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Forms of soluble iron in mouse stomach and duodenal lumen: significance for mucosal uptake

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Stomach contents of mice fed on a standard rodent breeding diet contained 29–733 μM-soluble non-haem-iron. A very variable percentage (3–100, mean 49·3 (SE 4·7), *n* 37) of this Fe was rapidly (half-life < 1–2 s) available for chelation by the strong Fe(II) chelator ferrozine, with little or no further Fe being available on addition of ascorbate. Ferrozine-available Fe could be detected in the duodenal lumen at concentrations up to 60 μM in vivo and after in vitro neutralization of stomach contents. No significant changes in quantity of stomach ferrozine-available Fe or soluble non-haem-Fe occurred in mice with adaptive enhancement of Fe absorption induced by chronic hypoxia. Electron paramagnetic resonance (e.p.r.) spectroscopy of the soluble portion of mouse stomach contents demonstrated a $g = 4{\cdot}3$ signal (rhombic Fe(III)) equivalent to up to 20% of soluble non-haem-Fe. The signal was unaffected by addition of excess ferrozine and increased on subsequent neutralization, suggesting redistribution of Fe from other e.p.r.-silent species. Solutions of Fe–nitrilotriacetate (NTA) (a synthetic Fe chelate used as a bioavailable, model Fe solution) were found to contain both rapidly and slowly ferrozine-available Fe (after addition of ascorbate) depending on pH, NTA:Fe ratio and the presence of Ca(II) ions. Fe–ascorbate mixtures (a model solution for Fe absorption studies) also contained ferrozine-available Fe. These results suggest the presence of Fe(II), rhombic Fe(III) and other e.p.r.-silent Fe species in the soluble fraction of mouse stomach contents. The ferrozine-available (Fe(II)) fraction is not limited by the reducing power in the diet, but by binding to ligands. Neutralization with bicarbonate leads to a loss of ferrozine-available Fe and increase in rhombic Fe(III) at the expense of both ferrozine-available and other e.p.r.-silent Fe species. The ferrozine-available Fe in mouse stomach and duodenal lumen can be related to Fe species present in model solutions used for in vitro studies of mucosal uptake mechanisms.

Duodenal iron uptake: Iron availability: Mouse

Recent studies of mucosal uptake of iron by mouse duodenum have suggested the existence of two separate cellular mechanisms of uptake (Raja *et al.* 1987*b*; Peters *et al.* 1988). One mechanism appears to represent uptake from unstable, unchelated Fe(II) or Fe(III) species present in fresh Fe–ascorbate solutions (a model bioavailable Fe solution) or Fe–nitrilotriacetate (NTA) solutions destabilized by, for example, addition of Ca(II) ions (Raja *et al.* 1987*b*; Simpson & Peters, 1987; Simpson *et al.* 1988). This mechanism is thought to be mediated by non-esterified fatty acids (Simpson & Peters, 1987; Simpson *et al.* 1988). Investigation of Fe–ascorbate solutions with the chelators ferrozine and mimosine and by physical techniques (Dorey *et al.* 1987) showed instability above pH 7·0 due to probable formation of small polymeric Fe(III) species, these not being available for transport by non-esterified fatty acids (Simpson & Peters, 1987). The second uptake mechanism relates to uptake of Fe from weakly chelated Fe(III) (e.g. Fe–NTA (1:2) but not Fe–EDTA (1:2)).

The question arises as to what forms of Fe are available in the duodenal lumen during physiological processes and which solutions represent good models for these forms of Fe.

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No direct information is available to answer this question. In mice, the duodenal lumen volume is too small for many forms of analysis. In addition, uptake of available Fe forms may be of high efficiency, hence the available forms would not be easy to detect. The unstable Fe species described above may have a lifetime of only a few minutes and no specific information is available regarding the effect of dietary ligands on these species.

It is also clear that some investigation of the forms of Fe present in mouse duodenal lumen during digestion of diet is required. Due to the rapidity of transit of dietary material through the duodenum (average residence time approximately 1 min; Raja *et al.* 1987*a*) two approaches are adopted in the present paper, namely: (1) investigation of the stomach contents with attempts to model changes occurring in the duodenum and (2) direct study of mouse duodenal contents. Model solutions, as used in previous in vitro Fe absorption studies, are also investigated.

