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The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables

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1. Non-haem iron absorption from a variety of vegetable meals was studied in parous Indian Women, using the erythrocyte utilization of radioactive Fe method.

2. The studies were undertaken to establish whether Fe absorption could be correlated with the chemical composition of the foodstuff.

3. Addition of the following organic acids commonly found in vegetables, improved the geometric mean Fe absorption from a basic rice meal as follows: from 0·028 to 0·085 with 1 g citric acid, from 0·031 to 0·081 with 15 mg ascorbic acid, from 0·048 to 0·095 with 1 g L-malic acid, from 0·041 to 0·096 with 1 g tartaric acid. The only exception was oxalic acid; the addition of 1 g calcium oxalate to cabbage (*Brassica oleraceae*) was associated with some depression in Fe absorption from 0·320 to 0·195.

4. There was a marked inhibition of the geometric mean absorption when 500 mg tannic acid was added to a broccoli (*Brassica oleraceae*) meal (0·015 *v.* 0·297). Sodium phytate (2 g) caused a similar, though less profound inhibition (0·035 to 0·152).

5. When 3 mg ferrous sulphate was added to different vegetables the geometric mean absorption varied widely. Vegetables of low Fe bioavailability were wheat germ (*Triticum aestivum*) 0·007, aubergine (*Solanum melongena*) 0·007, butter beans (*Phaseolus lunatus*) 0·012, spinach (*Spinacea oleraceae*) 0·014, brown lentils (*Lens culinaris*) 0·024, beetroot greens (*Beta vulgaris*) 0·024 and green lentils (*Lens culinaris*) 0·032. In contrast, bioavailability was moderate or good with carrot (*Daucus carota*) 0·098, potato (*Solanum tuberosum*) 0·115, beetroot (*Beta vulgaris*) 0·185, pumpkin (*Cucurbita mixta*) 0·206, broccoli 0·260, tomato (*Lycopersicon esculentum*) 0·224, cauliflower (*Brassica oleraceae*) 0·263, cabbage 0·320, turnip (*Brassica rapa*) 0·327 and sauerkraut 0·327.

6. All the vegetables associated with moderate or good Fe bioavailability contained appreciable amounts of one or more of the organic acids, malic, citric and ascorbic acids.

7. Poor Fe bioavailability was noted in vegetables with high phytate contents (e.g. wheat germ 0·007, butter beans 0·012, brown lentils 0·024 and green lentils 0·032).

8. The fact that a number of vegetables associated with low Fe-absorption turned bluish-black when Fe was added to them, suggested that the total polyphenol content in them was high. The vegetables included aubergine, spinach, brown lentils, green lentils and beetroot greens. When the total polyphenol content in all the vegetables tested was formally measured, there was a significant inverse correlation (*r* 0·859, $P < 0·001$) between it and Fe absorption. The inverse correlation between the non-hydrolysable polyphenol content and Fe absorption was *r* 0·901 ($P < 0·001$).

9. The major relevance of these findings is the fact that the total absorption of non-haem-Fe from a mixed diet may be profoundly influenced by the presence of single vegetables with either marked enhancing or inhibiting effects on Fe bioavailability.

In many parts of the world the average diet is predominantly cereal in nature, wheat, rice and maize forming the major staples. Although many of these diets appear to contain sufficient Fe (Apte & Iyengar, 1970; Hallberg, 1974), Fe bioavailability is poor (Martinez-

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* For reprints.

Torres & Layrisse, 1973). During digestion the non-haem-Fe in vegetables and cereals enters a 'common pool' where its absorption is influenced by various enhancers or inhibitors (Cook *et al.* 1972; Hallberg & Bjorn-Rasmussen, 1972). The major known enhancers of Fe absorption are ascorbic acid (Sayers *et al.* 1973, 1974*a*, *b*; Disler, Lynch, Charlton, Bothwell *et al.* 1975; Derman *et al.* 1977) and meat (Layrisse *et al.* 1973; Cook & Monsen, 1976). Ascorbic acid owes this ability to its reducing properties and to its ability to chelate Fe. Its promoting influence on Fe absorption is dose dependent (Sayers *et al.* 1973; Bjorn-Rasmussen & Hallberg, 1974; Derman, Bothwell, MacPhail *et al.* 1980) and in diets containing little or no meat, the ascorbic acid content of the components of a meal has, therefore, been regarded as the crucial factor in determining its Fe nutritive value. Its efficacy is illustrated by the report that the addition of cauliflower containing approximately 70 mg ascorbic acid to a vegetarian meal trebled the amount of Fe absorbed (Hallberg, 1981). Ascorbic acid is only one of the several organic acid constituents of fruit and vegetables however, and there is little information concerning the possible role of the others.

