Factors affecting the absorption of iron from cereals

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(*Received 4 January 1983 – Accepted 20 July 1983*)

1. Non-haem-iron absorption from a variety of cereal and fibre meals was measured in parous Indian women, using the erythrocyte utilization of radioactive Fe method.

2. The present study was undertaken to establish whether alteration of the phytate and polyphenol contents of sorghum (*Sorghum vulgare*) affected Fe absorption from sorghum meals, and to assess the influence of fibre on Fe absorption.

3. Removing the outer layers of sorghum grain by pearling reduced the polyphenol and phytate contents by 96 and 92% respectively. This treatment significantly increased the geometric mean Fe absorption from 0·017 to 0·035 (t 3·9, $P < 0·005$).

4. The geometric mean Fe absorption from a sorghum cultivar that lacked polyphenols (albino sorghum) was 0·043, which was significantly greater than the 0·019 absorbed from bird-proof sorghum, a cultivar with a high polyphenol content (t 2·83, $P < 0·05$).

5. Fe was less well absorbed from the phytate-rich pearlings of the albino sorghum than from the pearled albino sorghum (0·015 *v.* 0·035 (t 8·4, $P < 0·0005$)). Addition of sodium phytate to a highly Fe-bioavailable broccoli (*Brassica oleracea*) meal reduced Fe absorption from 0·185 to 0·037.

6. The geometric mean Fe absorption from malted sorghum porridge was 0·024 when 9·5 mg ascorbic acid were added and 0·094 when the ascorbic acid was increased to 50 mg (t 3·33, $P < 0·005$). This enhancing effect of 50 mg ascorbic acid was significantly depressed to 0·04 by tea (t 38·1, $P < 0·0005$).

7. Wheat bran significantly decreased the geometric mean Fe absorption from white flour from 0·116 to 0·043 (t 7·2, $P < 0·0005$).

8. Some of the constituents of the dietary fibre complex, such as apple pectin, guar gum, gum tragacanth and microcrystalline cellulose did not inhibit Fe absorption. On the other hand, hemicellulose and lignin decreased absorption. The geometric mean absorption of Fe given with hemicellulose was 0·079 *v.* 0·269 with microcrystalline cellulose (t 2·95, $P < 0·05$). Addition of cocoa, which contains approximately 280 g lignin/kg, reduced the geometric mean Fe absorption from milk from 0·075 to 0·035 (t 2·7, $P < 0·05$).

The diets of poorer populations in many parts of the world are predominantly cereal based, with rice, maize and wheat forming the major staples. Although these diets appear to contain sufficient iron (Apte & Iyengar, 1970; Hallberg, 1974), only 0·01, 0·03 and 0·05 of the Fe in rice, maize and wheat respectively have been found to be absorbed (Martinez-Torres & Layrisse, 1973). While it has been shown that some of these absorption values may be too low because of contamination with dirt containing insoluble Fe (Hallberg, 1981), there can be no question that cereal Fe is poorly bioavailable, and there is good evidence that this

* For reprints.

is because cereals contain substances that inhibit Fe absorption (Bothwell *et al.* 1979). Like the better-known cereal staples, sorghum (*Sorghum vulgare*) contains inhibitory substances and Fe absorption from sorghum porridge is very poor (Derman *et al.* 1980). Because all the non-haem Fe in a meal forms a common pool within the intestinal lumen, these inhibitory substances affect the absorption of not only the cereal Fe but also the other non-haem food Fe and, in addition, any added non-haem Fe (Hallberg, 1974, 1981). Their influence on Fe nutrition is therefore critical.

Three groups of substances found in cereals, namely phytates, polyphenols and fibre, have been proposed as inhibitors of Fe absorption. Many cereals contain large amounts of phytate (inositol hexaphosphate), which serves as the phosphate reserve for the plant seedling (Van Soest, 1978), and there is evidence that it may interfere with Fe absorption (Ellis *et al.* 1982), presumably by forming an insoluble Fe–phytate complex. Recent studies have also shown that a variety of plant polyphenols, particularly those occurring in the form of non-hydrolysable tannins, are potent inhibitors of Fe absorption (Disler *et al.* 1975; Gillooly *et al.* 1983). Recent interest in dietary fibre has revealed that certain fibre components influence the absorption of minerals (Cummings, 1978; Sandstead *et al.* 1978). Many of these fibres are indigestible polysaccharides and it has been claimed that even digestible polysaccharides, such as rice starch, may inhibit Fe absorption to some extent (Hallberg *et al.* 1978). In the present study an attempt was made to evaluate the relative roles of phytate, polyphenol and fibre on non-haem Fe absorption.