MATERIALS AND METHODS

Animals and diets

Male, To strain, 6–8-week-old (25–30 g) mice were used throughout and fed on a commercial pelleted rodent diet (Labsure diet ERD; Manea, Cambs) *ad lib.* except on the day of an experiment. This diet contained 3·1 (SE 0·3) nmol non-haem-Fe/g (n 3). Insignificant amounts ($< 0{\cdot}01$ nmol/g) of haem-Fe were present. The diet was made from maize, wheat, white-fish meal, soya-bean meal, wheat germ and dried Brewer's yeast, with a mineral and vitamin supplement including 50 mg ferrous sulphate/kg. The diet contained (g/kg): 207 protein, 33 oil, 24 fibre, the remainder being minerals, carbohydrate and ash (details as supplied by the manufacturer).

Hypoxia was induced by placing mice in a hypobaric chamber at a pressure of 50·7 kPa (0·5 atm) for 3 d. Food and water were given *ad lib.* Experiments with hypoxic mice were performed within 30 min of removal from the chamber, no diet being given during this period. Mice were starved overnight in wire-bottomed cages.

Investigation of Fe in stomach and duodenal contents

Mice were killed by anaesthetizing with methoxyfluorane (Metaphane; C-Vet, Bury St Edmunds, Suffolk) followed by cervical dislocation. Mouse stomachs were removed and opened on a plastic sheet. The lumen pH was determined using a Pye Unicam/Ingold MS E7 micro flat-headed pH electrode with a WPA Scientific Instruments (SEMAT (UK), London) CD60 pH meter calibrated to 0·1 unit over the pH range 1·0–7·0. The stomach contents were transferred to a weighed tube and centrifuged at 10000 ***g*** for 2 min. The supernatant fraction was removed, weighed and a portion (10–20 μl) added to a cuvette containing 1 ml 0·8 mM-ferrozine. The increase in absorbance at 562 nm was recorded until it stabilized, then 20 μl 0·1 M-sodium ascorbate were added and further change in absorbance was recorded.

Analytical procedures

Non-haem-Fe was determined using the method of Foy *et al.* (1967) except that ferrozine (Sigma Chemical Co., Poole, Dorset) was used as an Fe indicator. Analar reagents were used throughout (BDH Chemicals, Poole, Dorset) with appropriate blanks to correct for reagent Fe contamination. Electron paramagnetic resonance (e.p.r.) spectra of frozen (liquid nitrogen, stored at $-70°$), pooled supernatant fractions of stomach contents from up to four mice were recorded with a Varian x-band (9300 MHz) spectrometer. Samples were gas cooled to about $-173°$ in the Varian low-temperature accessory. Spectrometer settings were: microwave power 200 mW, modulation amplitude 10 G, modulation

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frequency 100 kHz, time constant 0·3 s, sweep rate 500 G/min. Peak intensities were estimated as equivalent Fe concentrations by comparison with Fe(III)–NTA (1:2) solutions in the same tube, after background signal subtraction.

Ferrozine-available Fe in duodenal contents was determined by removing the proximal 35 mm of small intestine from mice killed as described previously, and washing out the lumen with 1 ml 0·154 M-sodium chloride. The washings were centrifuged (10 s at 10000 ***g***) to remove insoluble material, then placed in a semi-microcuvette and the absorbance at 562 nm recorded before and after addition of 20 μl 10 mM-ferrozine and 20 μl 0·1 M-sodium ascorbate.

Availability of Fe in model solutions

Ferric chloride, Fe–NTA (1:2) and Fe–EDTA (1:2) in water were prepared by mixing 10 mM-$FeCl_3$ in 10 mM-hydrochloric acid, 10 mM-$NTA(Na)_3$ or 10 mM-$EDTA(Na)_3H$ (pH 7·4) where appropriate and diluting with water to give 20–200 μM-Fe. $FeSO_4$ was diluted from a 10 mM solution in 10 mM-HCl. The pH of these solutions was 3·3–4·8. The solutions and mixtures obtained by neutralization to pH 6·5–7·0 with small volumes of 0·5 M-sodium bicarbonate were investigated for availability of Fe to chelators by adding water, 2 mM (final)-sodium ascorbate and 200 μM-ferrozine (final); each as 0·02 vol. of a fifty-fold concentrated solution to give a final Fe concentration of less than 50 μM, then observing ferrozine–Fe(II) formation.

