British Journal of Nutrition (1988) **59**, 205–213

Iron absorption from fortified flat breads

BY MOHAMED EL GUINDI, SEAN R. LYNCH AND JAMES D. COOK*

Department of Medicine, University of Kansas Medical Center, Kansas City, Kansas 66103, USA

(Received 1 July 1987 – Accepted 6 October 1987)

1. Radio-iron absorption measurements were performed in healthy volunteer subjects to assess the availability of fortification Fe added to various bread products.

2. When ferrous sulphate was used as a fortifier, Fe absorption from a traditional Egyptian flat bread (Baladi) averaged only 16% of that observed with European bread. This difference was attributed to the high extraction flour used to prepare Baladi bread.

3. The inhibiting effect of Baladi bread was largely eliminated by adding EDTA to the flour before baking.

Wheat flour is the only food vehicle that has been used extensively for iron fortification at a national level. At the present time, fortification Fe in wheat flour and bakery products provides approximately 20% of the Fe consumed in North America and nearly 40% of that in Sweden. Although epidemiological surveys have never fully defined the efficacy of wheat-flour fortification, it is commonly assumed that it has contributed to the reduction in the prevalence of Fe deficiency in countries where food Fe fortification is mandatory (Hallberg *et al.* 1979; Cook *et al.* 1986).

A major advantage of employing bread products as a vehicle for Fe is the facilitating effect of wheat flour on Fe absorption as compared with other cereal foods. Fe absorption from Western wheat-based meals in Fe-deficient subjects varies between 20 and 30%, while only 2–4% of the Fe in rice and maize meals is assimilated (Cook *et al.* 1972; Bjorn-Rasmussen & Hallberg, 1974; Hallberg *et al.* 1977; International Nutritional Anemia Consultative Group (INACG), 1982). Because wheat is a dietary staple in Egypt where Fe deficiency is highly prevalent, the fortification of wheat bread is a logical intervention strategy in this country. However, there is reason to suspect that Egyptian flat breads (Baladi and Shami) differ in their effect on Fe availability compared with European bread consumed in countries where there is national fortification. Baladi bread is made with 82%-extraction flour produced within the country, whereas Shami bread is prepared with imported 72%-extraction flour. Absorption studies in humans indicate that higher-extraction flour substantially reduces the availability of added Fe (Widdowson & McCance, 1942; Bjorn-Rasmussen, 1974; Simpson *et al.* 1981). Furthermore, both types of Egyptian breads are baked at a much higher temperature than European bread. The present report describes studies performed to measure the availability of fortification Fe in commonly consumed Egyptian breads.

MATERIALS AND METHODS

Study design

Four separate studies were performed in groups of eight to eleven volunteer subjects. In each study, four Fe absorption tests were carried out as two successive sets of double-radioisotope measurements. Study 1 was performed to determine the validity of extrinsic radio-Fe labelling for evaluating the availability of Fe in Egyptian breads. Study 2

* For reprints

compared absorption from different types of bread eaten alone and study 3 examined the absorption of fortified flat breads consumed with typical Egyptian meals. Because absorption was poor in the first three studies, study 4 was performed to determine whether absorption from fortified Egyptian bread could be improved by adding EDTA to the fortifier, ferrous sulphate.

Subjects

A total of thirty-six volunteers, fifteen male and twenty-one female, between the ages of 19 and 35 years participated in these studies. Six were Fe-deficient as defined by a serum ferritin level less than 12 μg/l. None gave a history of disorders that might affect the absorption of Fe from the gastrointestinal tract and all stated that they were in good health. Written informed consent was obtained from each subject before entering the study and all experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

