

Absorption of 'fortification' iron

Bioavailability in man of different samples of reduced Fe, and prediction of the effects of Fe fortification

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1. The first part of this study was designed to determine the increase in the amount of iron absorbed by hypothetical groups of subjects on the border-line of Fe deficiency when they were given meals fortified with ferrous sulphate. Two levels of Fe fortification were used (1.6 and 8.9 mg/meal) and the increase in the amount of Fe absorbed by the 'border-line' groups was 0.13 and 0.7 mg respectively.

2. Subjects which are on the border-line of Fe deficiency may be considered the target group of an Fe-fortification programme. Therefore the experimental model used can provide valuable information on the effects on the prevalence of Fe deficiency which can be expected when such a programme is introduced.

3. In the second part of the study, the bioavailability in man of reduced Fe with different physico-chemical properties was measured. Special emphasis was given to the relationship between the values obtained in vitro and in vivo.

4. The bioavailability was expressed as absorbability relative to that of ferrous sulphate. In four samples of hydrogen-reduced Fe the bioavailability ranged from 90 to 13%. The reduced-Fe products differed considerably with respect to solubility in hydrochloric acid (8–94% dissolved after 30 min), particle size distribution (91–1% < 7 μm) and reactive surface area (< 0.1–2.7 m²/g).

5. The relationships found between physico-chemical information and bioavailability indicated that reactive surface area and dissolution rate were better predictors of the bioavailability than the particle size.

Fortification of foods with iron is currently used, or planned to be introduced, in many countries in order to reduce the high prevalence of Fe deficiency. Very little is known, however, about the amount of Fe which is absorbed, and the effects on the prevalence of Fe deficiency which can be expected when the Fe content of a diet is increased by fortification of, for example, cereal foods. The reason is that no method has been available to measure absorption of Fe from composite meals.

The purpose of Fe fortification is not primarily to treat but to prevent Fe deficiency. A long period of observation is therefore needed to be able to detect a decrease in the prevalence of Fe deficiency in a population after the introduction of Fe fortification of the diet or the improvement of an existing programme. Moreover, the cause of a change in the Fe status of a population may be difficult to establish as many factors may be involved, including altered food habits, the use of oral contraceptives or intra-uterine devices, and Fe prophylaxis to pregnant women.

The recently developed extrinsic-tracer method has made it possible to carry out valid measurements of the absorption of Fe from composite meals (Cook, Layrisse, Martinez-Torres, Walker, Monsen & Finch, 1972; Hallberg & Björn-Rasmussen, 1972; Layrisse & Martinez-Torres, 1972). This new method is based on findings

which indicate that there is an almost complete and rapid isotopic exchange between naturally-occurring non-haem-Fe in the food, and added extrinsic inorganic radio-Fe tracer. This isotopic exchange is considered to take place in a common pool of non-haem-Fe formed by the different kinds of non-haem-Fe in a meal (Björn-Rasmussen, Hallberg & Walker, 1972; Björn-Rasmussen, 1973*c*). One implication of the pool concept is that Fe added as fortification to certain foods in a meal will enter the non-haem-Fe pool. The only provisions are that the 'fortification' Fe is a non-haem-Fe compound and that it can be dissolved under the conditions prevailing in the gastrointestinal tract (Björn-Rasmussen, 1973*b*). Another important implication of the pool concept is that factors in the diet (e.g. foods, additives, drinks) which influence the absorbability of intrinsic non-haem-Fe from a meal will affect similarly the absorption of both the intrinsic non-haem-Fe and the added Fe (Björn-Rasmussen, 1973*a, c*; Layrisse, Martinez-Torres, Cook, Walker & Finch, 1973).

The absorption of 'fortification' Fe from a meal is affected by several factors, such as the composition of the meal, the amount of Fe present in the meal, the bio-availability of the Fe compounds used for fortification, and the Fe status of the subjects studied. Therefore, in order to be able to predict the effects of a fortification programme, knowledge is needed about all these factors as well as about the variation in the Fe requirements of the population.

The present paper describes studies on some of these factors. One part of the study was devoted to studies on the relationship between the level of fortification and absorption and the influence of the Fe status of the subjects. Another part of the study was devoted to determination of the bioavailability in man of reduced Fe with different physico-chemical properties. Special emphasis was given to the relationship between the values obtained *in vitro* and *in vivo*.

EXPERIMENTAL

Fe compounds studied

Six commercially available samples of reduced Fe (samples A-G) were studied more or less extensively. Samples A-D were prepared by electrolysis and sample F by the carbonyl process. The manufacturing process of sample G was unknown.

All the variants of sample E were made by a Swedish chemical manufacturer (Elektrokemiska AB, S-445 01 Surte, Sweden) in close collaboration with our laboratory. They were prepared by hydrogen reduction and were made especially for the present study. A variant of this sample of reduced Fe is in the process of being introduced commercially. Most of the variants were not labelled with radio-Fe (samples E1-6). Four samples were labelled with radio-Fe (samples E11-14). These samples were used in absorption studies designed to establish the relationship between bioavailability and dissolution rate.

To ensure homogeneous labelling the tracer was added to the Fe compound before H-reduction. By modifications of the manufacturing process, it was possible to prepare radio-Fe-labelled, reduced Fe with different solubility properties. Four such samples were made and used for studies on bioavailability and on dissolution rate.