Fe–NTA (1:2) incubation media in 20 mM-Hepes (pH 7·4), 0·1 M-NaCl, 0·1 M-mannitol, was prepared by mixing $FeCl_3$ and $NTA(Na)_3$ solutions as described previously with a two-fold concentrated Hepes–mannitol–NaCl solution and incubating for 5 min at 37°. Ferrozine and sodium ascorbate were added as described previously and absorbance at 562 nm recorded.

Statistical analysis

Values were tested for normality (Royston, 1983) and quoted as means with their standard errors, or median with interquartile limits, as appropriate. Groups of values were compared by Wilcoxon's Rank Sum test (Wetherill, 1967). Correlations were determined by linear regression.

RESULTS

Stomach contents

The stomach contents from mice, separated into soluble and insoluble material, are shown in Fig. 1. The normal feeding pattern for the mice used in the present study was to feed at 11.00–12.00 h and this is reflected by the increase in solids in the stomach noted at this time. Normal daily diet consumption was 4·24 (SE 0·37) g (n 20) with no correlation between body-weight and daily consumption (r 0·07, n 20, $P = 0·78$). Experiments in which 12 h-fasted mice were allowed to refeed, demonstrated a maximum stomach content of approximately 250 mg diet, suggesting a mean residence time of less than 2 h for diet in the stomach. In spite of this, Fig. 1 shows that short-term (4·5 h) withholding of diet does not significantly deplete the stomach, presumably due to reduced emptying on fasting. Even fasting for 16 h leaves mice with some material in the stomach (Fig. 1).

The quantity of diet solids in stomachs of hypoxic mice was not significantly different from that in normal mice fasted for up to 4·5 h. The percentage of total stomach contents which was soluble was variable (range 26·0–60·3, median 31·3, interquartile limits 27·8–38·9, n 11) and was not significantly affected by 4 h of fasting.

Stomach pH

Stomach pH may be a major influence on the availability of Fe for absorption (Hungerford & Linder, 1983). Stomach pH values for various groups of fed, fasted and hypoxic mice are

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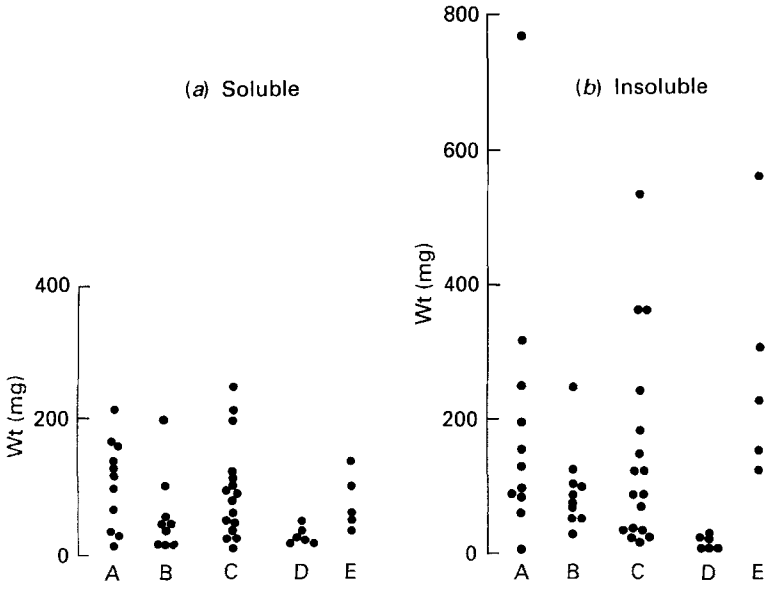

Fig. 1. Stomach contents of mice. Quantity of (*a*) soluble and (*b*) insoluble material in mouse stomach. Mice were (A) *ad lib.* fed till 09.00 hours,. analysed between 09.00 and 10.30 hours; (B) fasted from 09.00 hours, analysed between 13.30 and 14.30 hours; (C) *ad lib.* fed in a hypobaric chamber at a pressure of 50·7 kPa for 3 d, analysed between 09.00 and 10.30 hours; (D) fasted for 16 h before analysis at 09.00 hours; (E) *ad lib.* fed and analysed at 11.30–13.00 hours.

