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Evidence for regulatory control of iron uptake from ferric maltol across the small intestine of the rat

[1]Margery A. Barrand & Brian A. Callingham

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ

1 ^{59}Fe absorption from the novel iron compound, ferric maltol, was studied in rats pretreated twice daily for two weeks with non-radioactive ferric maltol in oral doses containing 7 mg elemental iron. Tissue accumulation of ^{59}Fe 2 h after administration of radioactive ferric maltol into the stomach was significantly lower in iron pretreated animals than in saline-treated controls.

2 ^{59}Fe uptake from ferric maltol into isolated fragments of ileum and of duodenum was of similar magnitude in control animals but in iron-treated animals, duodenal uptake was significantly lower than that of the ileum.

3 Absorption of ^{59}Fe was also investigated in anaesthetized rats after intestinal perfusion with saline (controls) or with 5 mM chenodeoxycholate to render the intestines more permeable.

4 Changes in permeability of the small intestine were monitored by estimating the amount of [^{14}C]-mannitol absorbed and fluid secreted with reference to the non-absorbable [^{3}H]-inulin in the perfusate effluents.

5 Despite the increased permeability of the intestines after bile salt treatment, there was little difference from control in the tissue accumulation of ^{59}Fe from ferric maltol 2 h after intraduodenal administration. However ^{59}Fe absorption from ferrous sulphate was significantly increased after bile salt treatment.

6 Gel filtration profiles of plasma made 5 and 60 min after intraduodenal administration of [^{59}Fe]-ferric [^{3}H]-maltol demonstrated that metal and ligand do not enter the circulation as the complex even when intestinal permeability is increased.

7 Uptake of ^{59}Fe was investigated in isolated fragments of rat small intestine after saline or bile salt perfusion. Although ^{59}Fe uptake from ferric maltol was somewhat greater in the bile salt-treated intestinal fragments, saturable kinetics were still observed. By contrast, ^{59}Fe uptake from ferrous sulphate: ascorbate was greatly enhanced by bile salt pretreatment and a very large diffusional component of uptake was evident.

8 It is concluded that iron uptake from ferric maltol may well be under endogenous regulatory control even in damaged intestines, so it is unlikely that this novel iron compound can bring about iron overload when administered orally.

Introduction

Since few regulatory mechanisms exist for eliminating body iron, normal control of iron status is exerted primarily at the point of intake i.e., at the intestinal mucosa. However, it is still not clearly understood how intestinal absorption of non-haem iron, which constitutes the bulk of dietary iron, is controlled. Three key steps i.e., uptake from the gut lumen, retention in the mucosal storage pool and transfer to the plasma appear to be involved in intestinal iron absorption. Should any one of these steps be by-passed or be abnormal, then changes in iron status, particularly iron overload, may result, as for example in haemochromatosis, where altered serosal iron transfer has been identified (McClaren *et al.*, 1988).

A novel iron compound, ferric maltol (3-hydroxy-2-methyl-4-pyrone), has been developed as a more palatable and less irritant alternative to ferrous compounds in the treatment of iron deficiency (Hider *et al.*, 1984). Since the ferric iron is bound with high affinity to maltol forming an uncharged complex with an oil:water partition coefficient sufficiently high (0.5) to allow diffusion across lipid membranes, it was originally supposed that ferric maltol might diffuse across the intestinal wall and donate its ferric iron directly to transferrin in the plasma. If this were the case, then danger of iron overload would be possible. However, saturable uptake kinetics for iron entry from ferric maltol have been observed in intact tissues both *in vivo* and *in vitro* (Barrand *et al.*, 1987; Levey *et al.*, 1988). Furthermore, reduction of the ferric iron to the ferrous state with consequent dissociation of metal and ligand probably take place immediately prior to iron absorption (Barrand *et al.*, 1990). The iron must thus be donated to the endogenous uptake carrier and thereby remains under regulatory control.

The present work provides further evidence that iron from ferric maltol may enter the normal regulatory uptake pathways in the intestine so that under iron replete conditions or when the intestine is damaged, excessive uptake of metal will not occur.