EXPERIMENTAL

Subjects

The parous Indian housewives who took part in the present study all belonged to a low socio-economic group living in municipal housing schemes in either Chatsworth or Merebank, near Durban. None of the subjects was pregnant or lactating and all were unpaid volunteers. It has previously been established that Fe deficiency is a common problem among the women of this community (Mayet *et al.* 1972; MacPhail *et al.* 1981).

Grain and commercial cereal products

Two types of sorghum grain were used. The first was a bird-proof sorghum, cultivar SSK-52 (Stoffberg, Transvaal). It was dark red-brown and had a high polyphenol content, whilst the second, albino sorghum, a white grain sorghum cultivar 766W (CIBA-Geigy), had a very low polyphenol content. Both sorghum varieties had a high phytate content. The polyphenols are located mainly in the outer layers of the sorghum grain and the phytate in the layers just below this. It is thus possible to remove most of the polyphenols and increasing amounts of phytate by removing the outer layers of the grain. This process, known as pearling, was carried out using a Miag laboratory rice pearler (Buhler-Miag, Muhlenbau and Industrie G.m.b.H., Braunschweig, West Germany). The pearled bird-proof sorghum used in the study was treated for 30 min. This removed 55% of the grain, leaving only 4% of the 16290 mg polyphenol/kg and 8% of the 5390 mg phytate/kg that had been present in the whole grain. Pearling the albino sorghum for 20 min removed 40% of the grain and left a grain containing 14% of the original phytate (1520 mg/kg) whilst the pearlings (the removed outer layers) had 9040 mg phytate/kg. The whole grain and the pearled grain were milled to a flour using a coffee mill. One other form of sorghum used in these studies was a commercially available breakfast food, Maltabella (Hinds Bros, Durban), which is prepared by milling malted sorghum and adding sodium chloride.

Preparation and administration of the meals

In each Fe absorption study, two meals were consumed on consecutive mornings after an overnight fast. No additional food or drink except water was permitted during the meal and for a period of 3 h afterwards.

Sorghum porridge. Sorghum (450 g; 30 g/subject) was made into a paste with 750 ml water. This was cooked in 2250 ml boiling water containing 10 g table salt for 20 min to make a porridge, of which each subject was given 200 g. Where tea was served with malted sorghum, 75 g tea leaves (Pot O'Gold: O.K. Bazaars Ltd, Johannesburg) were infused with 3000 ml boiling water to give 200 ml/subject.

Oat porridge. Oat meal (Jungle Oats, Cape Town; 450 g; 30 g uncooked meal per subject) was made into a paste with 500 ml water and added to 2250 ml boiling water containing 10 g table salt and cooked for 20 min to make a porridge. Each person was given 200 g cooked porridge together with 10 g sucrose.

Wheat flour and bran meal. Snow Flake cake flour (South African Milling Co, Johannesburg; 450 g; 30 g uncooked flour per subject) was made into a paste with 750 ml water and then cooked for 20 min in 2250 ml boiling salted water. Each subject received 200 g porridge. On the second day, 90 g wheat bran (6 g/subject) was added to 360 g Snow Flake cake flour (24 g/subject) and made into a porridge. Both meals were eaten with 10 g sucrose.

Broccoli meal. Each subject received a meal made up from 50 g uncooked broccoli. It was cooked in boiling water for 20 min and then homogenized to the consistency of a thick soup. Radioactive Fe was added just before serving; it was added alone on one morning and together with 2 g sodium phytate (sodium salt of inositol hexaphosphoric acid (BDH Chemicals Ltd, Poole, Dorset)) on the other.

Fibre meals. Several meals were prepared using constituents of the dietary fibre complex.

Individual 5 g portions of microcrystalline cellulose (Sigma cell type 50; Sigma Chemical Company, St Louis, Missouri) were weighed into glasses and 200 ml water containing 15 g sucrose and 3 mg Fe as $FeSO_4 . 7H_2O$ labelled with 3 μCi radioactive Fe was added to each and mixed well immediately before it was consumed.