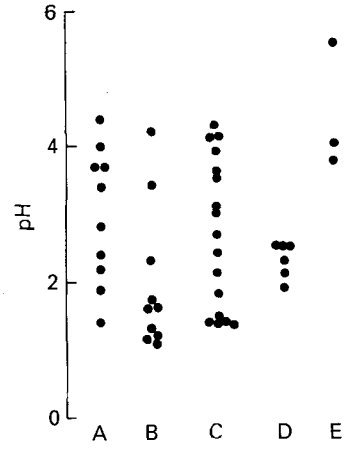

Fig. 2. pH of mouse stomach contents. Mice were (A) *ad lib.* fed till 09.00 hours, analysed between 09.00 and 10.30 hours; (B) fasted from 09.00 hours, analysed between 13.30 and 14.30 hours; (C) *ad lib.* fed in a hypobaric chamber at a pressure of 50·7 kPa for 3 d, analysed between 09.00 and 10.30 hours; (D) fasted for 16 h before analysis at 09.00 hours; (E) *ad lib.* fed and analysed at 11.30–13.00 hours.

shown in Fig. 2. A wide range of pH values was observed with no significant difference between 0–1·5 h fasted, 3·5–4·5 h fasted and hypoxic mice. There was a positive correlation between stomach pH and the quantity of solids found in the stomach (Figs. 1 and 2). This correlation was also evident when all normal and hypoxic stomach pH and solid content values were compared by linear regression (r 0·673, n 23, $P < 0·001$). This may be presumed to reflect the tendency of food to neutralize the stomach acid. The observation that 3·5–4·5 h

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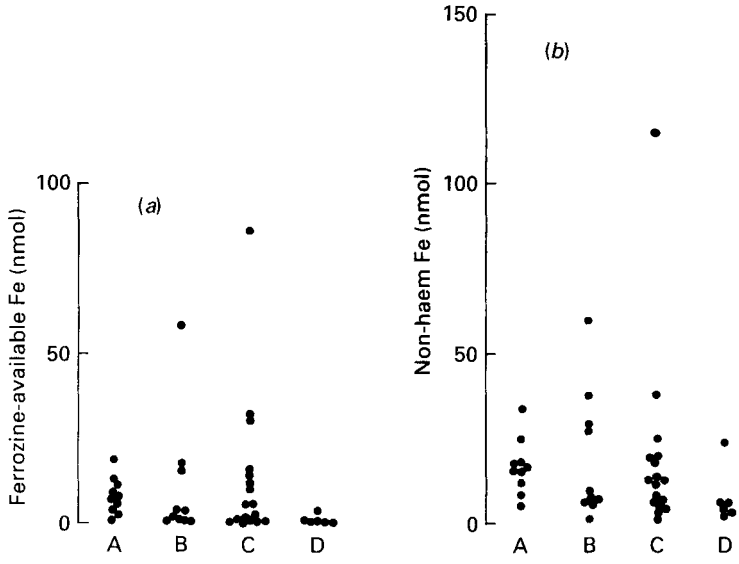

Fig. 3. (*a*) Rapidly ferrozine-available and (*b*) non-haem-iron levels (nmol) in soluble fraction of mouse stomach contents. Ferrozine-available Fe is Fe reacting within 5 s with 0·8 mM-ferrozine solution. Mice were (A) *ad lib.* fed till 09.00 hours, analysed between 09.00 and 10.30 hours; (B) fasted from 09.00 hours, analysed between 13.30 and 14.30 hours; (C) *ad lib.* fed in a hypobaric chamber at a pressure of 50·7 kPa for 3 d, analysed between 09.00 and 10.30 hours; (D) fasted for 16 h before analysis at 09.00 hours.

fasting does not greatly reduce solid content or pH suggests that the correlation is not a consequence of the time elapsed since feeding.