Methods

Absorption and distribution of ^{59}Fe in whole animals

Male Wistar rats, 150–200 g in weight, were given, by gavage twice daily for two weeks, ferric maltol in 500 μl of saline. Control animals received saline alone. In initial experiments, a further control group of animals received a saturated solution of maltol alone. Iron solutions for oral dosing were prepared just before use by mixing solid $FeCl_3$ and maltol powder at a molar ratio of 1:4 in sufficient saline to provide 7 mg elemental iron in each 500 μl dose. The final dose given contained 5 μCi of ^{59}Fe. The animals were killed by cervical dislocation 2 h later. Samples were taken of blood and liver together with urine, kidneys, femurs, spleen and the unabsorbed contents and washed segments of small intestine and their content of ^{59}Fe measured in a Packard Auto Gamma 500 counter. It has already been established that the ^{59}Fe content of the femurs provides a consistent index i.e. 1/12th of ^{59}Fe present in the total bone marrow (Barrand *et al.*, 1987).

[1] Author for correspondence at present address: MRC Clinical Oncology and Radiotherapeutics Unit, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH.

In initial experiments, the small intestines from animals after dosing for two weeks with ferric maltol or with saline were examined for overt signs of structural damage and biochemical abnormalities. Portions of duodenum were fixed overnight in 4% glutaraldehyde and processed for electron microscopy. Samples of small intestine were homogenised in 1 mM potassium phosphate buffer at pH 7.8 and assayed for monoamine oxidase activity by a radiochemical method (Callingham & Laverty, 1973) using [^{3}H]-5-hydroxytryptamine as substrate. Other samples of small intestine were homogenized in 2 mM HEPES buffer at pH 7.4 containing 50 mM mannitol and assayed for sucrase activity by a spectrophotometric method (Dahlqvist, 1968). Glucose uptake in the small intestine was assessed by the method described below for ^{59}Fe uptake into isolated fragments with 2-deoxy-D-[2,6-^{3}H]-glucose as substrate and [^{14}C]-mannitol as a marker of extracellular fluid volume.

For ^{59}Fe uptake studies in anaesthetized animals following intestinal perfusion (see below), the intestines were first washed through with saline alone (0.9% NaCl w/v), tied at each end and 500 μl volumes of the iron solutions (either ferric maltol prepared as above or $FeSO_4$ containing 5 μCi ^{59}Fe and 7 mg element iron) injected into the duodenum close to the pyloric end. The animals were maintained under anaesthesia for a further 2 h and then killed by anaesthetic overdose before being dissected as outlined above.

Perfusion of rat small intestine

Male Wistar rats, 150–350 g body weight, fasted for 18 h before use were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). The abdomen was opened by a midline incision and two cannulae (i.d. 3 mm) inserted into the small intestine, with inflow near the pylorus and outflow approximately 30 cm distal to this. The cannulae were tied in place avoiding obstruction of blood flow and the inflow connected to a peristaltic pump delivering warmed perfusion fluid at a rate of 0.6 ml min^{-1}. The intestines were flushed out initially with saline alone and then perfused for 30–45 min with saline containing 10 mM glucose, [^{14}C]-mannitol at 0.002 μCi ml^{-1} and [^{3}H]-inulin at 0.01 μCi ml^{-1} to obtain baseline levels and then for a further 45 min with the above perfusion mixture with or without 5 mM chenodeoxycholate. Each 5 min effluent was collected and measured both for volume and also for ^{14}C and ^{3}H content by liquid scintillation counting in a Packard 2000CA spectrometer with automatic external standardization. Fluid secretion and mannitol absorption relative to inulin was calculated per cm of small intestine using the amount of [^{3}H]-inulin as the nonabsorbable marker. For morphological evidence of tissue damage, samples of intestine were taken after perfusion, fixed overnight in 4% glutaraldehyde and processed for examination by electron microscopy.

Sampling and gel filtration of plasma

Blood samples were obtained via carotid cannulae from anaesthetized rats 5 and 60 min after intraduodenal administration of [^{59}Fe]-ferric [^{3}H]-maltol centrifuged at 6000 *g* for 2 min at 20°C and the plasma passed down 10 ml columns (PD-10) of Sephadex G-25 (Pharmacia, Uppsala, Sweden) previously equilibrated with 20 mM HEPES, pH 7.4 in 154 mM NaCl and eluted in 1 ml fractions. Each fraction was analysed for ^{59}Fe and for tritium content by liquid scintillation spectrometry.

