization in Haematology (1978) method and the unsaturated Fe-binding capacity was determined by the method of Herbert *et al.* (1967). The Fe content of digested samples of food was estimated by a modification (Bothwell *et al.* 1979) of the method of Lorber (1927). The serum ferritin concentrations were measured by radioimmunoassay using the method of Deppe *et al.* (1978).

The in vitro experiments were done using a modification of a method described previously (Bezwoda *et al.* 1978). In place of the bread used in the original method, a soft maize porridge was prepared using 70 g dry maize meal/l water. This was fortified with 3 mg Fe in the form of the appropriate radio-Fe compound. Equal weighed portions of the porridge were then incubated in duplicate for 30 min at room temperature in 5 ml HCl solutions

covering the pH range 0·5–6·0. Portions of the supernatant fraction were counted after centrifugation at 3000 *g* and the percentage Fe in solution was calculated.

Ethical considerations

Written consent was obtained from all subjects after the nature of the investigation had been explained to them by an Indian social worker. Before starting the study approval was obtained from the Committee for Research on Human Subjects of the Faculty of Medicine, University of the Witwatersrand, Johannesburg. With regard to radiation exposure, it was calculated that if the entire test dose of radioisotope was retained, the total radiation averaged over 13 weeks would be approximately 20% and 0·2% of the permissible whole-body burden for continuous exposure to ^{59}Fe and ^{55}Fe respectively (International Commission for Radiation Protection, 1960).

Statistical methods

Serum ferritin and Fe absorption values in individual experiments showed considerable variation. All values were therefore logarithmically transformed in order to correct for positive skew. Since all results were expressed as geometric means and standard deviation ranges, standard parametric statistical methods could be used.

RESULTS

Comparison of the absorption of Fe from maize-meal porridge fortified with $FeSO_4 . 7H_2O$ or with Fe(III)EDTA

In order to establish whether the absorption of Fe from $FeSO_4 . 7H_2O$ and Fe(III)EDTA differed, twelve subjects were given $FeSO_2 . 7H_2O$-fortified maize porridge on one morning and a similar Fe(III)EDTA-fortified porridge the following morning. Table 1 shows that the geometric mean Fe absorption (%) from the Fe(III)EDTA fortified meal (7·2) was significantly greater than from the $FeSO_4 . 7H_2O$ fortified meal (3·5) (t 5·5, $P < 0·001$).

The effect of inhibitors on Fe absorption from Fe(III)EDTA

The 2-fold increase in Fe absorption found in the first study suggested that Fe in the Fe(III)EDTA complex is protected from the inhibitors of Fe absorption present in maize porridge. It was therefore decided to determine whether known inhibitors of Fe absorption, such as bran and tea, had any effect on Fe absorption from Fe(III)EDTA.

In order to assess the effect of bran on Fe absorption, 9 subjects drank 100 ml water sweetened with sugar fortified with $FeSO_4 . 7H_2O$ on one morning while on the second morning 10 g bran was mixed with the Fe-fortified water (Table 2). Bran reduced the geometric mean absorption 11-fold, from (%) 16·5 to 1·5 (t 16·5, $P < 0·001$). In a second study, which was carried out in ten subjects, $FeSO_4 . 7H_2O$ was replaced by Fe(III)EDTA. There was no significant difference between the mean geometric absorption from Fe(III)-EDTA without bran (10·3) and with bran (8·4) (t 1·4, $P > 0·1$).

As tea is known to be a potent inhibitor of Fe absorption (Disler, Lynch, Charlton, Torrance *et al.* 1975), a similar study was performed in eight subjects using black tea instead of bran. The geometric mean Fe absorption from Fe(III)EDTA was reduced approximately 7-fold from 19·2 to 2·8, by the addition of tea (t 10·35, $P < 0·001$).