Small intestinal pH, measured at the mucosal surface, was found to be much more constant, namely: duodenum pH 6·46 (SE 0·03), *n* 16; jejunum pH 6·57 (SE 0·08), *n* 16; ileum pH 7·11 (SE 0·09), *n* 6. These values resemble those reported for the rat (Hungerford & Linder, 1983).

Stomach Fe

Total stomach Fe was found to vary greatly from one animal to another but no significant difference between the groups of mice was observed (Fig. 3). The majority of Fe was insoluble (median 8·9% soluble, interquartile limits 5·4–18·1%, *n* 37). The quantity of insoluble Fe, relative to the total solids in the stomach, was found to show a positive correlation (r 0·408, $P < 0·01$, *n* 37) with the stomach pH, as would be expected if lower pH values show a tendency to solubilize Fe. Soluble total Fe was significantly lower in 16 h starved mice compared with fed animals. There was no significant difference between soluble Fe in hypoxic and 4·5 h starved mice.

Fe rapidly available to the Fe(II) chelator ferrozine was observed in most stomachs (Fig. 3). In only four of thirty-seven stomachs was negligible ferrozine-available Fe present. In four of thirty-seven stomachs there was significant additional Fe available on addition of ascorbate (2 mM). Ferrozine-available Fe represented a variable percentage of soluble non-haem-Fe (3-100, mean 49·3 (SE 4·7), *n* 37). No significant differences in ferrozine-available Fe or soluble non-haem-Fe were detectable between the three groups 0–1·5 h fasted, 3·5–4·5 h fasted and hypoxic.

Duodenal ferrozine-available Fe

Ferrozine-availability of Fe in mouse duodenum was investigated both by direct analysis of duodenal contents and by in vitro neutralization of stomach contents (Table 1). In seven

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Table 1. *Rapidly ferrozine-available iron in mouse duodenal contents and neutralized stomach contents*

Mouse no.	Stomach rapidly ferrozine-available Fe (nmol/mg)	Rapidly ferrozine-available Fe in neutralized stomach contents (nmol/mg)	Duodenal rapidly ferrozine-available Fe (nmol/mg)
1	47	0	0
2	27	0	0·3
3	27	17	0·3
4	35	16	0·6
5	9	0	0·3
6	38	17	0·3
7	49	23	0·3
8	15	ND	0
9	11	ND	0
10	4	ND	0·1
11	113	ND	0·23
12	161	ND	0·04
13	ND	ND	0·27
14	ND	ND	0
15	250	40	ND
16	38	0	ND
17	20	0	ND

ND, not determined.

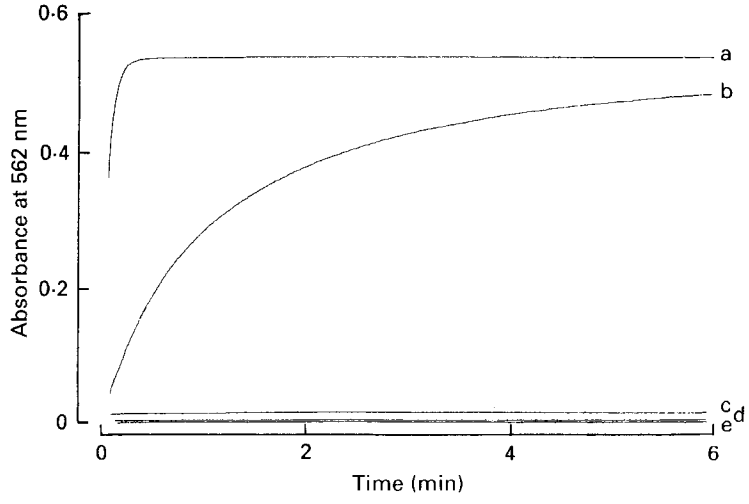

Fig. 4. Reaction of different iron complexes with ferrozine/ascorbate mixture. Fe–ligand solutions were mixed with 200 μM-ferrozine, 2 mM-sodium ascorbate at zero time and absorbance at 562 nm recorded. Temperature was 21° and Fe concentration 20 μM. (a) Fe–nitrilotriacetate (NTA) (1:2), pH 4·2; (b) Fe–NTA (1:2), pH 7·3; (c) Fe–EDTA (1:2), pH 4·9; (d) Fe–EDTA (1:2), pH 7·4; (e) no Fe blank. Low-pH mixtures were obtained by mixing equal volumes of ferric chloride in hydrochloric acid with trisodium salts of EDTA or NTA. Neutralized samples were obtained from acid solutions by adding small volumes of 0·5 M-sodium bicarbonate.