^{59}Fe uptake into isolated fragments of small intestine

This was carried out as described previously (Levey *et al.*, 1988) with tissue obtained both from rats dosed for two weeks with saline or with ferric maltol and from rats after intestinal perfusion. Briefly, intestinal fragments 30–50 mg in weight were immersed in buffer (composition, in mM, HEPES 16, NaCl 125, KCl 3.5, $CaCl_2$ 1, $MgSO_4$ 10, D-glucose 10, and pH 7.4) and incubated for 10 min at 37°C with the appropriate ^{59}Fe solutions containing also [^{3}H]-inulin (0.01 μC ml^{-1}) as extracellular fluid marker. Iron solutions for these *in vitro* studies were prepared by mixing $FeCl_3$ and maltol in 16 mM HEPES buffer at pH 7.4 at a ratio of 1:4 or by mixing $FeSO_4$ with ascorbic acid at a ratio of 1:5, $^{59}FeCl_3$ being added so as to achieve a final concentration of ^{59}Fe in the incubation media of 0.1–0.25 μCi ml^{-1}. Fragments, removed at intervals and digested in Soluene-350 (Packard, Reading, UK), were analysed for their ^{59}Fe and tritium content.

The amount of ^{59}Fe accumulated was calculated, after correction for extracellular fluid volume and wet weight of each tissue fragment, as a distribution ratio (tissue/medium) and subsequently converted to pmol min^{-1} mg^{-1} wet weight of tissue. Since ^{59}Fe uptake appears to be linear over 10 min (Levey *et al.*, 1988), initial rates of iron uptake may be estimated from the data.

Materials

D-[1-^{14}C]mannitol, 2-deoxy-D-[2,6-^{3}H]glucose, 5-hydroxy-[G-^{3}H] tryptamine creatinine sulphate, [^{3}H]inulin and $^{59}FeCl_3$ were purchased from Amersham International (Bucks, UK) and the pyrone, maltol (Veltol) obtained from Pfizer Ltd (Sandwich, Kent, UK). [^{3}H]-maltol was synthesized as described in Barrand *et al.* (1991). All other chemicals either were bought from Sigma Chemical Co Ltd (Poole, Dorset, UK) or were standard laboratory reagents of analytical grade.

Statistical methods

Statistical significance was determined by Student's *t* test (unpaired) comparing each group with its respective control.

Results

Ultrastructure, enzyme activities and glucose uptake capability of rat small intestine following two weeks treatment with oral ferric maltol

No gross differences in morphology of intestinal epithelial cells were observed between rats treated for two weeks with saline and with ferric maltol. There were no obvious signs of damage to the mitochondria or to the microvilli along the brush border of the epithelium, nor were there any gaps between the cells suggesting loss of intercellular contact (Figure 1). Activities of the outer mitochondrial membrane enzyme, monoamine oxidase, and of the brush border enzyme, sucrase, were similar in homogenates taken from the two groups of animals (monoamine oxidase activities of 68.2 ± 4.14 in the ferric maltol treated group compared with 72.8 ± 4.5 nmol product mg^{-1} protein h^{-1} in control tissue, $n = 6$ and sucrase activities of 36.2 ± 0.8 in the ferric maltol treated group compared with 34.0 ± 3.2 μg product mg^{-1} protein h^{-1} in control tissue, $n = 6$). Rates of initial uptake of [^{3}H]-deoxyglucose into isolated fragments of small intestine taken from each of the two groups of rats were similar (8.4 ± 1.1 in the ferric maltol treated group compared with 6.8 ± 1.1 pmol min^{-1} mg^{-1} wet tissue in the control group, $n = 12$).

^{59}Fe absorption following two weeks of oral ferric maltol

The amount of ^{59}Fe present in the blood and the accumulation of ^{59}Fe in liver, bone marrow, kidneys, spleen and urine was measured 2 h after stomach administration of [^{59}Fe]-ferric maltol at a dose containing 7 mg elemental iron. Previous studies (Bothwell *et al.*, 1979; Barrand *et al.*, 1987) have shown that the liver and bone marrow constitute the major sites of ^{59}Fe accumulation. Levels of ^{59}Fe significantly lower than control were found in the animals that had been