The reactive surface area of these labelled compounds was not measured because fairly large amounts were needed for the method used.

Effect of storage

Four samples of reduced Fe (samples C, E₂, F and G) were kept exposed to air at two temperatures and two relative humidities. There were no differences in dissolution rates before and after storage for 1 month.

Determination of dissolution rate

The dissolution rate of different Fe compounds was studied in hydrochloric acid at pH 1. Weighed amounts of the compounds, corresponding to 16 mg elemental Fe and 200 ml 0.1 M-HCl were shaken in a 500 ml flask at 23° using horizontal agitation (amplitude 60 mm, frequency 130 cycles/min). Duplicate 1 ml samples were withdrawn at various intervals during the first 30 min after the start of the agitation. The samples were immediately filtered through an acid-washed, fine-pore filter-paper and the Fe content of the filtrate was determined (International Committee for Standardization in Hematology, 1971). Each solubility study was done in duplicate.

The reproducibility of the method used to measure the dissolution rate was determined for both a readily-soluble compound (sample E₃) and a less-readily-soluble compound (sample D). Six determinations were made on each compound. The mean amount (\pm SD) of Fe dissolved at 30 min was $81 \pm 3\%$ for sample E₃ and $58 \pm 2.4\%$ for sample D. Thus the coefficient of variation at 30 min was 3.7 for sample E₃ and 4.1 for sample D.

Determination of particle size distribution and surface area

A suspension of the Fe powder in glycerol was analysed using a light microscope with a measuring ocular. Magnifications of $\times 100$ – $\times 400$ were used. A more dilute sample was prepared if the particles were not evenly dispersed and thus touching each other. The lengths of at least 200 particles were measured.

Determination of the reactive surface area was made using the 'BET' method (Brunauer, Emmett & Teller, 1938).

Absorption studies

Seventy-one male and female subjects aged between 18 and 50 years volunteered for the absorption studies. They all considered themselves healthy and they had no history of anaemia or gastrointestinal disease. The absorption studies were done in the morning in the laboratory, the subjects having fasted overnight. After ingestion of the test meal or test solutions no food or drink was allowed for 3 h. Before the absorption study, blood samples were taken for determination of haemoglobin, packed cell volume, plasma Fe concentration (International Committee for Standardization in Hematology, 1971), and total Fe-binding capacity (Herbert, Gottlieb, Lau, Fisher, Gevirtz, & Wasserman 1966). All individual values were within the normal range.

The absorption of non-haem-Fe from food was determined using the extrinsic-

tracer method (Hallberg & Björn-Rasmussen, 1972). Two isotopes were used, ^{55}Fe and ^{59}Fe . The absorption of the tracers was determined by the simultaneous use of a sensitive 3π whole-body counter (Arvidsson, Sköldbörn & Isaksson, 1972; Sköldbörn, Arvidsson & Andersson, 1972) and radioactivity in blood samples was determined as previously described (Eakins & Brown, 1966; Björn-Rasmussen, Hallberg, Isaksson & Arvidsson, 1974). Two series of studies were performed to determine first the effect of Fe fortification and second the bioavailability in man.

Studies on the effect of Fe fortification

This study was designed to estimate the increase in the amount of Fe absorbed when composite meals of 'lunch' or 'dinner' type were fortified with Fe. Unfortified meals and meals fortified with ferrous sulphate were served on different days to the same subjects, and the non-haem-Fe of the meals was labelled with two different isotopes. Two levels of Fe fortification were studied in two groups of subjects. The amount of Fe absorbed from reference doses of ferrous ascorbate was measured 2 weeks later as a basis for comparison of different subjects, and as a measure of the ability to absorb Fe (see p. 384).

Preparations of meals. The meals were composed of sixteen food items in which the relative amounts of the foodstuffs were similar to the average consumption at lunch or dinner in the Swedish population. The meals consisted of (g): milk products 134, vegetables and roots 82, fruits and jam 55, meat and fish 35, cereals 22, egg 5. All food components were carefully minced and mixed with an added inorganic radio-Fe tracer. The meals were weighed into aluminium dishes which were placed in a water-bath and then boiled for 2 h in an oven set at 200° . Some of the meals were fortified with FeSO_4 which was added as a solution and thoroughly mixed into the food before cooking. The total non-haem-Fe content of the unfortified meals was 2.4 mg and those of the two fortified meals were 4.0 and 11.3 mg, respectively. The unfortified meals were labelled with $4 \mu\text{Ci } ^{55}\text{FeCl}_3$ and the meals fortified with FeSO_4 were labelled with $2 \mu\text{Ci } ^{59}\text{Fe}$.

The absorption of haem-Fe was not measured. Portions of the labelled meals were taken for analysis of ^{55}Fe and ^{59}Fe . The Fe content of the meals was determined as previously described (Björn-Rasmussen *et al.* 1974). The reference doses contained 3 mg Fe as ferrous ascorbate and 30 mg ascorbic acid in 10 ml 0.1 M-HCl.

Serving procedure. One group of nineteen subjects were served two unfortified meals (A) and two meals fortified to the level of 4.0 mg Fe (B). The meals were served in the order ABBA or BAAB on four consecutive mornings. The meals fortified to the higher level, 11.3 mg (C), and the unfortified meals (A) were served, as described previously, to another group of eighteen subjects in the order ACCA or CAAC.