The effect of increasing doses of ascorbic acid on the absorption of Fe from Fe(III)EDTA

As the Fe in Fe(III)EDTA seemed to be protected to some extent from inhibitory ligands, the effect of a known promoter of Fe absorption, namely ascorbic acid, was studied. Two series of three experiments were done to find out whether increasing doses of ascorbic acid improved Fe absorption from Fe(III)EDTA. In the first set of experiments the Fe(III)EDTA

Table 1. *Comparison of absorption of iron from $FeSO_4 . 7H_2O$ and Fe(III)EDTA fortified maize porridge (5 mg Fe, 250 g porridge per subject) given to female subjects on two successive mornings*

(Mean values and standard deviations)

							Fe absorption (%)*					
	Haemoglobin (g/l)		Transferrin saturation (%)		Serum* ferritin (μg/l)		Reference† salt		$FeSO_4 . 7H_2O$ fortified porridge		Fe(III)EDTA fortified porridge	
No. of subjects	Mean	SD	Mean	SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	±1 SD
12	127	26	26·2	11·9	15·3	(5·3–44·2)	35·3	(20·9–59·6)	3·5	(2·5–4·9)	7·2	(3·9–13·2)

* Geometric means and SD ranges used because values were positively skewed.
† 3 mg Fe as ferrous ascorbate given in the fasting state.

Table 2. *The effect of inhibitors on the absorption of iron (5 mg) from $FeSO_4 . 7H_2O$ and Fe(III)EDTA fortified sugar (20 g) given in 100 ml water to female subjects on two successive mornings*

(Mean values and standard deviations)

								Fe absorption (%)*					
			Haemoglobin (g/l)		Transferrin saturation		Serum* ferritin (μg/l)		Reference† salt		Without inhibitor		With inhibitor	
Inhibitor	Fe compound	No. of subjects	Mean	SD	Mean	SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	±1 SD
10 g Bran	$FeSO_4 . 7H_2O$	9	137	9	25·2	8·0	33·0	(13·0–83·6)	22·5	(7·8–65·0)	16·5	(7·4–36·5)	1·5	(0·6–3·8)
10 g Bran	Fe(III)EDTA	10	120	22	22·9	14·1	10·7	(2·6–43·8)	43·3	(19·1–97·7)	10·3	(5·6–19·0)	8·4	(3·2–22·0)
Tea‡	Fe(III)EDTA	8	129	14	28·0	18·8	21·1	(6·3–70·7)	49·2	(27·7–87·5)	19·2	(8·0–45·9)	2·8	(1·3–6·2)

* Geometric means and SD ranges used because values were positively skewed.
† 3 mg Fe as ferrous ascorbate given in fasting state.
‡ 45 g tea leaves added to 1800 ml boiling water to give 200 ml/subject.

was administered in water alone, while in the second set it was administered in maize porridge. In each study the meal was given on one of the days with ascorbic acid and on the other, without ascorbic acid. At the completion of each absorption study, a further one was done in which the absorption of a reference dose of 3 mg Fe as ferrous ascorbate was measured. In this way it was possible not only to express the actual results obtained but also to standardize individual results to a 40% reference absorption, which has been used as a means of comparing the findings in individuals or groups with differing avidities for Fe (Hallberg *et al.* 1978).

When the unstandardized values were analysed (Table 3), it was clear that 25 mg ascorbic acid had no effect on the absorption of Fe from Fe(III)EDTA when administered in water or porridge (t 0·24, $P > 0·1$; t 1·03, $P > 0·1$ respectively). With the 50 mg dose there was a modest, but significant increase (t 2·84, $P < 0·05$) in Fe absorption when Fe(III)EDTA was given in water but none when it was given in porridge (t 0·38, $P > 0·1$). A dose of 100 mg ascorbic acid caused a more than 6-fold increase (t 6·23, $P < 0·01$) in Fe absorption when the Fe(III)EDTA was given in water and a 2-fold increase (t 5·71, $P < 0·01$) when it was given in porridge.