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mice, both approaches were adopted, six of them demonstrating detectable ferrozine-available Fe in the duodenum and four in the neutralized stomach contents. Three of these latter four showed further, slowly-available Fe. In all, ten of fourteen mice showed detectable ferrozine-available Fe in duodenal contents and five of ten showed such Fe in in vitro neutralized stomach contents. The quantities of duodenal lumen available Fe were close to the detection limits, but nevertheless the consistency of the findings shows that it is a real observation. Further support for the presence of rapidly ferrozine-available Fe in the duodenum comes from the neutralization experiments, although there was invariably some loss of stomach ferrozine-available Fe on neutralization.

Other Fe species in the stomach soluble fraction

Pooled stomach contents from several mice were used to search for other Fe species by e.p.r. Small $g = 4{\cdot}3$ signals (tetrahedral symmetry Fe(III)) were observed in all three samples studied, the signal intensity corresponding to 10–20% on non-haem-Fe present. This signal increased in intensity on in vitro neutralization of stomach contents to approximately 50% of non-haem-Fe present. Addition of ferrozine had no effect on the signal intensity although Fe–ferrozine complex formation was visible. These observations suggest that the soluble, non-ferrozine-available Fe consists of at least two species, one observable as $g = 4{\cdot}3$ Fe(III) and one e.p.r.-silent. Ferrozine-available Fe is also e.p.r.-silent and may, therefore, be assumed to be Fe(II) at the low pH of the stomach. The $g = 4{\cdot}3$ Fe(III) species increases at the expense of the other species on in vitro neutralization of stomach contents.

Analysis of model solutions

Model solutions Fe–NTA (1:2) (weakly chelated), Fe–EDTA (1:2) (strongly chelated), $FeCl_3$, $FeSO_4$ and Fe–ascorbate were investigated for availability of the Fe for rapid chelation by ascorbate/ferrozine. Fe–NTA (1:2) was rapidly available at pH 4·2 (stomach equivalent), but only slowly available (half-life 1 min) at pH 7·3 (Fig. 4). Fe–EDTA (1:2) was essentially unavailable at both pH 4·2 and 7·3 (Fig. 4). Fe–ascorbate has been studied previously (Dorey *et al.* 1987). Briefly the results show that the Fe is completely rapidly available up to pH 6·8. At higher pH the Fe is converted to slowly available forms.

$FeCl_3$ and $FeSO_4$ were found to be completely rapidly available at pH 3·3–4·0. Rapidly available Fe disappeared almost instantly from $FeCl_3$ on neutralization with bicarbonate, but slowly available Fe persisted for at least 25 min at 37°. The rate of availability of the slowly available Fe species declined over several minutes, the decline being slower at pH 6·5 than at pH 7·3. $FeSO_4$ demonstrated rapidly available Fe for only slightly longer than $FeCl_3$ after neutralization, with 15% of Fe still rapidly available after 70 s at 37°, irrespective of whether ascorbate was added with the ferrozine. This demonstrates that Fe(II) persists, albeit briefly, on neutralization of Fe(II) solutions in the presence of air. Slowly available Fe persisted in a much less time-dependent form than is observed with neutralized $FeCl_3$, suggesting formation of different species than those formed from $FeCl_3$ and $NaHCO_3$. When $FeSO_4$ was neutralized with a $NaHCO_3$–sodium ascorbate mixture (0·5 M and 1 mM respectively) to pH 6·5, relatively stable, rapidly chelatable forms of Fe were present.