The effect of 5 g apple pectin (250 grade; BDH Chemicals) was assessed in two studies. In the first, the jelly was prepared by dissolving 70 g pectin in 400 ml distilled water containing 140 ml ethanol, 210 g sucrose and 42 mg Fe as $FeSO_4 . 7H_2O$ labelled with 42 μCi $^{59}FeCl_3 . 6H_2O$. After removing the ethanol by boiling, the jelly was refrigerated overnight. Each subject received 70 ml of the jelly. In the second study the apple pectin jelly was prepared by adding 5 g apple pectin very slowly to 150 ml cold water and then adding 100 ml boiling water for each subject. The mixture was sweetened with 15 g sucrose and a drop of apple green food colouring was added to each bowl.

A separate guar gum meal was prepared for each subject. Guar gum (Jaguar, Stein Hall S A (Pty) Ltd; 5 g) was sprinkled over 250 ml cold water and allowed to settle overnight at room temperature. White sugar (15 g) and a drop of an apple green food colouring were used to sweeten and colour the mixture in each bowl.

A meal of gum tragacanth was prepared by slowly dissolving 70 g finely powdered gum tragacanth (Merck, Darmstadt, West Germany) in 2 l water. The dissolved material was refrigerated and before dividing it into 100-g meals the mixture was sweetened and flavoured by adding 200 g sugar and 5 ml caramel essence.

The effect of a highly purified hemicellulose preparation from psyllium seeds (*Plantago ovata*) was tested. Each meal was prepared separately by adding 200 ml water to 5 g Mucilose (Winthrop Laboratories, New York).

In a final experiment the effect of cocoa (Cadbury, Bournville, Birmingham) was assessed by measuring the change in Fe absorption which occurred when 10 g cocoa was added to

250 ml full-cream milk containing 3 mg Fe and three teaspoons of sugar. Although cocoa is not usually considered to be a high-fibre food, it contains (g/kg) 40 cellulose, 110 non-cellulosic polysaccharides and 280 lignin, giving it a total fibre content of 430 g/kg (Southgate *et al.* 1976).

The total Fe content of all the test meals was between 3 and 4·2 mg.

Isotopic and chemical methods. Immediately before the meal was eaten, 1 ml of a solution containing 3 mg Fe as $FeSO_4 . 7H_2O$ and 3 μCi radioactive Fe was thoroughly mixed with each portion. One meal was labelled with 3 μCi ^{59}Fe and the other with 3 μCi ^{55}Fe (Amersham International, Amersham, Bucks). After 2 weeks, blood for the determination of ^{59}Fe, ^{55}Fe, haemoglobin, serum Fe, unsaturated Fe-binding capacity and serum ferritin was obtained from all the subjects after they had fasted overnight. Each person then drank a standard 3 mg dose of Fe as a solution of $FeSO_4 . 7H_2O$ labelled with 3 μCi ^{59}Fe and containing 30 mg ascorbic acid. Only water was permitted during the following 3 h. Blood samples were obtained 14 d later and Fe absorption from the standard reference dose was determined from the increment in ^{59}Fe in the blood. This gave a measure of each person's absorption capacity and by expressing the absorption of Fe from the meals in relation to this reference dose it was possible to compare results in individuals of differing Fe nutritional status (Rossander *et al.* 1979; Hallberg, 1981).

Duplicate 10 ml blood samples and duplicate portions of standard Fe solutions were prepared for differential radioactive counting using a modification of the method of Eakins & Brown (1966). The activities of ^{55}Fe and ^{59}Fe in the processed samples were determined in Insta-Gel scintillant (Packard Instrument Co, Downers Grove, Illinois) using a liquid-scintillation spectrometer (Packard-Tri-Carb AAA spectrometer model no. 3375). The counting efficiency at optimal gain and window settings was 24% for ^{55}Fe and 53% for ^{59}Fe. 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 Packard auto gamma scintillation spectrometer model no. 5320, with a counting efficiency of 60%. The absorption values were calculated on the assumption that 100% of the absorbed radioactivity was present in the haemoglobin of circulating erythrocytes and that the blood volume for each subject was 65 ml/kg body-weight.