When the standardized absorptions of Fe from Fe(III)EDTA in water were compared with those obtained from Fe(III)EDTA-fortified porridge (Table 3), both in the absence of ascorbic acid, it was apparent that the addition of 250 g maize-meal porridge had little or no effect on the absorption from Fe(III)EDTA (F 1·3, $P > 0·1$). However, when 100 mg ascorbic acid was present, the standardized absorption (%) was significantly reduced from a geometric mean of 44 to 15·6 by the presence of maize porridge (t 1·93, $P < 0·05$).

Comparison between the absorption of intrinsically-labelled cereals and ^{59}Fe(III)EDTA

Three different meals were used to compare the absorption of intrinsically labelled food Fe and Fe(III)EDTA. Two experiments were done using maize poridge as in the previous studies. In the first of these experiments (Table 4) sugar fortified with ^{59}Fe(III)EDTA was sprinkled on the cooked maize porridge intrinsically labelled with ^{55}Fe and the porridge was eaten immediately. The geometric mean absorption from the extrinsic Fe(III)EDTA (%) (12·9) was more than double that from the intrinsic label (4·9) (t 3·74, $P < 0·005$). This suggested that the Fe in the form of Fe(III)EDTA was protected from inhibitory ligands in the maize, while the intrinsic Fe was not. However, since the two labels were not cooked together, the study was repeated and the ^{59}Fe(III)EDTA was added before cooking. When this was done there was no significant difference in the absorption of the intrinsic Fe and the extrinsic Fe administered as Fe(III)EDTA (t 2·05, $P > 0·1$). The observation was confirmed in another similar study (Table 4) in which ^{59}Fe(III)EDTA was added to a traditional intrinsically-labelled pea dhal before cooking. Although the absorption of the Fe in ^{59}Fe(III)EDTA was marginally greater in each subject there was no significant difference between the geometric mean absorptions (t 0·88, $P > 0·1$), and the ratio of the geometric mean absorption from extrinsic Fe to corresponding value for intrinsic food Fe was close to unity.

Excretion of Fe(III)EDTA in the urine

Since EDTA chelates are excreted by the kidney (Will & Vilter, 1954) a study was undertaken to assess whether Fe given orally as Fe(III)EDTA appeared in the urine. Fourteen fasting subjects with normal renal function drank 100 ml water sweetened with 10 mg sugar fortified with 5 mg Fe as ^{59}Fe(III)EDTA. The total urine output for the next 24 h was collected and the percentage of the dose of ^{59}Fe appearing in the urine was compared to that absorbed (Fig. 1). In all instances less than 1% of the administered dose appeared in the urine (geometric mean 0·32%, SD range 0·18–0·60) and serial sampling showed that all the radio-Fe was excreted in the first 24 h. A significant inverse relationship

Table 3. *The effects of increasing amounts of ascorbic acid (AA) on the absorption of iron from Fe(III)EDTA (5 mg Fe) given in either 100 ml water (A) or 250 g maize porridge (B) to fasting female subjects*

(Mean values and standard deviations)

No. of subjects	AA in meal (mg)	Feeding regimen	Fe:AA molar ratio	Haemoglobin (g/l)		Transferrin saturation (%)		Serum* ferritin (μg/l)		Fe absorption (%)*							
										Actual absorption				Reference† salt		Standardized to 40% reference absorption	
										No AA		AA				No AA	AA
				Mean	SD	Mean	SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	Geometric mean
11	25	A	1:1·5	134	20	24	14	25	(7–85)	6·4	(3·7–11·2)	6·6	(3·0–14·8)	37·4	(17·7–79·1)	7·2	8·0
10	50	A	1:3	133	12	36	23	31	(10–94)	7·7	(3·5–17·0)	11·0	(5·2–23·3)	26·8	(15·8–45·5)	9·8	16·8
9	100	A	1:6	134	26	25	15	14	(6–35)	7·0	(4·1–11·8)	47·7	(16·7–136·4)	41·5	(22·8–75·5)	6·8	44·0
12	25	B	1:1·5	142	12	26	14	19	(6–59)	6·4	(3·1–13·5)	6·9	(2·8–17·0)	25·4	(14·0–46·0)	8·5	8·9
11	50	B	1:3	137	23	28	11	33	(11–103)	6·3	(2·9–13·4)	6·0	(2·5–14·7)	20·4	(6·4–65·0)	12·0	12·0
9	100	B	1:6	138	13	29	11	21	(6–71)	6·1	(2·3–16·1)	12·0	(2·8–13·5)	30·5	(15·0–62·1)	8·0	15·6

* Geometric means and SD ranges used because values were positively skewed.