Investigation of the availability of Fe in Fe–NTA–Hepes–NaCl–mannitol mixtures, as used in studies of brush-border-membrane vesicle and non-esterified fatty acid-mediated Fe transport (Simpson & Peters, 1984; Simpson *et al.* 1988), showed that the availability of Fe for chelation, as measured by initial rates of ferrozine-Fe(II) formation after addition of ascorbate plus ferrozine, exactly parallels non-esterified fatty acid-mediated Fe uptake by vesicles (Fig. 5). In particular, the effects of varying the NTA:Fe ratio, Fe–NTA (1:2)

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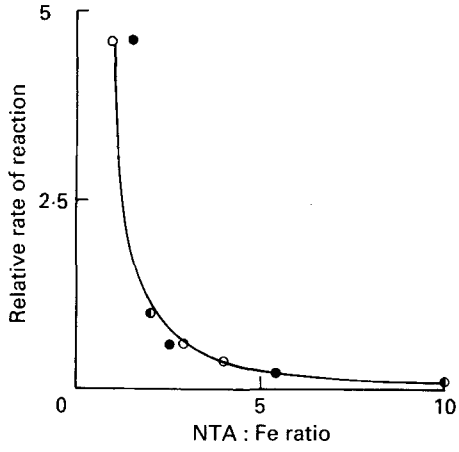

Fig. 5. Effect of nitrilotriacetate (NTA):iron ratio on rate of reaction with ferrozine/ascorbate mixture. Fe–NTA mixtures were prepared and incubated for 5 min at 21° (90 μM-Fe(III), 0·1 M-sodium chloride, 0·1 M-mannitol, 20 mM-Hepes, pH 7·4, various NTA concentrations (for details, see Simpson & Peters, 1984), then 200 μM (final)-ferrozine and 2 mM-sodium ascorbate added at zero time and absorbance at 562 nm recorded. Relative initial rates of reaction were determined and are plotted against NTA:Fe ratio (○). Also shown is the effect of a similar variation in NTA:Fe ratio on brush-border-membrane vesicle ^{59}Fe(III) uptake from similar solutions (●) (from Simpson & Peters, 1984).

concentration, and adding Ca(II) (not shown) all showed a correlation between availability of Fe for chelation by ferrozine/ascorbate and availability of Fe for non-esterified fatty acid-mediated transport. This correlation was further emphasized by the higher availability and transport seen with fresh Fe–ascorbate solutions compared with Fe–NTA solutions (Simpson *et al.* 1988).

DISCUSSION

The findings provide information about the concentrations and chemical forms of Fe present in mouse stomach and duodenal lumen during digestion of a commercial rodent diet capable of supporting normal mouse growth. It is clear that in mice, fasting for at least 16 h is necessary to largely clear the stomach of endogenous Fe. Our previous studies of mechanisms of mucosal Fe uptake have generally employed fed mice and one proposed mechanism of uptake, namely non-esterified fatty acid-mediated brush-border-membrane Fe transport (Simpson *et al.* 1988), may be sensitive to fasting (Simpson *et al.* 1986); others have also shown that fasting or semi-starvation can affect Fe absorption (Hopping & Ruliffson, 1963; Conrad *et al.* 1967; Forth & Schafer, 1987). However, the effects of fasting on the dietary constituents were not easy to exclude in such studies.

Many workers have injected or infused acid (i.e. pH 2) solutions into duodenal or jejunal lumen as a model for orally administered Fe leaving the stomach. Our studies show that in mice, the stomach pH is relatively high when large amounts of diet are present and thus, during normal dietary absorption after feeding, a higher pH (3–5) is more appropriate. Similar effects of food on stomach pH have been shown in man (James & Pickering, 1949).

Over the past 30 years many studies of Fe absorption have been performed and a wide range of Fe concentrations have been selected for these studies, e.g. Stremmel *et al.* (1987) used concentrations below 1 μM; Muir *et al.* (1984), Eastham *et al.* (1977) and Marx & Aisen (1981) favoured 1–20 μM; Cox & O'Donnell (1981), Simpson & Peters (1984, 1985), Cox & Peters (1979), Forth & Rummel (1973), Manis & Schachter (1964), Ruliffson &

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Hopping (1963), Huebers *et al.* (1974), Johnson *et al.* (1983), Hungerford & Linder (1983), Becker *et al.* (1979) and Berner *et al.* (1986) have favoured 20–500 μM; while still higher or more widely variable concentrations have been chosen by many (Greenberger *et al.* 1967; Helbock & Saltman, 1967; Pearson *et al.* 1967; Thomson & Valberg, 1971; Terato *et al.* 1973; Savin & Cook, 1980; Barton *et al.* 1983). The reasoning behind the choice of Fe concentrations may also be variable.