Haemoglobin concentrations were determined by the cyanmethaemoglobin technique. Serum Fe concentrations were measured using the International Committee for Standardization in Haematology (ICSH, 1978*a*) method, as were unsaturated Fe-binding capacities (ICSH, 1978*b*). Serum ferritin was measured using the ELISA radioimmunoassay of Conradie & Mbhele (1980). Polyphenols in the cereals were measured by the modified DMF-FAC procedure of Diaber (1975). The phytate content was measured by the method of Wheeler & Ferrel (1971), in which an acid extract of the grain was made and the phytate was precipitated as its ferric salt, and the phytate-phosphorus was determined as inorganic phosphate.

Fe absorption from the different meals was expressed in relation to the absorption of the standard reference dose of ferrous ascorbate and then corrected to a reference dose absorption of 0·40, which is the approximate amount absorbed by subjects who lack Fe stores although they are not yet anaemic (Hallberg, 1981). The Fe absorption and plasma ferritin concentrations were expressed as the geometric mean and standard deviation ranges, since values in individual experiments showed considerable variation with positive skew. The significance of differences between the absorption of the two isotopes used in each study was calculated using Student's *t* test for paired observations, except in the case of malted sorghum where the unpaired *t* test was used because the results were not based on direct comparison in the same individuals.

Ethical considerations

Approval for the studies was obtained from the Committee for Research on Human Subjects of the Faculty of Medicine, University of the Witwatersrand, Johannesburg. Written consent was obtained from all subjects after the nature of the investigation had been fully explained to them by an Indian social worker. Each subject took part in one experiment only. It was calculated that if each test dose were completely retained, the total whole body radiation dosage would be 143 mrems (Bothwell *et al.* 1979) which is 28% of the annual maximum permissible dose for members of the public (International Commission for Radiation Protection, 1960; South African Bureau of Standards, 1972). In practice, the percentage absorbed is much less, which would make the radiation exposure proportionately less.

RESULTS

Effects of polyphenols and phytates on Fe absorption from sorghum

In the first study, Fe absorption from whole bird-proof sorghum was compared with that from 55% pearled bird-proof sorghum in sixteen subjects (Table 1). Although absorption was poor in each case, removing most of the polyphenol and phytate by pearling did significantly improve Fe absorption from a geometric mean of 0·017 to 0·035 (t 3·9, $P < 0·005$). Since sorghum appeared to contain insignificant quantities of promoters of Fe absorption, such as ascorbic acid, it was felt that more clear-cut evidence of the effects of the removal of inhibitors might be obtained if a small dose of a known enhancer of Fe absorption were present in the meal. An amount of ascorbic acid equimolar with the added Fe (9·5 mg) was therefore included with each meal in subsequent studies. When the first experiment was repeated in twelve subjects using this approach, the geometric mean Fe absorption for whole grain was 0·024 *v.* 0·063 for pearled grain (t 3·4, $P < 0·01$). In a third experiment using fourteen subjects, the geometric mean Fe absorption from polyphenol-poor albino whole grain sorghum was found to be 0·043 *v.* 0·019 from the polyphenol-rich bird-proof sorghum (t 2·83, $P < 0·05$). The geometric mean Fe absorption from pearled albino sorghum in a further ten subjects was 0·035 *v.* 0·015 from a porridge prepared from the pearlings (t 8·4, $P < 0·0005$). Since the pearlings were rich in phytate, an experiment was carried out, on sixteen subjects, to assess the effects of sodium phytate on the absorption of Fe from a broccoli meal of high Fe-bioavailability. The addition of sodium phytate was associated with a significant decrease (t 6·13, $P < 0·0005$) in the geometric mean Fe absorption (0·185 *v.* 0·327).

The effect of ascorbic acid on Fe absorption from malted sorghum porridge was studied in further experiments. Increasing the dose of ascorbic acid from 9·5 to 50 mg was associated with a significant rise in the geometric mean Fe absorption from 0·024 (thirteen subjects) to 0·094 (eighteen subjects) (t 3·33, $P < 0·005$). The enhancing effect of the larger dose of ascorbic acid was effectively eliminated in seven subjects by serving the porridge with a cup of tea; the geometric mean Fe absorptions were 0·094 and 0·040 respectively (t 38·1, $P < 0·0005$).

The ability of ascorbic acid to overcome the effects of the inhibitors of Fe absorption present in cereals was shown also to extend to oats. In seven subjects the geometric mean absorption from oat porridge was 0·009 *v.* 0·057 after adding an equimolar amount of ascorbic acid (t 12·25, $P < 0·0005$).