† 3 mg Fe as ferrous ascorbate (molar ratio for Fe and AA of 1:3) given in fasting state.

Table 4. *The absorption of Fe from intrinsically-labelled food iron (^{55}Fe) and $^{59}Fe(III)EDTA$ (3 mg Fe) given in the same meal to fasting female subjects*

(Mean values and standard deviations)

	No. of subjects	Total Fe (mg)	Haemoglobin (g/l)		Transferrin saturation (%)		Serum* ferritin (μg/l)		Fe absorption (%)*						
									Reference† salt		Intrinsic label (^{55}Fe)		$^{59}Fe(III)EDTA$ label		
			Mean	SD	Mean	SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Ratio $^{59}Fe/^{55}Fe$
^{55}Fe Maize porridge (250 g) $^{59}Fe(III)EDTA$ sugar (20 g)	10	6·5	131	13	24·1	13·7	19·7	(6·8–56·9)	23·8	(12·8–44·3)	4·9	(2·1–11·7)	12·9	(5·6–29·7)	2·6
^{55}Fe Maize porridge (250 g) Cooked with $^{59}Fe(III)EDTA$	9	7·3	148	12	25·3	8·7	25·3	(8·1–79·0)	—	—	5·5	(1·5–20·5)	8·1	(3·5–18·6)	1·2
^{55}Fe Dhal cooked (200 ml) with $^{59}Fe(III)EDTA$	9	6·4	142	11	25·5	9·3	25·9	(16·5–40·6)	27·3	(12·1–61·4)	5·2	(2·3–12·0)	5·4	(2·1–13·9)	1·04

* Geometric means and SD ranges used because values were positively skewed.

† 3 mg Fe as ferrous ascorbate given in fasting state.

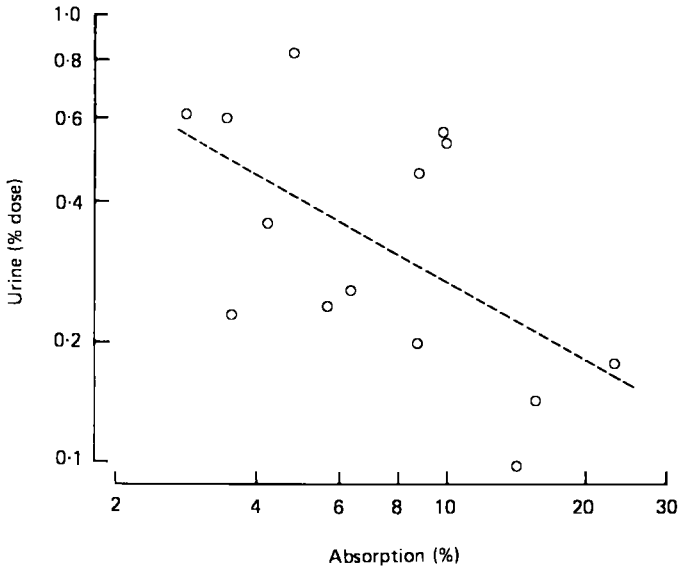

Fig. 1. The relationship between the excretion of ^{59}Fe in the urine collected over 24 h (% dose) and the absorption of ^{59}Fe from 3 mg Fe as ^{59}Fe(III)EDTA given in water to fourteen subjects after an overnight fast (r −0·58, $P < 0·05$).

was noted between the logarithm of the percentage of the dose absorbed and that excreted in the urine (r −0·58, $P < 0·05$).