Absorption measurements

All test meals were eaten between 07.00 and 09.00 hours after a 10 h fast. Only water was allowed for 3 h following the meals. Each meal was labelled with either 1 μCi $^{59}FeCl_3$ or 3 μCi $^{55}FeCl_3$, except in study 1 in which both tags were used in the first meal. On the 1st day of each study, 15 ml blood were drawn for measurements of serum ferritin (Miles *et al.* 1974) and background blood radioactivity. Test meals were administered on days 2 and 3. After 14 d, 25 ml blood were obtained to measure incorporated erythrocyte radioactivity, and the second pair of test meals was administered on the subsequent 2 d. Study 1 comprised only two test meals given on days 2 and 16. A further 25 ml blood were drawn 14 d after the final meal. Absorption from the second pair of meals was calculated from the increase in blood radioactivity between days 17 and 30. Assays for ^{55}Fe and ^{59}Fe were performed on duplicate 10 ml blood samples using a modification of the method of Eakins and Brown (Bothwell *et al.* 1979). Sufficient counts were obtained on each sample to reduce the net counting error for each isotope to less than ± 2% in subjects absorbing more than 1% of the test dose. Absorption was determined on the basis of the blood volume (Wennesland *et al.* 1959; Brown *et al.* 1962) assuming 80% erythrocyte incorporation (Hosain *et al.* 1967) of absorbed radio-Fe.

Test meals

For studies 1 and 2 each serving of Baladi bread was prepared by thoroughly mixing 100 g 82%-extraction stone-milled Egyptian flour with 65 ml water, 0.5 g salt and 1·5 g yeast. The dough was allowed to rise for 2 h. Each portion was then rolled into a disk approximately 180 mm in diameter and allowed to rise for an additional 1 h. The loaves were baked for 1 min at 470° in a muffle furnace. The loaf size was reduced by 25% in studies 3 and 4. Shami bread was prepared from 72%-extraction flour in the same way. The identical recipe and 72%-extraction flour were also used to make French bread, but the dough was made into rolls and baked at 250° for 5 min. The Fe contents of flour samples and portions of homogenized meals were determined by atomic absorption spectroscopy after they had been dry ashed.

The validity of the extrinsic-tag technique was examined in study 1. The first meal contained only a loaf of unfortified Baladi bread served with 14 g butter. Loaves were prepared individually to assure that the Egyptian flour was mixed with wheat that had been biosynthetically labelled with ^{55}Fe by hydroponic culture (Moore & Dubach, 1951); 1 g ^{55}Fe-labelled flour (Fe content 12 mg/kg, specific activity 0·25 μCi/μg) was carefully mixed into the dough of each loaf to provide 3 μCi ^{55}Fe per loaf. An extrinsic tag containing 1 μCi ^{59}Fe as $FeCl_3$ (0·1 mg Fe) in 1·0 ml 0·01 M-hydrochloric acid was also mixed into the dough. Thus an accurately measured quantity of each radio-Fe tracer was administered to each volunteer subject. The second meal in study 1 was designed to test the effect of baking on radio-Fe absorption. Only extrinsic tags were used. Sufficient $FeSO_4$ (Paniplus Co., Olathe, KS) was added to the flour to provide 2·2 mg additional Fe per loaf, increasing the total Fe content to 4·2 mg. The radio-Fe tag (3 μCi ^{55}Fe as $FeSO_4$ with 0·1 mg carrier Fe in 0·01 M-sulphuric acid) was mixed into the dough for each loaf before baking. The second extrinsic tag (0·1 mg Fe as $FeCl_3$ in 1 ml 0·01 M-HCl containing 1 μCi ^{59}Fe) was injected into the interior surface of the bread just before it was eaten.

The availability of Fe in various breads was examined in study 2 in which four test meals were given on separate days. The first three meals (A–C) consisted of a 120 g loaf of Baladi, Shami, or French bread with 14 g butter. Fortification Fe (3 mg Fe as $FeSO_4$ was added to the 100 g flour used to prepare each loaf. The extrinsic tag (0·1 mg Fe as $FeSO_4$ in 1·0 ml 0·01 M-H_2SO_4) was injected into each loaf just before serving. Meal D was the reference dose, i.e. a freshly prepared solution of 3 mg Fe as $FeSO_4$ and 18·9 mg ascorbic acid (molar ratio, 2:1) in 50 ml water.