Effect of various types of fibre on the absorption of Fe

The presence of wheat bran in a porridge prepared from white cake flour reduced the geometric mean Fe absorption from 0·116 to 0·043 (t 5·2, $P < 0·005$) (Table 2). A

Table 1. *The effect of the phytate and polyphenol contents of sorghum* (Sorghum vulgare) *on the absorption of iron (3 mg) as ferrous sulphate*

(Mean values and standard deviations)

No. of subjects	Haemoglobin (g/l)		Transferrin saturation (%)		Serum ferritin† (μg/l)		Fe absorption†				
							Reference salt‡				
	Mean	SD	Mean	SD	Geometric mean	±1 SD	Geometric mean	±1 SD	Test substance	Geometric mean‖	±1 SD
16	127	15	26·9	16·3	31·7	(13·3–75·3)	0·344	(0·020–0·590)	Whole bird-proof sorghum	0·017	(0·007–0·041)
									Pearled bird-proof sorghum	0·035 *	(0·015–0·081)
12	140	12	25·3	9·1	26·1	(10·7–63·7)	0·411	(0·257–0·657)	Whole bird-proof sorghum§	0·024	(0·007–0·073)
									Pearled bird-proof sorghum	0·063 *	(0·025–0·144)
13	122	17	22·9	17·1	11·9	(2·9–48·5)	0·293	(0·173–0·495)	Whole bird-proof sorghum§	0·019	(0·006–0·059)
									Whole albino sorghum§	0·043 *	(0·018–0·103)
10	129	15	25·2	10·6	18·1	(4·5–72·6)	0·340	(0·211–0·548)	Albino sorghum pearlings§	0·015	(0·009–0·024)
									Pearled albino sorghum§	0·035 *	(0·023–0·051)
16	132	17	24·9	13·1	25·0	(9·1–71·3)	0·349	(0·175–0·698)	Broccoli¶	0·185	(0·089–0·382)
									Broccoli + 2 g sodium phytate	0·037 *	(0·009–0·160)
13	135	12	25·9	6·5	16·1	(3·6–72·5)	0·450	(0·359–0·564)	Malted sorghum§	0·024	(0·011–0·051)
18	139	25	30·8	19·3	—	—	0·305	(0·135–0·687)	Malted sorghum + 50 mg ascorbic acid	0·094 *	(0·046–0·195)
7	129	16	27·6	13·2	9·1	(2·0–40·2)	0·363	(0·185–0·724)	Malted sorghum + 50 mg ascorbic acid + tea	0·040 *	(0·020–0·082)

* The difference was statistically significant ($P < 0·05$).
† Geometric means and SD ranges used because values were positively skewed.
‡ 3 mg Fe as ferrous ascorbate given in the fasting state.
§ Meal served with an equimolar amount of ascorbic acid (9·5 mg) to Fe.
‖ Individual results adjusted to 0·40 reference absorption.
¶ Broccoli (*Brassica oleracea*) included because of its high Fe bioavailability.

Table 2. *The effect of various types of 'fibre' on the absorption of iron (3 mg) as ferrous sulphate*

(Mean values and standard deviations)

No. of subjects	Haemoglobin (g/l) Mean	Haemoglobin (g/l) SD	Transferrin saturation (%) Mean	Transferrin saturation (%) SD	Serum ferritin† (μg/l) Geometric mean	Serum ferritin† (μg/l) ±1 SD	Fe absorption† Reference salt‡ Geometric mean	Fe absorption† Reference salt‡ ±1 SD	Test substance	Fe absorption† Geometric mean§	Fe absorption† ±1 SD
8	127	6	18·9	8·9	16·7	(5·4–51·9)	0·341	(0·148–0·785)	White flour	0·116 } *	(0·088–0·149)
									White flour + 6 g bran	0·043 } *	(0·030–0·061)
12	129	18	27·9	10·3	10·4	(2·8–38·5)	0·377	(0·237–0·600)	Cellulose (5 g)	0·356	(0·263–0·482)
									Apple pectin (5 g)	0·241	(0·152–0·383)
10	126	15	34·3	16·1	16·8	(4·6–61·5)	0·218	(0·127–0·376)	Guar gum (5 g)	0·392	(0·186–0·828)
									Apple pectin (5 g)	0·342	(0·184–0·635)
8	147	10	29·8	7·8	20·6	(10·5–40·8)	0·256	(0·170–0·387)	Gum tragacanth (2·5 g)	0·251	(0·108–0·582)
									Cellulose (5 g)	0·282	(0·146–0·547)
6	127	13	18·9	6·3	17·3	(6·3–47·6)	0·206	(0·123–0·290)	Hemicellulose‖	0·079 } *	(0·019–0·143)
									Cellulose (5 g)	0·269 } *	(0·091–0·468)
9	135	18	26·3	7·4	22·5	(9·4–54·2)	0·459	(0·256–0·828)	Milk	0·075 } *	(0·025–0·156)
									Milk + 10 g cocoa (279 g lignin/kg)	0·035 } *	(0·017–0·074)