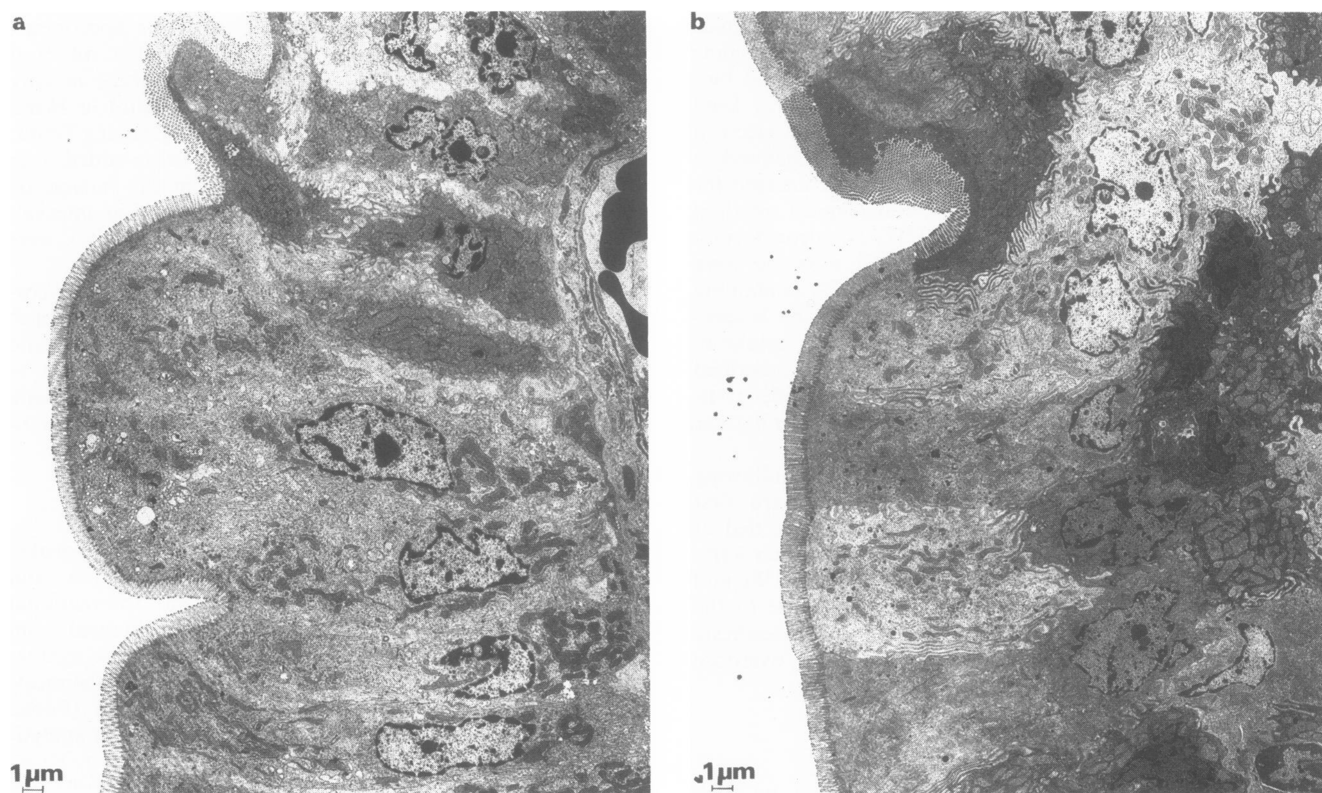

Figure 1 Electron micrographs of mucosal epithelium from the proximal part of the small intestine taken from rats treated orally for two weeks with twice daily doses of (a) ferric maltol (each dose containing 7 mg elemental iron) or (b) saline.

pretreated twice daily for two weeks with oral doses of ferric maltol containing 7 mg elemental iron before administration of the ^{59}Fe maltol (Table 1). In these experiments, the control animals received saline alone.

In initial experiments, control animals received either saline alone or maltol alone. It was found to be impossible, in the absence of iron, to dissolve maltol in amounts equivalent to those present in the ferric maltol doses. Animals therefore received oral doses of a saturated solution of maltol. This resulted in looseness of the stools, a feature not observed when ferric maltol was used for oral dosing. Free maltol is unlikely to be present within the gut lumen in any significant amounts when ferric maltol is administered. Both iron and maltol appear to be absorbed rapidly after dissociation in the gut lumen (Barrand *et al.*, 1991) and if not absorbed, ferric maltol remains as the complex which can be visualised by its deep red colour. Control experiments tracing the passage of the orally administered ferric maltol showed that it remained in the undissociated form throughout the small intestine. Since maltol alone has very different biochemical and physical properties from the ferric maltol complex, it did not appear to provide a very satisfactory control. In all subsequent experiments, only controls receiving saline alone were used.