Fe absorption from fortified Baladi bread consumed with Egyptian meals was evaluated in study 3. Meals A and B, representing a typical lunch or dinner meal, contained 125 g green beans (*Phaseolus vulgaris*), 17 g minced onion, 2·8 g margarine and 18 g tomato paste cooked in 40 ml water. They were served with 100 g boiled rice and an Fe-fortified Baladi bread loaf. The total Fe content of each meal was 5·5 mg. Water was given *ad lib.* with meal A and 180 ml hot tea with meal B. Meal C was similar to an Egyptian breakfast and comprised 90 g fava beans (*Vicia fava*), 10 g lentils (*Lens esculenta*), 5·2 g vegetable oil, 3·5 g calcium hydroxide and 160 ml water. The total Fe content was 5·9 mg. The meals were tagged as described previously. Meal D again consisted of the reference dose.

Study 4 was undertaken to determine whether the low absorption from Egyptian breads observed in the first three studies could be improved by adding EDTA with the fortification Fe. Fe-EDTA is less affected by inhibitory factors than $FeSO_4$ (MacPhail *et al.* 1981). It was not necessary to prepare Fe-EDTA since all soluble non-haem Fe compounds present in most meals enter a common pool before being absorbed (Bothwell *et al.* 1979). The percentage absorption of Fe from the pool is determined by the interaction of enhancing and inhibiting ligands with the whole Fe pool and is independent of the form in which the Fe enters it. EDTA acts on the common non-haem pool and we have previously demonstrated that the same level of Fe absorption is obtained by adding Na_2EDTA:Fe to a meal in a molar ratio of 1:1 as that achieved when radiolabelled Fe-EDTA is used (T. A. Morck, S. R. Lynch and J. D. Cook, unpublished results). Four separate meals were served. The first three consisted of a Baladi bread loaf and 14 g butter. For meals A and B, 3·0 mg fortification Fe as $FeSO_4$ containing 3 μCi ^{55}Fe or 1 μCi ^{59}Fe were added to each 100 g flour while making the dough. Na_2EDTA (200 mg/kg flour) was also added to meal B. For meal C, unfortified flour was used; 2·2 mg Fe as $FeSO_4$ with 1 μCi ^{59}Fe and 14·6 mg Na_2EDTA were mixed with the butter just before serving the meal. Meal D was the reference dose.

Statistical analysis

Because of the highly skewed distribution of Fe absorption values when expressed as a percentage of the administered dose, individual values were converted to logarithms for statistical analysis and the results reconverted to anti-logarithms to recover the original units (Cook *et al.* 1969). All values for Fe absorption and meal ratios are reported as geometric means. When absorption for any pair of test meals was compared in the same subject, a paired *t* test was used to determine whether the mean difference in log absorption values differed significantly from 0.

Table 1. *Iron absorption from unfortified and fortified Baladi bread**

					Fe absorption (% of dose)					
					Unfortified		Fortified			
Subject no.	Sex	Age (years)	Packed cell volume	Serum ferritin (μg/l)	Intrinsic label (A)	Extrinsic label (B)	Before baking (C)	After baking (D)	Absorption ratio B:A	C:D
1	♂	22	0·42	96	0·96	0·83	1·38	0·93	0·86	1·48
2	♂	23	0·47	61	1·02	0·76	0·26	0·71	0·74	0·37
3	♂	22	0·46	109	1·61	1·30	0·76	0·70	0·80	1·08
4	♂	22	0·44	79	2·58	2·61	2·85	1·81	1·01	1·57
5	♂	22	0·46	101	2·68	2·57	1·75	0·81	0·95	0·31
6	♂	23	0·50	43	2·81	3·22	3·61	1·73	1·14	2·09
7	♂	24	0·46	81	3·07	2·70	1·40	1·32	0·87	1·06
8	♂	19	0·44	107	7·66	8·03	10·22	5·97	0·48	1·71
Mean†	—	22	0·46	81	2·28	2·10	1·70	1·32	0·92	1·29
−2 SE	—	—	—	—	1·42	1·21	0·79	0·80	0·84	0·52
+2 SE	—	—	—	—	3·66	3·65	3·67	2·19	1·02	1·16

* For details see pp. 206–207.
† Geometric mean except for age and packed cell volume.