Inhibitors of Fe absorption present in vegetable foods may also exert a profound influence on Fe nutrition. Sodium phytate has been reported to have such an effect (Turnbull *et al.* 1962; Hallberg, 1981), and it occurs in a number of plants as a phosphorus reserve storage compound (van Soest, 1978). Another established inhibitor is the tannin in tea (Disler, Lynch, Charlton, Torrance *et al.* 1975; Disler, Lynch, Torrance *et al.* 1975); other tannins or polyphenols are present in many vegetable foods, but there is little information concerning any influence they might exert. Therefore, a detailed study of Fe absorption from a number of vegetables was undertaken in order to obtain some insight into the relative contributions of these various factors to the bioavailability of dietary non-haem-Fe.

EXPERIMENTAL

Subjects

A group of 180 parous Indian housewives took part in these studies. None of the subjects was pregnant or lactating and all were unpaid volunteers. Their ages ranged between 21 and 76 years (mean 39 years) and all belonged to a low socio-economic group living in municipal housing schemes in Chatsworth and Merebank, near Durban. 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).

Preparation and administration of the meals

The absorption of radio-labelled-Fe that had been mixed with the vegetables listed in Tables 3 and 4 (see pp. 337 and 338), or with rice, was measured. In each absorption study two meals were consumed on consecutive mornings after an overnight fast. Only water was permitted during the meal and for a period of 3 h afterwards.

Vegetable meals. Each subject received a meal that was equivalent to 50 g of the uncooked vegetable. Preparation was as follows: 750 g portions of the vegetables were weighed (after peeling in the case of pumpkin, carrots, potato, beetroot and turnip) and cooked in 1500 ml boiling water with 5 g table salt (sodium chloride) for 20 min. Lentils were cooked in 2500 ml water for 40 min. The cooked vegetable was homogenized in a Waring blender to the consistency of a thick soup, and each subject consumed 100 g of it after the radio-labelled-Fe had been mixed in.

Rice meal. Polished parboiled rice (1 kg) was boiled for 20 min in 2000 ml water containing 20 g table salt. After boiling, 75 g yellow margarine and 150 g sucrose were added. Each person received 215 g of the 'pudding' with the radio-labelled-Fe solution thoroughly stirred in, on one of the two occasions together with the organic acid under investigation. However, tannic acid was given as a drink with sugar and water, since a dark colour developed when added to the meal that made the meal appear unpalatable.

Measurement of Fe absorption

Fe as $FeSO_4 . 7H_2O$ (3 mg) in solution was thoroughly mixed with each individual's serving. On one morning it was labelled with 3 μCi ^{59}Fe and on 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 each subject after she had fasted overnight. Each person then drank a solution of 3 mg Fe as $FeSO_4 . 7H_2O$ labelled with 3 μCi ^{59}Fe to which 30 mg ascorbic acid had been added. Only water was permitted during the following 3 h. Blood samples were obtained 14 d later, and the absorption of this Fe was determined from the increment in ^{59}Fe in the blood. By expressing the absorption of Fe from the meals relative to the absorption of this reference iron salt, a basis for comparing results in individuals of differing Fe nutritional status was established (Rossander *et al.* 1979; Hallberg, 1981).

Radioisotopic, chemical and statistical methods

Duplicate 10 ml blood samples and duplicate portions of standard Fe solutions were prepared for differential radioactive counting using 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) which automatically corrected for quenching. The counting efficiency at optimal gain and window settings was 24% for ^{55}Fe and 42% for ^{59}Fe. The ^{59}Fe activity in 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 no. 5320, with a counting efficiency of 60%. The absorption values were calculated on the basis that 100% of the absorbed radioactivity was present in the haemoglobin of the circulating erythrocytes, and that the blood volume for each subject was 65 ml/kg body-weight. The calculated food Fe absorptions were then corrected to an absorption of 0·40 for the reference Fe salt as follows:

$$\text{corrected food Fe absorption} = \frac{\text{calculated food Fe absorption} \times 0{\cdot}40}{\text{calculated reference Fe salt absorption}}.$$

A value of 0·40 was chosen as the reference point, since it represents approximately the amount absorbed by subjects who lack Fe stores but are not yet anaemic (Hallberg, 1981); the corrected food Fe absorptions would therefore be expected to provide a sensitive indicator of the relative bioavailability of Fe in different meals. The Fe absorptions and the ferritin concentrations were expressed as geometric means and standard deviation ranges, since individual experiments showed considerable variation, with positive skewing. The significance of differences between the corrected absorptions of the two isotopes used in each study was calculated using Student's *t* test for paired observations.