The appearance of radio-Fe in the urine provided a means whereby intraluminal exchange of Fe could be studied. A similar study was therefore carried out in ten subjects using two isotopes of Fe. Equimolar quantities of ^{55}Fe(III)EDTA and $^{59}FeSO_4 . 7H_2O$ were mixed into a 250 g maize porridge after cooking. The mean for absorption of ^{55}Fe: absorption of ^{59}Fe from the meal was 1·22 (±0·11) which was similar to the value of 1·28 (±0·08) found in the urine (t 2·19, $P > 0·05$). Since these results suggested that there was some exchange between the isotopes an in vitro experiment was performed using a modification of a method described by Bezwoda *et al.* (1978) in order to try and verify the point. The solubilization of ^{59}Fe from portions of cooked maize meal, fortified with $^{59}FeSO_4 . 7H_2O$ or ^{59}Fe(III)EDTA, at various pH values is shown in Fig. 2. The percentage ^{59}Fe appearing in the supernatant fraction at pH values greater than those normally found in the stomach was lowest when $^{59}FeSO_4 . 7H_2O$ was used alone and highest when ^{59}Fe(III)EDTA was used alone. However, if an equimolar quantity of unlabelled Fe(III)EDTA was added to the $^{59}FeSO_4 . 7H_2O$ fortified maize the percentage ^{59}Fe appearing in the supernatant fraction increased markedly, shifting the curve to approximately midway between that produced by ^{59}Fe(III)EDTA alone and $^{59}FeSO_4 . 7H_2O$ alone.

DISCUSSION

The prevalence of Fe deficiency has been shown to be approximately 26% in the population from which the Indian women who volunteered for the absorption studies was drawn (Mayet *et al.* 1972). The current findings tend to confirm this in that in none of the groups did the geometric mean of the serum ferritin concentration exceed the normal adult female value of 35 μg/l (Jacbos *et al.* 1972). In addition, the over-all mean absorption (%) of 3 mg Fe as ferrous ascorbate was 31·0 (14·3–67·6), which can be taken as further evidence that the over-all group was avid for Fe (Kuhn *et al.* 1968). This background of Fe deficiency gave

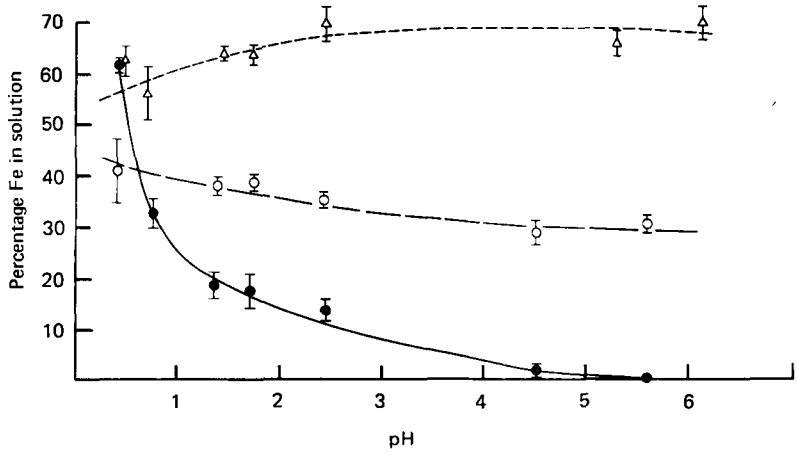

Fig. 2. The percentage ^{59}Fe appearing in the supernatant fraction after incubation of portions of maize porridge fortified with Fe at various pH values. (●——●) $^{59}FeSO_4 . 7H_2O$; (△——△) ^{59}Fe(III)EDTA; (○——○) $^{59}FeSO_4 . 7H_2O$ and Fe(III)EDTA. Results are the mean values with their standard errors represented by vertical bars of three experiments done in duplicate.

added relevance to the in vivo Fe absorption findings, since it is to target populations such as this that Fe-fortification programmes should be directed. However, a major deterrent to the mounting of such programmes is the fact that the fortification-Fe is as poorly absorbed as is the intrinsic Fe present in cereal staples (Layrisse *et al.* 1973). In addition, ionizable Fe salts can produce a number of undesirable colour changes in food and can adversely affect the storage properties of certain foods (International Nutritional Anemia Consultative Group, 1977). It is against this background that the current findings should be seen.