Table 2. *Iron absorption from different fortified breads**

					Fe absorption (% of dose)						
Subject no.	Sex	Age (years)	Packed cell volume	Serum ferritin (μg/l)	French bread (A)	Shami bread (B)	Baladi bread (C)	Reference (D)	Absorption ratio B:A	C:B	C:A
1	♂	23	0·47	177	2·07	2·31	1·08	11·68	1·11	0·46	0·52
2	♂	20	0·50	29	3·82	7·68	1·80	40·23	2·01	0·23	0·47
3	♂	20	0·51	97	5·60	2·76	0·67	23·11	0·49	0·24	0·11
4	♀	22	0·40	19	13·76	3·76	1·17	33·17	0·27	0·31	0·08
5	♀	29	0·47	16	24·22	8·60	1·10	64·73	0·35	0·12	0·04
6	♀	23	0·45	9	38·32	27·88	7·67	56·03	0·72	0·27	0·20
7	♀	26	0·38	11	43·47	33·31	6·02	67·27	0·76	0·18	0·13
8	♀	23	0·44	9	44·88	20·40	6·46	91·47	0·45	0·31	0·14
Mean†		23	0·45	25	13·50	8·60	2·17	41·03	0·64	0·25	0·16
−2 SE		—	—	—	5·77	4·10	1·10	25·54	0·40	0·19	0·09
+2 SE		—	—	—	31·60	18·04	4·31	65·92	1·01	0·33	0·29

* For details, see pp. 206–207.
† Geometric mean except for age and packed cell volume.

RESULTS

Labelling technique (*study* 1). There was no appreciable difference in absorption of the intrinsic and extrinsic radio-Fe tags from Baladi bread (Table 1). Mean absorption was 2·3 % from the intrinsic label and 2·1 % from the extrinsic tag, giving a mean absorption ratio of 0·92 (not significantly different from 1·00; t 1·45, $P = 0{\cdot}18$). In the second pair of test meals, absorption of Fe added to the dough was slightly higher than that of Fe added to the bread after it had been baked (1·7 *v.* 1·3 %, meals C and D respectively), but the

Table 3. *Iron absorption from fortified Baladi bread consumed with typical Egyptian meals**

Subject no.	Sex	Age (years)	Packed cell volume	Serum ferritin (μg/l)	Fe absorption (% of dose): Baladi bread: Rice and vegetables + water (A)	Rice and vegetables + tea (B)	Fava beans† (C)	Reference (D)	Absorption ratio: A:D	B:D	C:D
1	♀	24	0·42	54	0·33	0·07	0·45	9·26	0·03	0·01	0·04
2	♂	23	0·46	167	0·62	0·20	0·35	11·00	0·05	0·01	0·03
3	♂	27	0·51	74	0·63	0·05	0·46	16·11	0·03	0·01	0·02
4	♂	31	0·47	63	2·12	0·47	0·76	18·03	0·11	0·02	0·04
5	♀	22	0·43	19	2·27	1·60	2·72	41·33	0·05	0·03	0·06
6	♀	34	0·44	17	2·81	1·37	13·36	49·98	0·05	0·03	0·06
7	♀	23	0·40	48	4·65	2·67	1·68	18·03	0·25	0·14	0·09
8	♀	22	0·43	8	4·68	1·86	2·91	20·61	0·22	0·09	0·14
9	♀	34	0·41	7	18·47	8·15	19·82	85·21	0·21	0·09	0·23
Mean‡		27	0·44	32	2·07	0·68	1·80	23·18	0·09	0·03	0·08
−2 SE		—	—	—	0·90	0·21	0·67	14·23	0·06	0·02	0·04
+2 SE		—	—	—	4·76	2·14	4·80	37·77	0·20	0·07	0·13

* For details, see pp. 206–207.
† *Vicia fava*.
‡ Geometric mean except for age and packed cell volume.