A blood sample was taken 2 weeks after the last serving for determination of ^{55}Fe and ^{59}Fe , and the total retention of ^{59}Fe was measured by whole-body counting. Then, on two consecutive mornings, reference doses of Fe ascorbate were given. These doses were labelled with ^{59}Fe . After a further 2 weeks the retention of the reference doses of Fe was measured by whole-body counting.

Bioavailability studies

Four different variants of a sample of H-reduced Fe (samples E11-14) were studied. The reduced-Fe sample was labelled with ^{55}Fe . The bioavailability was studied in wheat rolls fortified with the labelled, reduced-Fe samples. During preparation of the wheat rolls 0.5 mg reduced Fe, labelled with $3 \mu\text{Ci } ^{55}\text{Fe}$, was added to 30 g '60% extraction' wheat flour. At the same time, trace amounts of FeCl_3 labelled with $1 \mu\text{Ci } ^{59}\text{Fe}$ were also added. The baking procedure was the same as that described previously (Björn-Rasmussen *et al.* 1972). The total amount of Fe in each wheat roll was 2.2 mg, of this amount 0.5 mg Fe was added reduced Fe, and 1.7 mg Fe was non-haem-Fe in the wheat flour.

Subjects in four groups were each served two wheat rolls, together with tap water. The wheat rolls served to each group contained one of the four labelled, reduced-Fe compounds (samples E11-14).

The bioavailability of one of the Fe compounds (sample E12) was also determined in another group of eight subjects using a different experimental design. To reduce the possibility of an isotopic exchange between the ^{55}Fe -labelled, reduced Fe and the ^{59}Fe -labelled Fe salt, the two different tracers were added to two separate batches of wheat rolls (*A* and *B* respectively) which were served separately in the order *ABBA* on four consecutive days. The two batches of wheat rolls, *A* and *B*, were baked in the same way as the other wheat rolls used in this study, and were made to contain the same amount of elemental Fe (0.5 mg/wheat roll).

RESULTS

Solubility studies

The dissolution rates of eight samples of reduced Fe at pH 1 and of the radio-Fe labelled samples of reduced Fe (samples E11-14) are shown in Fig. 1. The amount of Fe dissolved after 10, 20 and 30 min was also determined for samples E1-6.

Particle size and surface area

The particle size distribution and the reactive surface area was determined for all the Fe compounds which were not labelled with radio-Fe. The results are given in Table 1. The relationship between the amounts of Fe dissolved at intervals after the start of agitation and both the reactive surface area and the median particle size are shown in Fig. 2.

Effect of fortification of a composite meal

One group of nineteen subjects was served a 'lunch' meal on four consecutive mornings. Two of the meals were not fortified and contained 2.4 mg non-haem-Fe/meal. The other two meals were fortified with FeSO_4 so that the total Fe content was 4.0 mg/meal. The mean (\pm SEM) amounts of Fe absorbed from the unfortified and fortified meals were 5.6 ± 1.1 and 6.5 ± 1.4 % respectively. The difference in absorption was not significant but the amount of Fe absorbed increased significantly

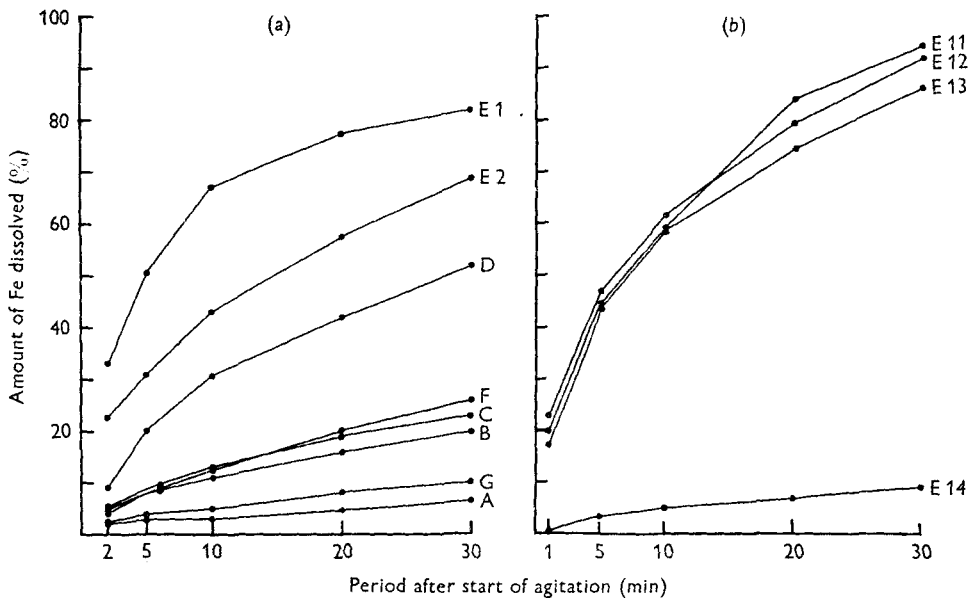


Fig. 1. Dissolution rates (amount of iron dissolved in 0.1 M-hydrochloric acid (%)) at intervals after the start of agitation (min) of (a) six different commercially available samples of reduced Fe (samples A-G) and two different samples of hydrogen-reduced Fe (samples E1 and E2) and (b) four different samples of radio-Fe-labelled-reduced Fe (samples E11-14). For details of samples, see p. 376 and for details of experimental procedures, see p. 377. Mean values for six determinations/sample.