Haemoglobin concentrations were determined by the cyanmethaemoglobin technique. Serum Fe concentrations and unsaturated Fe-binding capacities were measured using the methods of the International Committee for Standardisation in Haematology (1978*a*, *b*). Serum ferritin was measured using the ELISA radioimmunoassay technique of Conradie & Mbhele (1980). The total and extractable polyphenol contents of vegetables were measured by a modification of the method of Singleton & Rossi (1965).

Ethical considerations

Approval for the studies to be performed 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 was completely retained, the total 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 makes the radiation exposure proportionately smaller.

RESULTS

The effect of various organic acids on Fe absorption from a rice meal

As expected, Fe absorption from the unsupplemented rice was low, ranging between 0·028 and 0·049 (Table 1). In the first study the amount of ascorbic acid present in an equivalent weight of potatoes (i.e. 15 mg) was found to increase Fe absorption to 0·081. An equimolar amount of citric acid (BDH Lab. Chemicals Div., Poole, Dorset) produced a mean absorption of 0·099, but this was not significantly higher than from the unsupplemented rice (t 1·51, $P > 0·1$). However, a highly-significant improvement occurred when the amount of citric acid was increased to 1 g, the absorption trebling from 0·028 to 0·085 (t 4·7, $P < 0·0005$). When 1 g tartaric acid (Analytical Grade; Merck, Darmstadt) was substituted, the mean absorption increased from 0·041 to 0·096 (t 2·5, $P < 0·025$), and with 1 g L-malic acid (Sigma Chemical Co., St Louis) it rose from 0·048 to 0·095 (t 2·8, $P < 0·025$).

In a final experiment, 1 g calcium oxalate (BDH Chemicals, Poole, Dorset) was added to cabbage and the effect on the absorption of Fe was examined. There was a moderate depression from a geometric mean of 0·320 (SD 0·192–0·532) to 0·195 (SD 0·103–0·370) (t 3·1, $P < 0·01$).

Effect of sodium phytate and of tannic acid on Fe absorption

The effect of two possible inhibitors of Fe absorption was tested by adding them to broccoli (Table 2). In the first experiment, 2 g sodium phytate reduced the geometric mean Fe absorption from 0·152 to 0·035 (t 4·8, $P < 0·0005$), while in the second experiment 500 mg of the polyphenolic compound tannic acid diminished it from 0·297 to 0·0150 (t 6·0, $P < 0·0001$).

Factors affecting the absorption of Fe from a number of vegetables

There was a wide range in Fe absorption from the seventeen vegetables studied (Table 3), the lowest being wheat germ (0·007) and the two highest turnip (0·327) and sauerkraut (0·327). Since it seemed likely that the variations might, in part, be due to differences in the concentrations of inhibitors and enhancers of Fe absorption in individual vegetables, an attempt was made to relate the absorption findings to the concentrations of phytate and organic acids contained within them according to published tables (Diem & Lentner, 1970; Paul & Southgate, 1976) (Table 4).

The vegetables could be divided into two distinct groups with regard to Fe absorption. In the first, which included wheat germ (0·007), aubergine (0·007), butter beans (0·012), spinach (0·014), brown lentils (0·024), beetroot greens (0·024) and green lentils (0·032), the bioavailability of Fe was poor. In the second group the bioavailability was moderate or good, namely carrot (0·098), potato (0·115), beetroot (0·185), pumpkin (0·206), broccoli

Table 1. *The effect of different organic acids on the absorption of Fe (3 mg) as $FeSO_4 . 7H_2O$ from a basic rice meal (215 g/subject) in fasting female subjects*

(Mean values and standard deviations)