Table 4. *Iron absorption from Baladi bread fortified with ferrous sulphate or Fe-EDTA**

Subject no.	Sex	Age (years)	Packed cell volume	Serum ferritin (μg/l)	Fe absorption (% of dose): Baladi bread: $FeSO_4$ (A)	Fe-EDTA: Before baking (B)	Fe-EDTA: After baking (C)	Reference (D)	Absorption ratio: B:A	C:A	B:C
1	♀	28	0·43	72	0·41	1·37	1·52	13·52	3·34	3·70	0·09
2	♂	29	0·43	133	0·45	1·63	1·55	17·68	3·62	3·44	1·05
3	♀	27	0·39	58	0·75	2·03	2·11	17·43	2·70	2·81	0·96
4	♀	35	0·36	37	1·03	3·10	3·36	40·74	3·00	3·26	0·92
5	♀	26	0·41	16	1·51	8·38	11·21	64·28	5·54	7·42	0·74
6	♀	25	0·40	26	1·62	3·41	13·51	8·90	2·10	2·16	0·97
7	♀	24	0·38	8	2·05	13·41	27·17	106·47	6·54	13·25	0·49
8	♀	23	0·43	44	2·63	3·41	5·43	31·06	1·29	2·06	0·62
9	♀	30	0·43	33	3·18	4·03	14·61	62·48	1·26	4·59	0·27
10	♀	20	0·42	15	4·30	6·22	8·81	26·27	1·44	2·04	0·70
11	♀	21	0·42	31	6·26	11·82	9·37	45·38	1·88	1·49	1·26
Mean†		26	0·41	32	1·59	4·10	5·41	30·83	2·58	3·41	0·76
−2 SE		—	—	—	0·93	2·58	3·04	19·57	1·84	2·33	0·59
+2 SE		—	—	—	2·71	6·51	9·63	47·58	3·61	4·99	0·98

* For details, see pp. 206–207.
† Geometric mean except for age and packed cell volume.

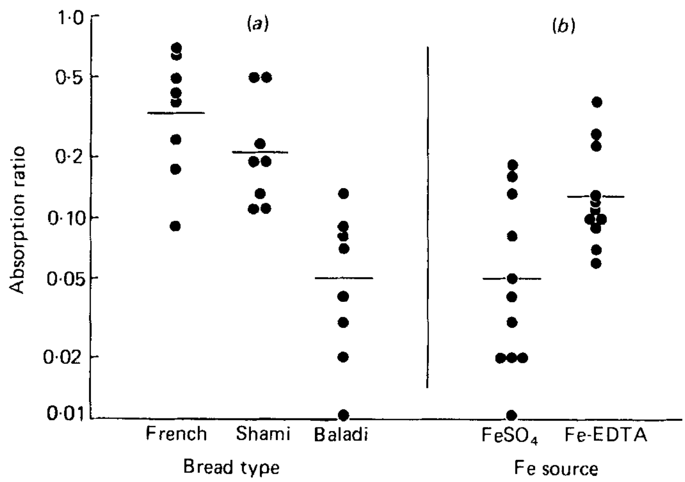

Fig. 1. Effect of type of bread and fortification iron on Fe absorption, expressed as a ratio with respect to reference dose absorption. (*a*) Fortified with ferrous sulphate for all meals; (*b*) Baladi bread was used for both studies. The horizontal bars represent the geometric mean values. Note that by expressing absorption relative to the reference dose, an identical absorption ratio was obtained for Baladi bread fortified with $FeSO_4$ in the two studies.

difference was not statistically significant (t 1·17, $P = 0·28$). It should be noted that by increasing the Fe content of the bread from 1·8 mg (meal B) to 4·0 mg (meal D), there was a modest decrease in percentage absorption, although this difference was not significant (t 1·06, $P = 0·32$).