The chelator EDTA forms a very stable complex with Fe and because of this the Fe(III)EDTA complex might be expected to be protected to some extent from the ligands in cereals which inhibit Fe absorption. The results of the present investigation confirmed that Fe(III)EDTA was well absorbed and utilized for haemoglobin formation and that its absorption was not inhibited by bran or maize porridge. Nevertheless more powerful inhibitors of absorption such as tea were able to decrease the absorption of Fe from Fe(III)EDTA. The absorption of Fe(III)EDTA was also altered by the addition of ascorbic acid. In the absence of inhibitors (Table 3A) ascorbic acid did promote Fe absorption from Fe(III)EDTA in a similar dose-dependent manner to that which has been previously demonstrated with simple Fe salts (Sayers *et al.* 1973; Sayers, Lynch, Charlton & Bothwell, 1974; Sayers, Lynch, Charlton, Bothwell *et al.* 1974; Bjørn-Rasmussen & Hallberg, 1974). Thus, when present at sufficiently-high concentration ascorbate was able to compete successfully for the Fe in Fe(III)EDTA. There were, however, quantitative differences between the behaviour of Fe(III)EDTA and simple Fe salts. For example, in one study (Table 3) the absorption (%) of Fe from Fe(III)EDTA alone was 7·7 compared with 26·8 from the ferrous ascorbate reference salt. When ascorbate was added to give a molar ratio of 3:1 for ascorbate and Fe (which is the same as that in the reference salt) absorption increased to only 11. This result suggests that perhaps one-sixth of the Fe was transferred to ascorbate in the presence of a 3-fold molar excess of ascorbic acid. Fe absorption from Fe(III)EDTA was increased to the same level as that from the reference salt only when the molar ratio for ascorbate and Fe was 6:1. Under such circumstances all the Fe was presumably in the form of ascorbate. In the presence of maize there was no improvement

in the absorption of Fe from Fe(III)EDTA until a molar ratio for ascorbate and Fe of 6:1 was present and even then the increase was a modest one, from a standardized absorption (%) of approximately 8–15·6. These results suggest that although ascorbate Fe is very well absorbed its absorption was reduced considerably (from 44 to 15) by the presence of maize porridge. On the other hand, the standardized absorption of EDTA-complexed Fe, although only 6–10, was not significantly affected by the presence of the maize porridge. In this context, the unchanged absorption of Fe from Fe(III)EDTA in maize porridge when the molar ratio for ascorbate and Fe was 1:3 is not surprising if it is accepted that only one-sixth of the Fe was present as the ascorbate complex and that only approximately 12% of this was absorbed (Table 3). These results suggest that competitive binding of Fe occurs in the gastrointestinal tract and that the amount of Fe absorbed depends on the relative affinities for Fe of the various ligands which either promote or inhibit Fe absorption.