Thus, most rodent diets contain total non-haem-Fe levels equivalent to stomach concentrations in the range 1–10 mM, but the presence of saturable uptake in the range 50–500 μM has suggested this as the physiological range of concentrations available for absorption. In vitro studies have favoured lower Fe concentrations because of the danger of tissue damage by the Fe. Ignorance of the molecular mechanism of Fe uptake, has limited chemical investigation of possible available Fe in the intestinal lumen, to a variety of in vitro studies of Fe release to Fe(II) chelators or studies of Fe dialysability, and to studies of Fe solubility or dialysability after mixture with gastric juice or in jejunal aspirates.

Extensive studies of the reaction of Fe salts with human gastric juice (Davis *et al.* 1967; Wynter & Williams, 1968; Webb *et al.* 1973) have not provided information on the availability of the chemical forms studied, simply that a major mucoprotein component of gastric juice can combine with and solubilize Fe after neutralization. Analysis of dietary Fe in jejunal aspirates from humans has been attempted (Glover & Jacobs, 1971), but only crude molecular weight estimates of Fe species present were made. These techniques provide no information about transient or unstable species likely to be present during the rapid transit and neutralization of stomach contents in the duodenal lumen, nor do they provide information on the availability of the observed Fe species for uptake by the duodenal mucosa.

Release of Fe to chelators in vitro has been studied after varying periods of incubation with α,α-bipyridyl or tripyridyltriazine after addition of reducing agents and sometimes strong acid (Kohler *et al.* 1936; Shackleton & McCance, 1936; Sanford, 1960; Jacobs & Greenman, 1969; Narasinga Rao & Prabhavathi, 1978; Lock & Bender, 1980). The term ionizable Fe has frequently been used to describe the chelatable Fe. These early studies do not, however, provide information on the presence of Fe suitable for absorption by non-esterified fatty acid-mediated mechanisms in the duodenum. Ferrozine was recently used by Reddy *et al.* (1986) to assess Fe forms present in in vitro digests of one particular diet, namely pinto beans (*Phaseolus vulgaris*). They found that the diet did not have sufficient reducing power to produce physiologically significant Fe(II) concentrations, unlike the rodent diet employed by us, which clearly does contain significant Fe(II), at least in the low pH of the stomach.

The findings further show that rapidly chelatable Fe persists in the duodenum, the main site of Fe absorption (Wheby & Crosby, 1963) and that in vitro neutralized stomach contents can also contain, at least transiently, such Fe. This rapidly chelatable Fe is also available for absorption by non-esterified fatty acid-mediated brush-border-membrane Fe transport (Simpson & Peters, 1987), suggesting that this proposed mechanism of Fe uptake can play a part in normal uptake of dietary Fe by mice. Our findings also suggest that the physiological concentration of Fe available for absorption in mouse duodenum is in the range 10–100 μM.

Hypoxia provides a means of increasing Fe absorption in mice without dietary manipulation, thus the contribution of mucosal regulation to control of Fe absorption can be studied without the complicating effect of changes in diet composition. No difference in chemically available forms of stomach Fe was detected in stomachs of hypoxic mice, which show enhanced Fe absorption (Simpson & Peters, 1986). This suggests that an increased

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capability of the hypoxic mouse mucosa to take up available forms of dietary Fe can explain enhanced Fe absorption in these mice, and that no change in availability of dietary Fe occurs in the hypoxic mouse stomach.

The present findings provide identification and quantification of Fe species, present during dietary digestion and absorption, which can be related to Fe complexes used in model studies of Fe uptake by duodenal mucosa. These species include transient forms of Fe which are available for uptake by a proposed, non-esterified fatty acid-mediated, mucosal uptake mechanism, suggesting that this mechanism can operate in dietary Fe absorption.

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