Comparison of bread products (*study* 2). The availability of Fe from the three bread products was quite different (Table 2). Absorption from French bread (meal A) was high, averaging 13·5%. It fell to 8·6% when the same dough was baked at a higher temperature (Shami bread, meal B), although the difference was not statistically significant (t 1·84, $P = 0·10$). However, when 82%-extraction flour was used (Baladi bread) absorption decreased dramatically to 2·2% (meal C). This reduction was significant when compared with both Shami bread (mean ratio 0·25, t 9·34, $P < 0·001$) and with French bread (mean ratio 0·16, t 5·89, $P < 0·001$).

Effect of Egyptian meals (*study* 3). Absorption from fortified Baladi bread consumed with typical Egyptian meals was similar to that from the bread eaten alone. Mean absorption from the dinner meal (A) was 2·1% (Table 3), giving a mean absorption ratio relative to the reference dose of 0·09, which is close to the value of 0·05 observed with Baladi bread in study 2. Similarly, absorption from the breakfast meal (C) averaged 1·8%, giving a ratio relative to the reference dose of 0·08. Absorption fell significantly from 2·1% (A) to 0·68% (t 4·77, $P = 0·001$) when tea was consumed with the dinner meal (B).

Form of fortification Fe (*study* 4). A substantial improvement in availability was demonstrated when EDTA was added to Baladi bread (Table 4). When the Fe was added to the dough before baking, absorption from bread containing EDTA averaged 4·1% compared with 1·6% from Fe_2SO_4 alone. This 2·5-fold increase in absorption was highly significant (t 5·37, $P < 0·001$). Even better absorption, 5·4%, was obtained when the Fe and EDTA were added to the bread after it was baked (C), although the difference (C *v* B) was not significant (t 2·07, $P = 0·06$).

A more reliable comparison of Fe-absorption in these studies is obtained by calculating the ratio, absorption from bread:reference dose absorption (Fig. 1). The absorption ratio for Baladi bread averaged 0·05 compared with 0·33 and 0·21 observed with French and

Shami breads respectively. The addition of EDTA to the fortification Fe increased the absorption ratio from 0·05 to 0·13. The composite mean reference absorption was 30·5%. Since 40% reference-dose absorption is commonly assumed to represent Fe-deficiency (Hallberg, 1980), absorption values would be 30% higher in Fe-deficient individuals.

DISCUSSION

A reduction in the high prevalence of Fe deficiency in the developing world will require the provision of additional Fe, either by Fe supplementation or fortification. Supplemental or medicinal Fe has been used as a short-term approach during pregnancy and occasionally in school-age children. However, the efficacy of Fe supplementation has been questioned in recent years. The regular distribution of Fe tablets requires sophisticated health delivery systems which are often lacking in Third World countries. In addition, compliance is often a major obstacle to this approach because of the gastrointestinal side effects associated with medicinal Fe. The most effective strategy for reducing the prevalence of Fe-deficiency is dietary Fe fortification.

There are a number of constraints to developing an effective programme of Fe fortification at a national level (Cook & Reusser, 1983). The most critical is the availability of a suitable food vehicle. The food must reach a high proportion of the population, have no potential for idiosyncratic intake, and be equally available to people of differing socio-economic status. Further, it must be inexpensive, centrally processed to permit government regulation, and support relatively high absorption of the added Fe. In Egypt wheat flour meets these requirements. It is heavily subsidized within the country and reaches virtually all segments of the population. Wheat consumption averages 180 kg/person per year and provides nearly 50% of the average daily intake of both energy and protein. Half the population eats Baladi bread, at an average consumption of three 169 g loaves daily. Shami and French breads are also popular, with consumption levels averaging five 125 g loaves/d. Wheat flour is clearly an excellent vehicle for food Fe fortification in Egypt.