								Fe absorption*					
		Haemoglobin (g/l)		Transferrin saturation (%)		Serum ferritin (μg/l)		Reference salt†		Rice meal		Rice meal with organic acid	
Organic acid	No. of subjects	Mean	SD	Mean	SD	Geometric mean‡	±1 SD	Geometric mean‡	±1 SD	Geometric mean‡	±1 SD	Geometric mean‡	±1 SD
—	25	126	18	26·2	14·9	14·7	(4·3–50·3)	0·320	(0·195–0·525)	0·031	(0·016–0·059)	—	—
Ascorbic acid 15 mg	9	146	8	35·3	11·6	42·6	(19·9–91·0)	0·242	(0·142–0·413)	—	—	0·081	(0·033–0·196)
Citric acid 36 mg	7	113	19	17·7	17·6	6·7	(1·8–25·5)	0·318	(0·197–0·515)	0·049	(0·023–0·101)	0·099	(0·052–0·186)
Citric acid 1 g	9	125	15	25·6	12·1	18·2	(4·5–73·0)	0·272	(0·169–0·436)	0·028	(0·016–0·051)	0·085	(0·047–0·154)
Tartaric acid 1 g	11	132	12	22·0	10·4	18·8	(4·3–82·9)	0·367	(0·177–0·761)	0·041	(0·013–0·133)	0·096	(0·037–0·248)
Malic acid 1 g	8	129	8	24·8	9·7	11·5	(5·4–24·6)	0·321	(0·221–0·466)	0·048	(0·026–0·091)	0·095	(0·053–0·169)

* Individual results adjusted to a 0·40 reference absorption.

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

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

Table 2. *The effect of sodium phytate and of tannic acid on the absorption of Fe (3 mg) from 100 g broccoli puree in fasting female subjects*

(Mean values and standard deviations)

	Haemoglobin (g/l)		Transferrin saturation (%)		Serum ferritin (μg/l)			Fe absorption*	
No. of subjects	Mean	SD	Mean	SD	Geometric mean†	±1 SD	Vegetable	Geometric mean†	±1 SD
19	129	19	24·6	13·1	23·3	(7·0–77·1)	Broccoli	0·152	(0·068–0·343)
							Broccoli+2 g sodium phytate	0·035	(0·019–0·121)
8	127	13	27·4	7·4	23·0	(11·4–46·5)	Broccoli	0·297	(0·130–0·513)
							Broccoli+ 500 mg tannic acid	0·015	(0·003 – 0·067)

* No reference absorption studies were done and the absorption results are, therefore, uncorrected.

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

Table 3. *The absorption in fasting female subjects of Fe (3 mg) as $FeSO_4 . 7H_2O$ from 100 g puree made up from different vegetables*

(Mean values and standard deviation ranges)

							Fe absorption*				
	Haemoglobin (g/l)		Transferrin saturation (%)		Serum ferritin (μg/l)		Reference salt†		Vegetable		
No. of subjects	Mean	SD	Mean	SD	Geometric mean‡	±1 SD	Geometric mean‡	±1 SD		Geometric mean‡	±1 SD
6	110	48	20·3	7·5	17·7	(4·1–76·3)	0·418	(0·228–0·767)	Wheat-germ	0·007	(0·003–0·014)
7	129	6	20·6	8·6	9·4	(4·1–21·9)	0·439	(0·248–0·799)	Aubergine	0·007	(0·002–0·026)
6	110	48	20·3	7·5	17·7	(4·1–76·3)	0·418	(0·288–0·767)	Butter beans	0·012	(0·007–0·020)
7	135	7	29·7	6·7	49·2	(27·3–88·7)	0·271	(0·181–0·406)	Spinach	0·014	(0·006–0·032)
11	121	17	22·0	11·9	7·7	(1·7–34·1)	0·348	(0·147–0·825)	Brown lentils	0·024	(0·005–0·105)
4	139	6	22·3	7·7	21·2	(11·4–39·6)	0·229	(0·104–0·503)	Beetroot greens	0·024	(0·003–0·175)
11	121	17	22·0	11·9	7·7	(1·7–34·1)	0·348	(0·147–0·825)	Green lentils	0·032	(0·009–0·109)
9	129	25	25·4	8·2	24·7	(14·9–40·7)	0·339	(0·193–0·597)	Carrot	0·096	(0·052–0·187)
9	146	8	35·3	11·6	42·6	(19·9–91·0)	0·218	(0·138–0·343)	Potato	0·115	(0·057–0·233)
25	136	16	22·6	9·5	19·1	(7·3–50·3)	0·286	(0·126–0·673)	Beetroot	0·185	(0·075–0·458)
7	129	6	20·6	8·6	9·4	(4·1–21·9)	0·439	(0·248–0·779)	Pumpkin	0·206	(0·157–0·270)
8	134	11	32·0	13·1	17·3	(7·0–42·6)	0·295	(0·165–0·526)	Tomato	0·224	(0·110–0·456)
8	139	9	25·9	7·1	31·5	(10·8–91·4)	0·246	(0·164–0·369)	Broccoli	0·260	(0·139–0·486)
18	133	45	25·1	9·2	23·6	(9·7–57·4)	0·217	(0·099–0·476)	Cauliflower	0·263	(0·089–0·455)
6	130	9	24·0	10·4	13·0	(6·6–26·1)	0·589	(0·354–0·981)	Cabbage	0·320	(0·192–0·532)
10	146	18	23·9	9·5	18·6	(6·2–55·0)	0·489	(0·353–0·679)	Turnip	0·327	(0·192–0·556)
6	139	3	27·2	6·3	29·3	(12·3–70·0)	0·306	(0·172–0·543)	Sauerkraut	0·327	(0·208–0·512)