Most Fe salts which are added as fortificants to cereals have been shown to enter a 'common non-haem-Fe pool' and are absorbed to the same extent as the intrinsic food Fe (Layrisse & Martinez-Torres, 1971; Cook *et al.* 1972; Björn-Rasmussen & Hallberg, 1972). Although Fe(III)EDTA appears to form a common pool with food Fe in some situations it is preferentially absorbed in others. Thus when Fe(III)EDTA was cooked with either maize porridge or a pea-based dhal, absorption of extrinsic Fe(III)EDTA:absorption of intrinsic food Fe was close to unity. However, absorption of Fe from Fe(III)EDTA added to pre-cooked maize porridge was 3-fold higher than that of the intrinsic maize Fe, which was presumably due to incomplete mixing. It should be noted that even when the relative absorption value was close to unity the absorption of the radio-Fe added as Fe(III)EDTA was always marginally greater. A similar deduction can be made from results obtained by Layrisse and co-workers (Layrisse & Martinez-Torres, 1977; Martinez-Torres *et al.* 1979). Statistical analysis of their values using Student's t test for paired values indicates the absorption from Fe(III)EDTA (5 mg and 50 mg Fe) given in a maize-soyabean meal was significantly greater than from the intrinsic Fe (t 3·54, $P < 0{\cdot}005$; t 5·04, $P < 0{\cdot}005$ respectively). This may indicate that the Fe in the Fe(III)EDTA complex may frequently not exchange completely with the intrinsic Fe or indeed with added Fe salts. Evidence that there is indeed an exchange between the Fe in the EDTA complex and other Fe salts was provided by the demonstration of both ^{55}Fe and ^{59}Fe in the urine of subjects given porridge fortified with ^{55}Fe(III)EDTA and $^{59}FeSO_4 . 7H_2O$. Since none of the Fe absorbed from $^{59}FeSO_4 . 7H_2O$ is excreted in the urine, the appearance of almost equal quantities of the two isotopes in the urine implies that almost complete exchange must have occurred in the lumen of the gut. This exchange process was confirmed in vitro by the enhanced solubilization of ^{59}Fe from $^{59}FeSO_4 . 7H_2O$-fortified maize porridge by the addition of unlabelled Fe(III)EDTA. The relatively good absorptions seen when Fe(III)EDTA was used to fortify cereal-based meals can probably be ascribed to the fact that Fe in the form of the Fe(III)EDTA complex remains in solution and relatively unaffected by inhibitory ligands in food even at pH 5. Thus in the region of the duodenum and jejunum, where most Fe absorption takes place, the Fe is in a highly-soluble form and formation of insoluble, poorly-absorbed Fe complexes is prevented. There is one final point concerning the exchange of Fe between Fe(III)EDTA and intrinsic food Fe which merits comment. In one experiment $FeSO_4$ was given with porridge on one morning and Fe(III)EDTA was fed on the other. Since both Fe compounds form a common pool with food Fe it would have been expected that Fe absorption on the 2 days would have been the same. It was, however, twice as great when the fortificant was Fe(III)EDTA. This suggests that the mechanism whereby Fe(III)EDTA forms a common pool with intrinsic Fe differs from simple Fe salts. The apparent enhancement of intrinsic food Fe absorption by Fe(III)EDTA, which was also previously noted by Layrisse & Martinez-Torres (1977), could be due to an exchange of

isotope between intrinsic ^{55}Fe and ^{59}Fe in the EDTA chelate. The intrinsic ^{55}Fe which complexes with EDTA remains in solution and is well absorbed. On the other hand, in the instance of simple Fe salts no 'solubilizing' chelate is present and the Fe is bound to inhibitory ligands in the food and is as poorly absorbed as the intrinsic Fe.

The fact that small amounts of radio-Fe appeared in the urine after ingestion of labelled Fe(III)EDTA indicates that some of the Fe was probably absorbed intact in the form of the Fe(III)EDTA complex, since Fe and other EDTA chelates have been shown to be rapidly excreted by the kidney (Will & Vilter, 1954). In the present study all radio-Fe was excreted in 18–24 h and in no subject did the amount exceed 1% of the administered dose. An inverse relationship was noted between the Fe absorbed and the Fe appearing in the urine (Fig. 1) which suggests that less Fe was absorbed as the chelate when the mucosa was avid for Fe. At the same time, it was noteworthy that most of the Fe was probably absorbed in a non-chelated form, even by individuals who were not Fe-deficient. Whether the Fe dissociates from the complex before entering the mucosa or within the mucosa is not clear.

The results of the present study confirm the potential usefulness of Fe(III)EDTA as an Fe fortificant (Layrisse & Martinez-Torres, 1977; Martinez-Torres *et al.* 1979). It can be added to food with little or no colour change and is well absorbed even in the presence of known inhibitors. If it can be shown that the long-term administration of Fe(III)EDTA does not adversely affect trace mineral metabolism, then it may well fulfil an important role in Fe-fortification programmes.

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