* The difference was statistically significant ($P < 0·05$).

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

‡ 3 mg Fe as ferrous ascorbate given in the fasting state.

§ Individual results adjusted to 0·40 reference absorption.

‖ Mucilose (Winthrop Laboratories, New York).

comparison was then made in twelve subjects between the absorption of Fe in apple pectin jelly and in microcrystalline cellulose slurry. The corrected geometric mean Fe absorptions were high on both occasions, being 0·241 and 0·356 respectively. Although the difference was statistically significant (t 5·41, $P < 0·0005$), this may have been due to the method of preparation, since the geometric mean Fe absorption from apple pectin jelly was higher when ethanol was not used in preparing it. In the next study the alcohol-free preparation was compared with guar gum in ten subjects and the geometric mean Fe absorption values were 0·342 and 0·392 respectively (t 1·03, $P > 0·1$). In eight subjects there was no significant difference between the geometric mean Fe absorption from gum tragacanth (0·251) and from cellulose (0·282; t 0·44, $P > 0·1$). However, when Mucilose was substituted for the gum tragacanth in another six subjects, the mean geometric Fe absorption was significantly lower (0·079 *v.* 0·269) compared with the cellulose (t 2·95, $P < 0·05$). In a final experiment in nine subjects, the addition of cocoa to milk reduced the geometric mean Fe absorption significantly from 0·075 to 0·035 (t 2·7, $P < 0·05$).

DISCUSSION

This study was carried out on a female population in which Fe deficiency, usually of mild extent, is common (Mayet *et al.* 1972; MacPhail *et al.* 1981). The diets consumed by these people of Indian origin are in many respects similar to those in India, where cereals and legumes are important staples (Narasinga Rao & Prabhavathi, 1982). The bioavailability of the Fe in these staples is low and this has stimulated a good deal of research into the reasons and ways of overcoming it.

One potent inhibitor of Fe absorption is bran (Björn-Rasmussen, 1974), and this was confirmed in the present study. It has been suggested that its action may be due to some factor or factors present in vegetable fibre. Reinhold *et al.* (1981) have shown that the fibre present in wheat and maize binds Fe. The effect cannot be ascribed entirely to its phytate content, since the capacity of bran to bind Fe (Reinhold *et al.* 1981) and to inhibit Fe absorption persists even after the phytate has been removed (Morris & Ellis, 1980; Simpson *et al.* 1981). However, there is reason to believe that phytates can inhibit Fe absorption since both diferric and tetraferric phytate are poorly bioavailable (Ellis & Morris, 1979), as is the Fe in various vegetables with high-phytate contents, such as wheatgerm, butter beans (*Phaseolus lunatus*) and lentils (*Lens culinaris*) (Gillooly *et al.* 1983).

Certain polyphenols also exert an inhibitory effect on Fe absorption. An important example is the tannin in tea (Disler *et al.* 1975; Rossander *et al.* 1979) but many other vegetable foodstuffs contain similar polyphenols in quantities which correlate inversely with the bioavailability of the Fe they contain (Gillooly *et al.* 1983). Narasinga Rao & Prabhavathi (1982) have postulated that decortication of legumes should enhance Fe absorption, since most of the tannins reside in the seed coat.

The present study systematically examined the effect on Fe absorption of the polyphenol and phytate in sorghum. When amounts of both compounds were reduced to low levels by pearling there was a significant increase in Fe absorption from 0·017 to 0·035. This difference was still present when absorption was enhanced by adding ascorbic acid, a known enhancer of Fe absorption (Sayers *et al.* 1973, 1974*a*, *b*; Derman *et al.* 1977). When only an equimolar amount (9·5 mg) was added, absorption increased from 0·024 to 0·063. A much greater enhancement of Fe absorption from 0·024 to 0·094 was found when 50 mg ascorbic acid was added to a meal of malted sorghum, confirming that the effect was dose related (Derman *et al.* 1980). This enhancement was compromised by taking tea with the meal, which decreased absorption to 0·040. These results illustrate the interplay of inhibitory and promoting ligands for Fe on its bioavailability.