Although previous studies in human beings have demonstrated relatively high absorption when available forms of Fe are added to wheat products (International Nutritional Anemia Consultative Group, 1982), there are reasons to suspect that availability may be substantially less with Egyptian flat breads. Baladi bread is prepared from high-extraction flour, and has a high content of bran which is known to inhibit Fe absorption (Bjorn-Rasmussen, 1974). Also, the high baking temperatures used for Egyptian flat breads may render added Fe less available for absorption. In the present studies, the absorption from Shami bread was only about 65% that observed from French bread, but the difference was not statistically significant. However, a dramatic fourfold reduction was observed with Baladi bread, almost certainly due to its higher content of bran. The effect of higher baking temperature was marginal.

A major constraint to implementing Fe-fortification programmes in developing countries is the nature of the diet. Although availability from a fortified food eaten alone may be high, absorption is affected by other common pool ligands when the food is part of a meal. Most diets in developing countries are characterized by a low content of substances that promote Fe absorption, such as meat and ascorbic acid, and a high content of vegetable and cereal foods. The present study demonstrates that Fe absorption from fortified Baladi bread is no lower when eaten with food than when eaten alone, except when the meal includes tea which produces a threefold reduction in absorption. Tea, the most potent inhibitor of non-haem-Fe absorption yet identified (Disler *et al.* 1975; Rossander *et al.* 1979), is widely consumed in Egypt and this may be a far greater impediment to an effective fortification programme than the inhibiting effect of high-extraction flour. Bread is eaten both alone and with meals, but tea is almost always part of the meal. We did not examine the effect of a meal on Fe-absorption from Shami or French breads, but it is likely that an inhibitory meal would mask the observed differences in Fe absorption from the various breads.

One approach to reducing the inhibiting effect of a cereal- or vegetable-based diet on Fe absorption is to include an enhancing substance such as ascorbic acid with the fortification Fe (International Nutritional Anemia Consultative Group, 1982). Unfortunately, ascorbic acid is unstable and is ineffective in bread because oxidation occurs during baking (Sayers *et al.* 1974). EDTA is more stable and has similar enhancing properties. NaFe–EDTA (molar ratio, EDTA:Fe of 1:1) may be absorbed adequately even from strongly inhibitory meals (Garby & Areekul, 1974; Layrisse & Martinez-Torres, 1977; Viteri *et al.* 1978; MacPhail *et al.* 1981). However, unlike ascorbic acid, an increasing molar ratio of EDTA:Fe leads to reduced Fe absorption (Cook & Monsen, 1976). The use of a fortifier consisting of EDTA and Fe is therefore potentially valuable, especially in diets that do not already contain appreciable quantities of EDTA. NaFe–EDTA has been used to fortify a variety of vehicles including fish sauce in Thailand (Garby & Areekul, 1974), sugar in Guatemala (Viteri *et al.* 1978), and the seasoning, masala, in South Africa (MacPhail *et al.* 1981). The findings in the present report suggest the addition of Fe together with EDTA should also be considered in Egypt. Fe absorption from meals containing EDTA was nearly three times greater than that from those with $FeSO_4$ alone. Although EDTA is unstable at high temperatures, only minimal reduction in absorption occurred when it was added to Baladi bread before baking. Presumably exposure to these temperatures was too brief to have a major deleterious effect. The use of Fe–EDTA could offset the strongly inhibitory effects of the typical Egyptian diet.

The present work was supported by AID Cooperative Agreement DAN-0227-A-00-2104-00.

REFERENCES

Bjorn-Rasmussen, E. (1974). *Nutritional Metabolism* **16**, 101–110.

Bjorn-Rasmussen, E. & Hallberg, L. (1974). *Nutritional Metabolism* **16**, 94–100.