Table 1 Tissue accumulation of ^{59}Fe 2 h after gavage with [^{59}Fe]-ferric maltol dose

	Ferric maltol treated	*Controls*
Marrow	23.1 ± 1.4**	30.1 ± 1.5
Liver	53.2 ± 1.5*	156.1 ± 35.0
Blood	27.09 ± 2.8*	33.46 ± 2.1
Spleen	2.73 ± 0.63	3.78 ± 0.56
Kidney	1.4 ± 0.14*	3.15 ± 0.7
Urine	0.42 ± 0.14	0.42 ± 0.07

[^{59}Fe]-ferric maltol, containing 7 mg of elemental iron was administered to rats, previously treated, twice daily, for two weeks with oral doses of either unlabelled ferric maltol containing 7 mg of elemental iron or saline (controls). Values shown are the mean ± s.e.mean of data from 6 rats in each group and are given as μg of administered ^{59}Fe recovered in each tissue. Significant differences between the groups are shown by use of Student's *t* test (** $P < 0.01$ and * $P < 0.05$).

^{59}Fe uptake into isolated fragments of ileum and duodenum from rats treated for two weeks with oral doses of ferric maltol

In control, saline-treated animals, rates of ^{59}Fe uptake from ferric maltol into isolated fragments of ileum and of duodenum were of similar magnitude (Figure 2b). In the iron-treated animals however, rates of ^{59}Fe uptake were significantly lower in duodenal fragments ($P < 0.01$, Figure 2a). In a previous study on iron deficient animals (Callingham & Barrand, 1987) the converse was observed i.e., rates of iron uptake were significantly higher into duodenal fragments than into ileal fragments. Data from these experiments are shown in Figure 2c for comparison.

Permeability changes in perfused small intestines

Virtually no [^{14}C]-mannitol was absorbed from the intestines during perfusion with saline/glucose alone. However, 5 to 10 min after changing the perfusion medium to one containing 5 mM chenodeoxycholate, [^{14}C]-mannitol absorption increased, levelling off 20 to 30 min later (Figure 3a). Changes in fluid secretion showed a similar time course (Figure 3b). Extensive damage to the intestines was observed histologically (Figure 4) after perfusion with 5 mM chenodeoxycholate. The goblet cells were ruptured with their content of mucus extruded and epithelial cells had been stripped away leaving the villus tips denuded of epithelium. These permeability and histology changes are similar in pattern to those reported by others (Erickson *et al.*, 1987).

^{59}Fe absorption from ferric maltol and ferrous sulphate following intestinal perfusion

Despite the increased permeability of the intestines perfused with the bile salt, there appeared to be little difference between

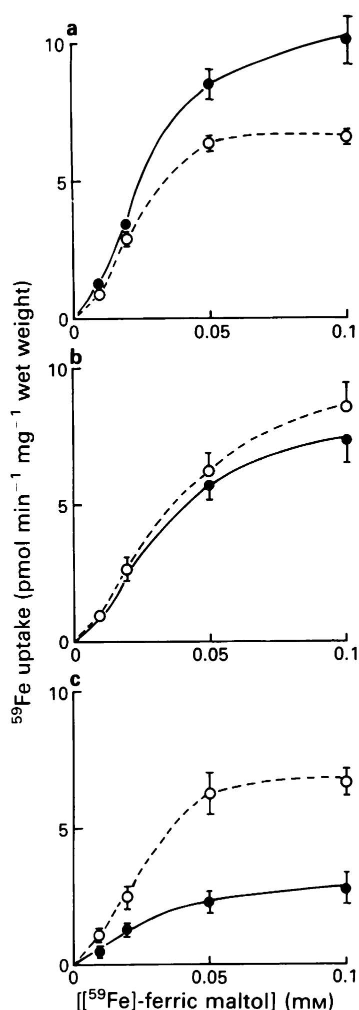

Figure 2 The effect of ferric maltol concentration on the initial rates of uptake of ^{59}Fe into isolated fragments of ileum (●) and of duodenum (○) taken from (a) rats given twice daily oral doses of ferric maltol containing 7 mg of elemental iron for two weeks, (b) saline-treated control rats and (c) rats made iron-deficient by feeding from weaning on a low iron diet. Each value represents the mean of data from 4–6 separate experiments, quadruplicate determinations being obtained from each experiment: vertical bars show s.e.mean. Values are given as pmol of iron min^{-1} mg^{-1} wet weight of tissue. The rates of uptake were significantly different in (a) and (c) ($P < 0.01$) at [^{59}Fe]-maltol concentrations above 0.05 mM.