* Individual results adjusted to a 0·40 reference absorption.

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

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

Table 4. *Phosphorus, polyphenol and organic acid contents of vegetables*

	Phosphorus (mg/kg)*	Polyphenols (mg/g)		Organic acids (mg/kg edible portion)*			
		Total	Extractable	Malic	Citric	Oxalic	Ascorbic
Wheat germ	11180	8·4	2·0	0	3400	0	0
Aubergine	150	3·4	0·4	170	0	70	50
Butter beans	3200	3·5	0·6	—	—	—	0
Spinach	510	6·9	1·1	90	800	4600	500
Brown lentils	3800	6·8	1·8	0	0	0	0
Beetroot greens	40	6·6	2·3	0	0	9200	300
Green lentils	3800	6·3	2·9	0	0	0	0
Carrot	360	0·9	0·2	24	900	330	100
Potato	530	0·9	0·7	0	5100	60	200
Beetroot	330	2·4	0·9	0	1100	3400	100
Pumpkin	440	0·7	0·6	150	0	0	90
Tomato	270	0·6	0·3	150	3900	80	230
Broccoli	780	3·2	2·8	120	2100	0	1130
Cauliflower	560	1·6	0·9	390	2100	0	780
Cabbage	270	0·7	0·6	0	0	0	460
Turnip	300	0·9	0·6	230	0	0	360
Sauerkraut	180	0·6	0·4	0†	0†	0†	140

* Values from Diem & Lentner (1970) and Paul & Southgate (1976).
† Lactic acid 1600 mg/kg.

(0·260), cauliflower (0·263), tomato (0·224), cabbage (0·320), turnip (0·327) and sauerkraut (0·327). All except one of the second group of vegetables contained reasonable quantities of one or more of the organic acids, malic, citric and ascorbic acids. The exception was sauerkraut, which contains a large amount of lactic acid. The role of organic acids was further defined in another experiment in which broccoli was given with and without the cooking water in which it was boiled, since a significant proportion of the acids in the broccoli would be dissolved in the water. Haematological information on the ten housewives who took part in this study were as follows: mean haemoglobin concentration 14·0 g/l (SD 0·95), mean transferrin saturation 27·1% (SD 6·9), geometric mean serum ferritin concentration 41·3 μg/l (SD 12·8–133·7) and geometric mean reference absorption 0·205 (SD 0·106–0·398). When the broccoli was given with the cooking water the corrected geometric mean Fe absorption was 0·253 (SD 0·148–0·432) compared with 0·115 (SD 0·068–0·193) when the water was discarded (t 4·1, $P < 0·005$). No relationship between oxalate content and Fe absorption emerged when the three vegetables that contained large amounts of oxalate were examined: Fe absorption was poor from spinach and beetroot greens and good from beetroot. On the other hand the P and, therefore, presumably the phytate contents, were high in wheat germ, butter beans and lentils, in all of which the Fe bioavailability was low.