An albino sorghum cultivar containing phytate but no polyphenol was used to study the

relative inhibitory effects of these two compounds on Fe absorption. The significantly higher absorption (0·043) from this grain than from the bird-proof cultivar (0·019) suggested that the polyphenol in the latter cultivar was a more potent inhibitor. Evidence that phytate was also an inhibitor was obtained by pearling the albino sorghum to give fractions of high and negligible phytate content and showing that Fe was absorbed better from the latter (0·035 *v.* 0·015). More direct evidence was obtained by adding 2 g sodium phytate to a broccoli meal; it decreased Fe absorption from 0·185 to 0·037. This effect may explain the low Fe absorption of 0·009 from oatmeal, a cereal having a particularly high phytate content (approximately 10 g/kg). Addition of ascorbic acid, equimolar to added Fe, increased absorption to 0·057 indicating that ascorbate can overcome the inhibitory effect of phytate.

Since pearling also causes a reduction in the fibre content of cereal grains, an attempt was made to identify components of fibre that inhibit Fe absorption. Previous evidence that fibre may be of importance rests on the observations of an inverse exponential correlation between the bran content of bread and the absorption of Fe (Björn-Rasmussen, 1974). Certain components of fibre have the property of cation exchange (Eastwood & Mitchell, 1976) which may be responsible for the occurrence of a negative calcium balance observed by Cummings (1978) in students given an increased amount of whole wheat and bran. The major fibre of many vegetable foods is cellulose, which does not bind cations (Van Soest, 1978) and which in the present study did not appear to inhibit Fe absorption: in three experiments the absorption of Fe given with microcrystalline cellulose was 0·356, 0·282 and 0·269. It has been postulated that the Fe binding of the neutral detergent fibre of wheat (0·38 mg Fe/g) is related to the unsubstituted uronic groups of hemicellulose (Reinhold *et al.* 1981). The neutral detergent fibre contains only the insoluble plant cell walls and not the more soluble wall constituents, such as guars and pectins (Van Soest, 1978), which would not be expected to affect Fe absorption. This prediction was substantiated in the present study. Neither guar gum, which is a plant mucilage contained in the endosperm of the fodder legume, *Cyamopsis tetragonolobus*, nor gum tragacanth, another mucilaginous type fibre, inhibited Fe absorption; the geometric mean absorptions were 0·392 and 0·251 respectively compared with a Fe absorption of 0·282 from cellulose. Although Monnier *et al.* (1980) claimed apple pectin inhibited Fe absorption no such evidence was obtained in the present study: the mean Fe absorptions in two groups of women were 0·241 and 0·342 respectively. However, less Fe was absorbed from Mucilose, which is a highly refined form of hemicellulose consisting of pentosans, hexosans and galactans obtained from psillium (*Plantago ovata*) (Fernandez & Phillips, 1982), than from cellulose (0·079 *v.* 0·269). This finding suggests that hemicellulose may act as an inhibitor of Fe absorption. Because of the extremely high lignin content of cocoa we examined its effect on Fe absorption. Addition of 10 g cocoa to 250 ml milk decreased Fe absorption from 0·075 to 0·035. It is possible that this decrease in Fe absorption was due to the lignins, some of which have a polyphenolic structure, including groups of the gallic acid type. Similar compounds account for the inhibitory effect of tea on Fe absorption.

The results obtained in the present study suggest that the low bioavailability of Fe when sorghum is consumed is due to several factors, including the presence of the inhibitory substances phytate and polyphenols, and insufficient quantities of promoting substances such as ascorbic acid. Some components of dietary fibre may also contribute to the poor bioavailability of Fe in sorghum meals. Of the various constituents of the fibre complex that were tested only lignin and hemicellulose were shown to have an inhibitory effect on Fe absorption. While these latter results are in agreement with in vitro observations (Fernandez & Phillips, 1982), their relevance to Fe nutrition remains to be defined.

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Printed in Great Britain