Table 1. Reactive surface area and median particle size for six different commercially available samples of reduced iron (samples A-D, F and G) and of six different variants of hydrogen-reduced Fe (samples E1-6)

Sample	Reduction process	Shape	Reactive surface area (m ² /g)	Median particle size (μm)	Particle size distribution (%)					
					≤ 7 μm	14 μm	21 μm	28 μm	35 μm	> 3 μm
A	Electrolytic	Irregular	< 0.1	35.0	1	10	17	14	17	41
B			< 0.1	42.0	4	23	9	6	7	51
C			< 0.1	3.4	72	7	7	2	3	9
D			0.40	5.1	69	20	7	1	2	1
E1	Hydrogen	Nodular	2.70	1.7	91	7	1	1	—	—
E2			1.81							
E3			2.00							
E4			0.90							
E5			0.97							
E6			1.03							
F	Carbonyl	Spherical	0.20	3.4	85	10	3	2	—	—
G	Unknown (probably electrolytic)	Irregular	< 0.1	11.9	24	41	28	5	2	—

* For details of samples, see p. 376.

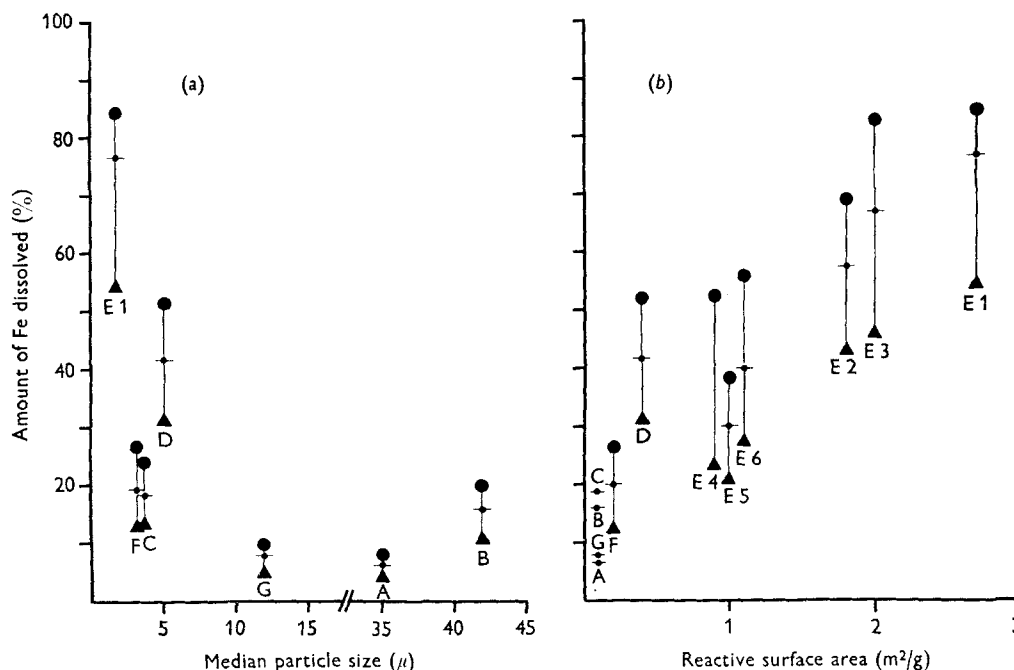


Fig. 2. The relationship between dissolution rates (amount of iron dissolved in 0.1 M-hydrochloric acid at 10 (▲), 20 (●) and 30 (●) min intervals after the start of agitation) and (a) the median particle size (μ), and (b) the reactive surface area (m^2/g) for six different commercially available samples of reduced Fe (samples A-G), and for six variants of one sample prepared by hydrogen reduction (samples E1-6). For details of samples, see p. 376 and for details of experimental procedures, see p. 377.

from 0.13 to 0.26 mg. The mean (\pm SEM) amount of Fe absorbed from the reference dose (3 mg Fe as ferrous ascorbate) was $38.5 \pm 4.2\%$ (Table 2).

Four 'lunch' meals were served on four consecutive mornings to another group of eighteen subjects. These meals were identical to the meals given to the previous group. Two of the meals were not fortified and the other two were fortified with $FeSO_4$ so that the total Fe content was 11.3 mg/meal. The mean (\pm SEM) amounts of Fe absorbed from the unfortified and fortified meals were 6.9 ± 1.1 and $7.7 \pm 1.4\%$ respectively. The difference between the two mean absorption values was significant ($P < 0.05$; paired *t* test). The amount of Fe absorbed from the fortified meals was about five times higher than that absorbed from the unfortified meals, 0.87 and 0.17 mg respectively ($P < 0.01$). The mean (\pm SEM) amount of Fe absorbed from the reference dose was $36.5 \pm 4.4\%$ (Table 2).

Bioavailability of reduced Fe

Four groups of subjects were served wheat rolls baked from flour fortified with one of four different variants of a ^{55}Fe -labelled, reduced Fe compound (samples E11-14). The same wheat rolls contained $^{59}FeCl_2$ added as an extrinsic tracer of the amount of Fe absorption from the non-haem-Fe pool.