Bothwell, T. H., Charlton, R. W., Cook, J. D. & Finch, C. A. (1979). *Iron Metabolism in Man*, 1st edn, pp. 401–410. Oxford: Blackwell Scientific.

Brown, E., Hooper, J., Hodges, J. L., Bradley, B., Wennesland R. & Yamaushi, M. (1962). *Journal of Clinical Investigation* **41**, 2182–2190.

Cook, J. D., Layrisse, M. & Finch, C. A. (1969). *Blood* **33**, 421–429.

Cook, J. D., Layrisse, M., Martinez-Torres, C., Walker, R., Monsen, E. & Finch, C. A. (1972). *Journal of Clinical Investigation* **51**, 805–815.

Cook, J. D. & Monsen, E. R. (1976). *American Journal of Clinical Nutrition* **29**, 614–620.

Cook, J. D. & Reusser, M. E. (1983). *American Journal of Clinical Nutrition* **38**, 648–659.

Cook, J. D., Skikne, B. S., Lynch, S. R. & Reusser, M. E. (1986). *Blood* **68**, 726–731.

Disler, P. B., Lynch, S. R., Charlton, R. W., Torrance, J. D. & Bothwell, T. H. (1975). *Gut* **16**, 193–200.

Garby, L. & Areekul, S. (1974). *Annals of Tropical Medicine and Parasitology* **68**, 467–476.

Hallberg, L. (1980). In *Methods in Hematology*: *Iron*, pp. 116–133 [J. D. Cook, editor]. New York: Churchill Livingstone.

Hallberg, L., Bengtason, C., Garby, L., Lennartsson, J., Rossander, L. & Tibblin, E. (1979). *Bulletin WHO* **57**, 947–954.

Hallberg, L., Bjorn-Rasmussen, E., Rossander, L. & Suwanik, R. (1977). *American Journal of Clinical Nutrition* **30**, 539–548.

Hosain, F., Marsaglia, G. & Finch, C. A. (1967). *Journal of Clinical Investigation* **46**, 1–9.

International Nutritional Anemia Consultative Group (1982). *Report of the International Nutritional Anemia Consultative Group* (*INACG*). Washington DC and New York: The Nutrition Foundation.

Layrisse, M. & Martinez-Torres, C. (1977). *American Journal of Clinical Nutrition* **30**, 1166–1174.

MacPhail, A. P., Bothwell, T. H., Torrance, J. D., Derman, D. P., Bezwoda, W. R., Charlton, R. W. & Mayet, F. (1981). *British Journal of Nutrition* **45**, 215–227.

Miles, L. E. M., Lipschitz, D. A., Bieber, C. P. & Cook, J. D. (1974). *Analytical Biochemistry* **61**, 209–224.

Moore, C. V. & Dubach, R. (1951). *Transactions of the Association of American Physicians* **64**, 245–256.

Rossander, L., Hallberg, L. & Bjorn-Rasmussen, E. (1979). *American Journal of Clinical Nutrition* **32**, 2482–2489.

Sayers, M. H., Lynch, S. R., Charlton, R. W., Bothwell, T. H., Walker, R. B. & Mayet, F. (1974). *British Journal of Nutrition* **31**, 367–375.
Simpson, K. M., Morris, E. R. & Cook, J. D. (1981). *American Journal of Clinical Nutrition* **34**, 1469–1478.
Viteri, F. E., Garcia-Ibanez, R. & Torun, B. (1978). *American Journal of Clinical Nutrition* **31**, 961–971.
Wennesland, R., Brown, E., Hopper, J., Hodges, L., Guttentag, O. E., Scott, K. G., Tucker, I. N. & Bradley, B. (1959). *Journal of Clinical Investigation* **38**, 1065–1077.
Widdowson, E. M. & McCance, R. A. (1942). *Lancet* **i**, 588–591.

Printed in Great Britain