The results of a further experiment raised the possibility that the polyphenols present in certain vegetables might also exert a profound effect on Fe absorption. A 10 g ferric chloride/l solution was added to individual vegetables, since it has previously been shown that when ferric salts are added to a solution containing polyphenols a bluish-black discoloration develops due to the formation of coloured Fe complexes (Seikel, 1964; Disler, Lynch, Torrance *et al.* 1975). Marked darkening of aubergine, spinach, green and brown lentils and beetroot greens was noted, and the bioavailability of the Fe in each had been found to be low. In contrast, a vegetable such as pumpkin, from which Fe was well absorbed, did not darken noticeably when Fe was added. The total polyphenol content

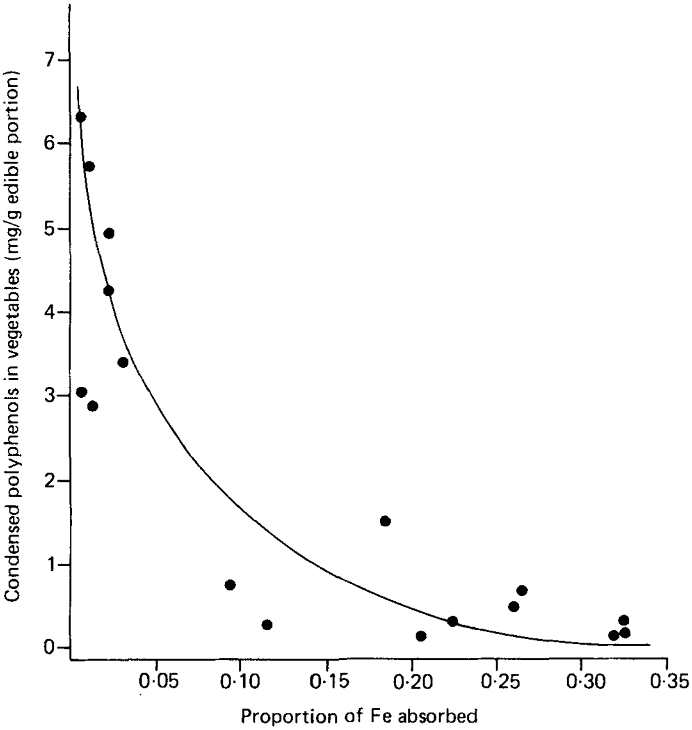

Fig. 1. The inverse correlation between the condensed polyphenol content of vegetables (total – extractable polyphenols) and Fe absorption from them. The line is derived from the least squares regression after square-root transformation (r – 0·90).

of all the vegetables was therefore measured (Table 4) and a significant inverse correlation with Fe absorption was noted (r – 0·859; $P < 0·001$). It seemed that the effect was due to condensed rather than to hydrolysable polyphenols, since the inverse correlation was even closer (r – 0·901; $P < 0·001$) when the amounts of extractable polyphenols had been subtracted (Fig. 1). In these analyses square-root conversion of the values was used as this normalized the values which were otherwise positively skewed.

DISCUSSION

Fe deficiency has been shown to be a common problem in the Indian population in which this series of studies was undertaken, with a 26% prevalence in the females (Mayet *et al.* 1972; MacPhail *et al.* 1981). These findings were confirmed by our present results, which showed that the geometric mean serum ferritin level in most of the groups of women was a good deal less than the normal value of 35 μg/l.

In each experiment, 3 mg inorganic Fe labelled with radioactive-Fe was added to the meal and the absorption of the isotope measured. The results reflected the absorption of not only the added Fe but also the intrinsic rice or vegetable Fe, since it has previously been well established that there is free isotopic exchange within the lumen of the gastrointestinal tract under such circumstances (Bothwell *et al.* 1979; Hallberg, 1981).

In the first series of studies, different organic acids were added to a rice meal to examine their individual effects. Citric, tartaric and malic acids in 1 g quantities were each found to increase Fe absorption significantly. These findings may well have relevance to Fe nutrition since citrus fruits, such as oranges, lemons and grapefruit, contain a minimum of