The absorption of ^{55}Fe and ^{59}Fe tracers was compared in each subject. The values

Table 2. *Absorption in man of non-haem-iron from composite meals,* unfortified (containing 2.4 mg Fe and labelled with ^{55}Fe) (A) or fortified with ferrous sulphate (containing 4.0 and 11.3 mg Fe and labelled with ^{59}Fe) (B1 and B2 respectively)†*

(Mean values with their standard errors)

No. of subjects		Haemoglobin (g/l)	Absorption of non-haem-Fe from:				Increase in Fe absorption (B-A) (mg)	Fe absorption from reference dose‡ (%)	
♀	♂		A		B1				
			%	mg	%	mg			
8	11	143 ± 2	5.6 ± 1.1	0.13 ± 0.03	6.5 ± 1.4	0.26 ± 0.05	0.88 ± 0.06	0.13 ± 0.03	38.5 ± 4.2
			A		B2				
7	11	143 ± 3	6.9 ± 1.1	0.17 ± 0.03	7.7 ± 1.4	0.87 ± 0.15	0.92 ± 0.05	0.69 ± 0.13	36.5 ± 4.4

* For details of composition, see p. 378.
 † For details of experimental procedures, see p. 379.
 ‡ 3 mg Fe as ferrous ascorbate.

Table 3. *Bioavailability in man of four variants of a sample of ^{55}Fe -labelled, hydrogen-reduced Fe when given together with $^{59}\text{FeCl}_3$ in two wheat rolls/subject served at one bread meal (1) and when the two isotopes were added to two separate batches of wheat rolls (A and B) and served at four bread meals on four consecutive days in the order ABBA (2)*

(Mean values with their standard errors)

Sample*	Method of serving†	No. of subjects		Haemoglobin (g/l)		$^{59}\text{FeCl}_3$ (%)		^{55}Fe -labelled, reduced Fe compound (%)		$^{55}\text{Fe} : ^{59}\text{Fe}$	
		♀	♂	Mean	SE	Mean	SE	Mean	SE	Mean	SE
		E11	1	3	3	138	8	18.5	7.7	15.6	6.8
E12	1	4	2	147	4	21.8	5.8	19.2	4.7	0.90	0.02
E13	1	3	3	149	6	10.6	2.8	7.0	1.9	0.66	0.02
E14	1	4	4	145	3	2.5	0.3	19.9	2.1	0.13	0.01
E12	(2)	2	6	133	6	12.2	3.2	14.5	3.4	1.10	0.10

* For details, see p. 376.

† For details of procedures, see p. 379.

for the absorption of each tracer and the values for the absorption ratio, $^{55}\text{Fe} : ^{59}\text{Fe}$, are given in Table 3. The mean (\pm SE) value for $^{55}\text{Fe} : ^{59}\text{Fe}$ for the group receiving sample E11 was 0.82 ± 0.02 and the corresponding values for samples E12, E13 and E14 were 0.90 ± 0.02 , 0.66 ± 0.02 and 0.13 ± 0.01 respectively.

A fifth group of eight subjects was served four wheat rolls, one wheat roll/d on four consecutive days. Two of the wheat rolls each contained 0.5 mg ^{55}Fe -labelled, reduced Fe powder (sample E12) and the other two each contained 0.5 mg Fe as $^{59}\text{FeSO}_4$. The mean (\pm SE) amounts of the ^{55}Fe and ^{59}Fe tracers absorbed were 14.5 ± 3.4 and 12.2 ± 3.2 % respectively. The mean value for $^{55}\text{Fe} : ^{59}\text{Fe}$ was 1.10 ± 0.10 (Table 3).

DISCUSSION

Effect of an Fe-fortification programme: methodological considerations

In predicting the effects of an Fe-fortification programme several factors have to be considered, such as the bioavailability of the Fe compound used, the amount of Fe added, the composition of frequently consumed meals and the Fe status of the target population. A factorial experimental design is highly efficient in establishing the relative role of various factors involved. One factor known to influence Fe absorption markedly is the Fe status of the subjects. The usual haematological criteria are too poorly correlated with absorption to be of practical use. The best method developed so far is to use the absorption of a small dose of an Fe salt as a measure of the ability of the individual to absorb Fe. The absorption from this reference dose will thus serve as a basis of comparison for different groups of subjects. It may also serve as an indicator of the Fe status of the subjects, and thus as a basis for setting a rough border-line between subjects with normal Fe status and those with Fe deficiency. At our laboratory, using the technique described in this paper, we have considered an Fe absorption value of 30% from the 3 mg reference dose to be a border-line value. This value is considered to be the lowest absorption value in the Fe-deficient section of the population. The hypothetical population with this absorption value may thus be regarded as the target group for an Fe-fortification programme.

The absorption of Fe from a particular meal may be expressed as the linear regression line of the amount of Fe absorbed from the reference dose and the amount of Fe absorbed from the food. The three regression lines shown in Fig. 3 represent the unfortified meals and the meals fortified to 4.0 and 11.3 mg Fe respectively. The values for the slopes of the regression lines obtained in this study is thus determined both by the Fe status and by the composition of the meals and the level of Fe fortification. These regression lines can then be used to estimate the expected increase in the amount of Fe absorbed when the amount of Fe absorbed from the reference dose is 30%, i.e. in the border-line subjects who have the lowest level of Fe absorption in the Fe-deficient section of the population. When an additional 1.6 mg Fe is provided in this type of meal, the amount of Fe absorbed could be estimated to increase by 0.13 mg in this group of subjects. When an additional 8.9 mg Fe is provided the amount of Fe absorbed could increase by 0.7 mg.

This study was a first attempt to develop a method for predicting the effects of Fe fortification. The present results are given as an example of this new method of analysing food-fortification studies.

When changing the composition of the meals the values for the slopes of the regression lines will be changed. It is therefore important to use several different meals with a composition similar to the average consumption in the population. In a recent study done at our laboratory it was shown that the way in which the food is served influences the absorption of Fe (Björn-Rasmussen, Hallberg, Magnusson, Rossander, Svanberg & Arvidsson, 1976). Therefore, attempts should be made to serve the meals in as realistic a way as possible.

The accuracy of the calculations depends mainly on the extent of the correlation

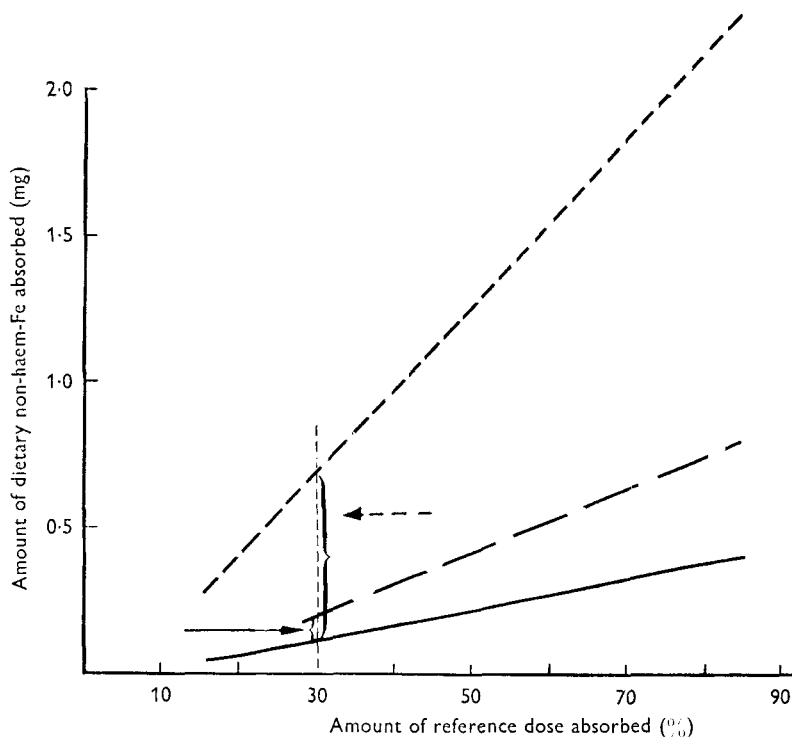


Fig. 3. Linear relationship between the absorption of non-haem-iron from a meal of 'lunch' or 'dinner' type (unfortified (2.4 mg Fe) (—) or fortified to the level of 4.0 (---) or 11.3 (— · —) mg Fe) and the absorption of a reference dose of 3 mg Fe as ferrous ascorbate. Fortification of the meal to 4.0 and 11.3 mg Fe increased the Fe absorption by 0.13 (→) and 0.70 (— →) mg respectively. Regression coefficients were: unfortified meals, 0.70 (n 37); meals fortified to 4.0 mg Fe, 0.62 (n 19); meals fortified to 11.3 mg Fe, 0.81 (n 18). For details of meals and experimental procedures, see pp. 378, 379.

between the amount of Fe absorbed from the meals and that absorbed from the reference doses. In a recent study from our laboratory it was shown that the extent of correlation was high when the reference doses and the meals were given on consecutive days, and was lower when the meals and the reference doses were given 2 weeks apart (Björn-Rasmussen *et al.* 1976), particularly for women. This may be the reason for the low correlation found in the present study, where the meals and the reference doses were given with an interval of 2 weeks. The correlation coefficient for the regression of the amount of Fe absorbed from the unfortified meals *v.* the amount of Fe absorbed from the reference doses was 0.70 for all subjects and 0.91 for the men only; the corresponding values for the meals fortified to 4 mg Fe were 0.62 and 0.79, and for the meals fortified to 11.3 mg Fe were 0.81 and 0.88 respectively.

It is well known that when increasing oral doses of Fe are given in the form of an Fe salt the total amount of Fe absorbed will increase, while the relative amount of Fe absorbed will decrease. The finding in the present study that the relative amount of Fe absorbed did not decrease but instead increased when the level of fortification of a composite meal was increased was therefore quite unexpected. In a previous study

the effect of a threefold increase in the level of Fe fortification was measured in three composite meals served on 2 d to normal subjects. No significant decrease in the relative amount of Fe absorbed was found when the total amount of Fe in the meals was increased from 16.4 to 25.5 mg/d (Björn-Rasmussen *et al.* 1974). The present finding of an increase in the relative amount of Fe absorbed when the level of fortification of the meal served was increased thus agrees with this previous study. The probable explanation for these findings is that the properties of the non-haem-Fe pool are altered by the addition of a readily-soluble Fe salt. When the amount of Fe added to a meal is further increased the relative amount of Fe absorbed decreases, as found by Layrisse *et al.* (1973) who added Fe to a level of 60 mg/meal.