10 g citric acid/kg, while malic acid is found in the deciduous fruits such as plums, peaches and apples, in concentrations ranging from 3 g/kg to greater than 10 g/kg (Diem & Lentner, 1970; Paul & Southgate, 1976). Tartaric acid is not present in vegetables but is found in concentrations ranging from 1630–2340 mg/kg in white wines (see Diem & Lentner, 1970) and may play a part in the high bioavailability of the Fe they contain. Although the concentrations of individual organic acids in vegetables are not notably high, they usually contain more than one of them, and much of the considerable variation in the bioavailability of the Fe given with different vegetables in the present study could be ascribed to differences in the content of malic, citric and ascorbic acids (Table 4). Carrots, potato, beetroot, pumpkin, broccoli, cauliflower, tomato, cabbage and turnip were associated with good Fe bioavailability and contained one or more of the organic acids in reasonable concentrations. Ascorbic acid appeared to be the major promoting agent in cabbage, and malic acid in pumpkin, but in other vegetables it was not possible to dissect out the individual effects of the different acids. The good absorption of Fe from sauerkraut may have been due to its high lactic acid content, since this acid has previously been shown to be an effective promoter (Derman, Bothwell, Torrance *et al.* 1980). The high bioavailability of Fe in some of these vegetables was underlined by the fact that the absorption from cabbage, turnip and sauerkraut was as much as 80% of that of a 3 mg reference dose of ferrous ascorbate.

Meat is an important dietary constituent for adequate Fe nutrition, since it not only potentiates the absorption of the non-haem-Fe in the meal, but it also contains haem-Fe which is well absorbed since it is not vulnerable to any inhibitory ligands that may be present (Layrisse *et al.* 1969; Cook & Monsen, 1976; Monsen *et al.* 1978). The low meat intake of many populations has, therefore, been regarded as one of the factors responsible for the widespread occurrence of dietary Fe deficiency, and the critical importance for Fe nutrition of the ascorbic acid content of such diets has been stressed. In the present series of experiments, however, it was possible to show that ascorbic acid is just one of several organic acids which potentiate non-haem-Fe absorption, although it is the most potent of them.

The one organic acid that was found to have a moderate depressing effect on Fe absorption was oxalic acid. However, its role, if any, in modifying the bioavailability of Fe in vegetables was not clearly elucidated. While the addition of calcium oxalate, which is one of the two major salts of oxalic acid in vegetables (Diem & Lentner, 1970; Paul & Southgate, 1976), to broccoli caused some depression in Fe absorption, there was a wide range of absorption from vegetables containing large amounts of oxalate. The bioavailability of the Fe in these vegetables therefore appeared to depend more on the presence of other inhibitors and enhancers. Two factors possibly responsible for the poor bioavailability of Fe in some of the vegetables were identified. Sodium phytate and the polyphenol, tannic acid, were shown in separate experiments to inhibit the absorption of vegetable Fe, which is in line with previous findings (Hallberg & Solvell, 1967; Disler, Lynch, Charlton, Torrance *et al.* 1975). It therefore seemed probable that the presence of certain phytates and polyphenols in food might have a similar effect and, indeed, Fe absorption was found to be uniformly poor in those vegetables such as wheat germ, butter beans and lentils that contain large amounts of P, presumably mostly in the form of phytates (van Soest, 1978). As far as polyphenols are concerned, we have previously shown that tea, which contains tannates, is a potent inhibitor of Fe absorption (Disler, Lynch, Charlton, Torrance *et al.* 1975). Suggestive evidence that polyphenols, of which tannates are an example, might be important inhibitors of vegetable Fe absorption, was obtained when Fe was added to two vegetables, namely aubergine and spinach. The bluish-black colour that developed suggested that they might contain significant amounts of phenolic compounds (Seikel, 1964), and that

Fe was poorly absorbed despite the presence of adequate organic acids and little phytate. It was then observed that the other vegetables, including brown and green lentils and beetroot greens, that showed marked colour changes on the addition of Fe, were also associated with poor Fe bioavailability. The total and extractable phenol contents of all the vegetables on which Fe absorption measurements had been done were therefore determined, and a strong inverse correlation between the total polyphenol content and Fe absorption was found ($r - 0{\cdot}859$, $P < 0{\cdot}001$). The fact that the inverse correlation was even greater when the extractable polyphenol content had been subtracted from the total value ($r - 0{\cdot}901$; $P < 0{\cdot}001$) suggested that it was the non-hydrolysable condensed polyphenols that were responsible for the inhibitory effect on Fe absorption.

The present findings have a relevance wider than that of the bioavailability of the Fe contained within the individual vegetables tested. The Fe content of vegetables varies considerably, and in some is low. However, the effect of the inhibitors and enhancers of Fe absorption which they contain is not confined to their endogenous Fe, but extends to the whole pool of non-haem-Fe in the particular meal of which they form a part. In this context vegetables such as broccoli, cauliflower, cabbage and turnip exert a positive effect on Fe nutrition, while the opposite is the case with wheat germ, aubergine, butter beans, lentils and spinach.

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