Bioavailability of reduced Fe

The absorption of labelled, reduced Fe and of other labelled Fe compounds added to foods has previously been studied in man (Steinkamp, Dubach & Moore, 1955; Elwood, 1968; Höglund & Reizenstein, 1969). Results of such studies indicate that different Fe compounds differ in availability. The great variation in absorption between different subjects and the marked influence of the composition of meals on Fe absorption makes it very difficult to interpret the results and to make valid comparisons between different Fe compounds.

The bioavailability of an Fe compound to be used for fortification should preferably be compared with that of readily-soluble, simple Fe salt, e.g. FeSO₄, given to the same subjects and in the same type of meal. Earlier studies on the bioavailability of 'fortification' Fe have mainly been carried out in animals. Unfortunately, however, it is difficult to translate the results of such studies to humans. The only direct measurement of bioavailability of 'fortification' Fe in man is that reported by Cook, Minnich, Moore, Rasmussen, Bradley & Finch (1973) who compared the absorption of Fe from FeSO₄ with that from a specially-prepared, reduced Fe.

The present measurements of the bioavailability of reduced Fe are based on the concept of a non-haem-Fe pool in a meal (Hallberg & Björn-Rasmussen, 1972). Fe added as an easily-soluble, radio-Fe-labelled salt to a fortified meal will exchange with, and uniformly label the intrinsic non-haem-Fe. The dissolved fraction of the radio-Fe-labelled, reduced Fe will similarly exchange with the other non-haem-Fe in the pool. The value for the ratio, the amount of absorption of the radio-Fe originating from the reduced Fe: the amount of absorption of radio-Fe from the tracer added to the food will then be a direct measure of the bioavailability of the reduced Fe under the conditions studied.

Theoretically, however, the tracer used to label the intrinsic Fe might also exchange with some of the undissolved and unavailable part of the reduced Fe. It may also be adsorbed onto the surface of the Fe particles. This would then lead to falsely low Fe absorption values and to over-estimation of the bioavailability. This was the reason for choosing two experimental models in the present studies on the bioavailability of reduced Fe. In the first model the Fe in the food and the reduced Fe sample added to the food were labelled with two different isotopes and were given in the same meal, as described previously (see p. 379). In the second model the same amount of Fe in

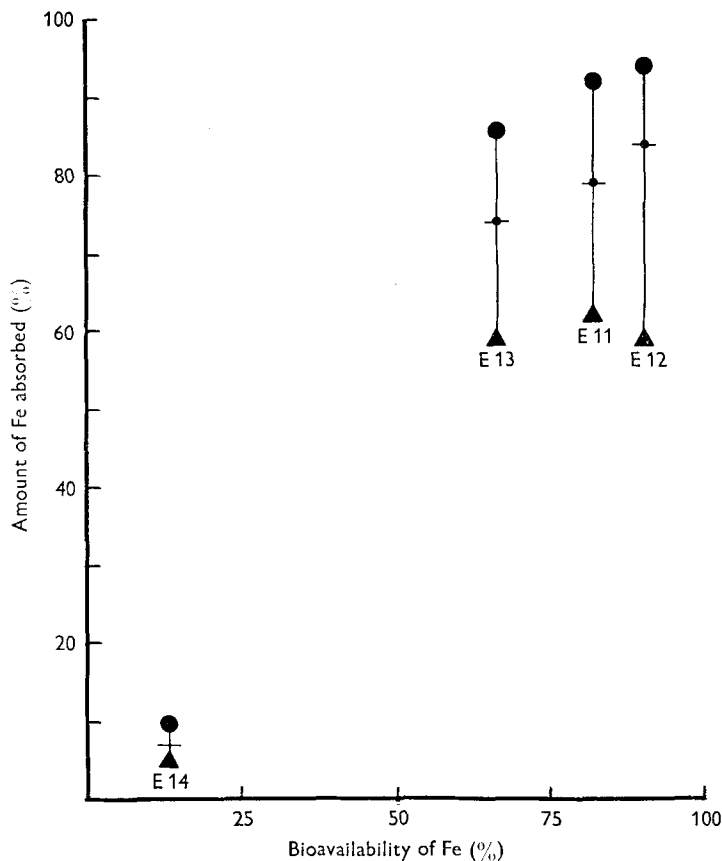


Fig. 4. The relationship between dissolution rates (amount of iron dissolved in 0.1 M-hydrochloric acid at 10 (▲), 20 (◐) and 30 (●) min intervals after the start of agitation) and the bioavailability (% Fe absorbed) of different variants of a sample of radio-Fe-labelled, hydrogen-reduced Fe samples (E11-14). For details of samples, see p. 376 and for details of determination of bioavailability, see p. 379.

the form of FeSO_4 and as a reduced-Fe compound, each labelled with a different isotope, was given in wheat rolls served on different days. No difference in the mean Fe absorption ratio was found whether the two tracers were served together on the same day, as in the first model, or separately on different days, as in the second model. It can be concluded therefore that there is no obvious isotopic exchange between the extrinsic tracer and the undissolved part of the H-reduced Fe compound. The simpler experimental design in which the labelled, reduced Fe and the extrinsic, readily-soluble tracer are given in the same wheat roll can thus be used without introducing any measurable systematic errors. Actually, the accuracy of this simpler model is greater as it is unaffected by the variation in Fe absorption on different days.

Reduced Fe can be manufactured in several different ways. Different samples of reduced Fe vary considerably with respect to physico-chemical properties such as particle size, reactive surface area and solubility. This was also true for the compounds

used in this study (Table 1). Unfortunately, it was only possible to obtain labelled samples of the H-reduced Fe compound (sample E) which was manufactured in Sweden. No direct information about the bioavailability of any other Fe compound could thus be obtained.

It is reasonable to assume that there is some relationship between dissolution rate and bioavailability. The present findings with the labelled compounds (samples E₁₁–₁₄) support this assumption (Fig. 4). Two samples, E₁₃ and E₁₄, were made coarser than samples E₁₁ and E₁₂ and sample E₁₄ was made less soluble than samples E₁₁ and E₁₂, in order to facilitate a study on the relationship between bioavailability and solubility. Determination of the dissolution rate might therefore be used both as a screening method in the search for 'Fe-fortification' compounds with suitable bioavailability properties and to study the effect of storage.

The relationship between dissolution rate and mean particle size (Fig. 2) suggested that samples B, C and A, G respectively were almost equally soluble. However, the particle size differed markedly and, moreover, the difference in particle size between the samples C, D, E₁ and F was small but the solubility differed markedly. It can be concluded therefore that the particle size is a poor predictor of the solubility and of the bioavailability of an 'Fe-fortification' compound. On the other hand, a close relationship was found between the reactive surface area and the dissolution rate (Fig. 2). This finding indicates that the reactive surface area is a better predictor of the dissolution rate and of the bioavailability in man than is the particle size.

Two of the labelled, reduced-Fe compounds (samples E₁₁ and ₁₂) were readily soluble and also had a bioavailability which was close to that of FeSO₄. The unlabelled compound (sample E₁), prepared by the same procedure, had the same physico-chemical properties. In the study by Cook *et al.* (1973) a specially-prepared reduced-Fe compound was used which also had a high bioavailability in man. However, no information was given on the chemical properties of this Fe compound.

The general conclusions that can be drawn from the present study are that there are considerable variations between different kinds of reduced Fe, not only in particle size distribution but also in reactive surface area and dissolution rate. The present findings indicate that such variations are associated with differences in bioavailability in man. The reactive surface area of reduced Fe and the dissolution rate seem to be better predictors of bioavailability than the particle size.

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REFERENCES

- Arvidsson, B., Sköldbörn, H. & Isaksson, B. (1972). *Proc. 3rd int. Conf. med. Phys. including med. Engng* **41**, 2.
- Björn-Rasmussen, E. (1973*a*). *Scand. J. Haemat.* **11**, 391.
- Björn-Rasmussen, E. (1973*b*). *Scand. J. Gastroent.* **8**, 645.
- Björn-Rasmussen, E. (1973*c*). *Am. J. clin. Nutr.* **26**, 1311.
- Björn-Rasmussen, E., Hallberg, L., Isaksson, B. & Arvidsson, B. (1974). *J. clin. Invest.* **53**, 247.
- Björn-Rasmussen, E., Hallberg, L., Magnusson, B., Rossander, L., Svanberg, B. & Arvidsson, B. (1976). *Am. J. clin. Nutr.* **29**, 772.
- Björn-Rasmussen, E., Hallberg, L. & Walker, R. B. (1972). *Am. J. clin. Nutr.* **25**, 317.
- Brunauer, S., Emmett, P. H. & Teller, E. (1938). *J. Am. chem. Soc.* **60**, 309.
- Cook, J. D., Layrisse, M., Martinez-Torres, C., Walker, R., Monsen, E. & Finch, C. A. (1972). *J. clin. Invest.* **51**, 805.
- Cook, J. D., Minnich, V., Moore, C. V., Rasmussen, A., Bradley, W. E. & Finch, C. A. (1973). *Am. J. clin. Nutr.* **26**, 861.
- Eakins, J. D. & Brown, D. A. (1966). *Int. J. appl. Radiat.* **17**, 391.
- Elwood, P. C. (1968). *Rep. Publ. Hlth med. Subj., Lond.* No. 117.
- Hallberg, L. & Björn-Rasmussen, E. (1972). *Scand. J. Haemat.* **9**, 193.
- Herbert, V., Gottlieb, C. W., Lau, K-S., Fisher, M., Gevirtz, N. R. & Wasserman, L. R. (1966). *J. Lab. clin. Med.* **67**, 855.
- Höglund, S. & Reizenstein, P. (1969). *Blood* **34**, 496.
- International Committee for Standardization in Hematology (1971). *Br. J. Haemat.* **20**, 451.
- Layrisse, M. & Martinez-Torres, C. (1972). *Am. J. clin. Nutr.* **25**, 401.
- Layrisse, M., Martinez-Torres, C., Cook, J. D., Walker, R. & Finch, C. A. (1973). *Blood* **41**, 333.
- Sköldbörn, H., Arvidsson, B. & Andersson, M. (1972). *Acta radiol. Suppl.* **313**, 233.
- Steinkamp, R., Dubach, R. & Moore, C. V. (1955). *Arch. intern. Med.* **95**, 181.