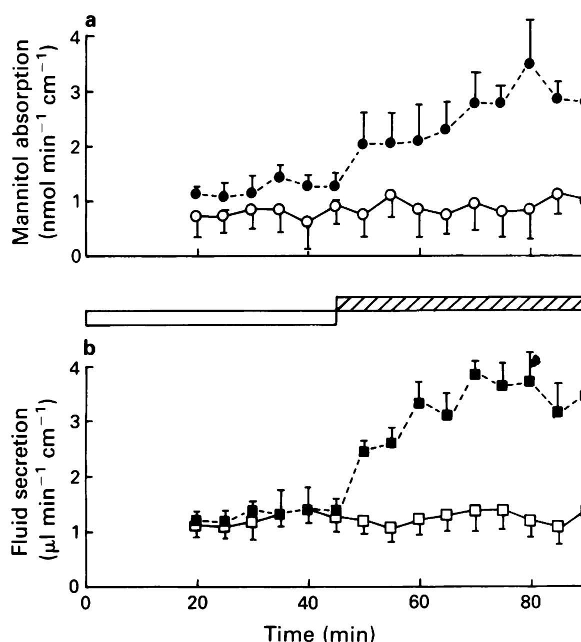

Figure 3 The effects of bile salt perfusion through the intestine of anaesthetized rats on mannitol absorption (a) and fluid secretion (b) in the intestines. Rats were perfused in pairs. For the first 45 min (indicated by the open bar), both rats received saline containing 10 mM glucose. During the second 45 min (indicated by the hatched bar), the experimental rat (closed symbols) was perfused with 5 mM chenodeoxycholate whilst the control rat continued to receive saline/glucose. Each value represents the mean of data taken from 4 separate experiments, i.e., 8 rats; vertical bars show s.e.mean. Duplicate determinations of the effluent composition were taken at each time point for calculation of mannitol absorbed in nmol min^{-1} cm^{-1} lengths of intestine and of fluid secreted in µl min^{-1} cm^{-1} lengths of intestine.

experimental and control animals in the amount of ^{59}Fe accumulated in the tissues 2 h after intraduodenal administration of ferric maltol at a dose containing 7 mg elemental iron (Table 2). When equivalent doses of ferrous sulphate were administered, significant increases in tissue ^{59}Fe accumulation were seen in the bile salt-treated animals. ^{59}Fe absorption from ferrous sulphate was however very much lower than that from ferric maltol. This may be explained on the basis of the lower bioavailability of the ferrous iron. Precipitates of iron could be seen adhering to the mucosal lining at the end of the 2 h exposure. Since the intestines were tied off, movement of iron down the gut was prevented, thus accentuating the differences between ferric maltol which can hold iron in soluble form and ferrous sulphate which rapidly precipitates at neutral pHs.

Disposition of ^{59}Fe and ^{3}H in plasma samples after [^{59}Fe]-ferric [^{3}H]-maltol administration into perfused intestines

Gel filtration studies were carried out on plasma samples taken 5 and 60 min after intraduodenal administration of ^{59}Fe complexed to [^{3}H]-maltol. ^{59}Fe eluted from the gel column at the void volume, a position corresponding to the high molecular weight proteins, whereas ^{3}H was detectable only in the fractions containing smaller molecular weight material (Figure 5). This pattern was the same in plasma taken at 5 and at 60 min, whether or not the intestines had been rendered leaky by bile salt perfusion.

Table 2 Tissue accumulation of ^{59}Fe 2 h after intraduodenal administration of [^{59}Fe]-ferric maltol and [^{59}Fe]-ferrous sulphate

	Ferric maltol		*Ferrous sulphate*	
	Control	*Treated*	*Control*	*Treated*
Marrow	48.3 ± 9.1	65.8 ± 11.2	18.9 ± 2.8	**30.1 ± 3.5
Liver	626 ± 142	702 ± 63.0	78.4 ± 16.1	***274 ± 28.0
Kidney	37.1 ± 10.5	55.3 ± 11.2	2.1 ± 0.7	***14.0 ± 7.0

Either [^{59}Fe]-ferric maltol or [^{59}Fe]-ferrous sulphate containing 7 mg of elemental iron was administered into the duodenum of anaesthetized rats previously perfused for 45 min through the intestines with saline alone (controls) or with 5 mM chenodeoxycholate in saline. Values shown are the mean ± s.e.mean of data from 6 rats in each group and are given as µg of administered ^{59}Fe recovered in each tissue. Statistical analysis by Student's t test shows significance compared with control values as *** $P < 0.001$ and ** $P < 0.01$.

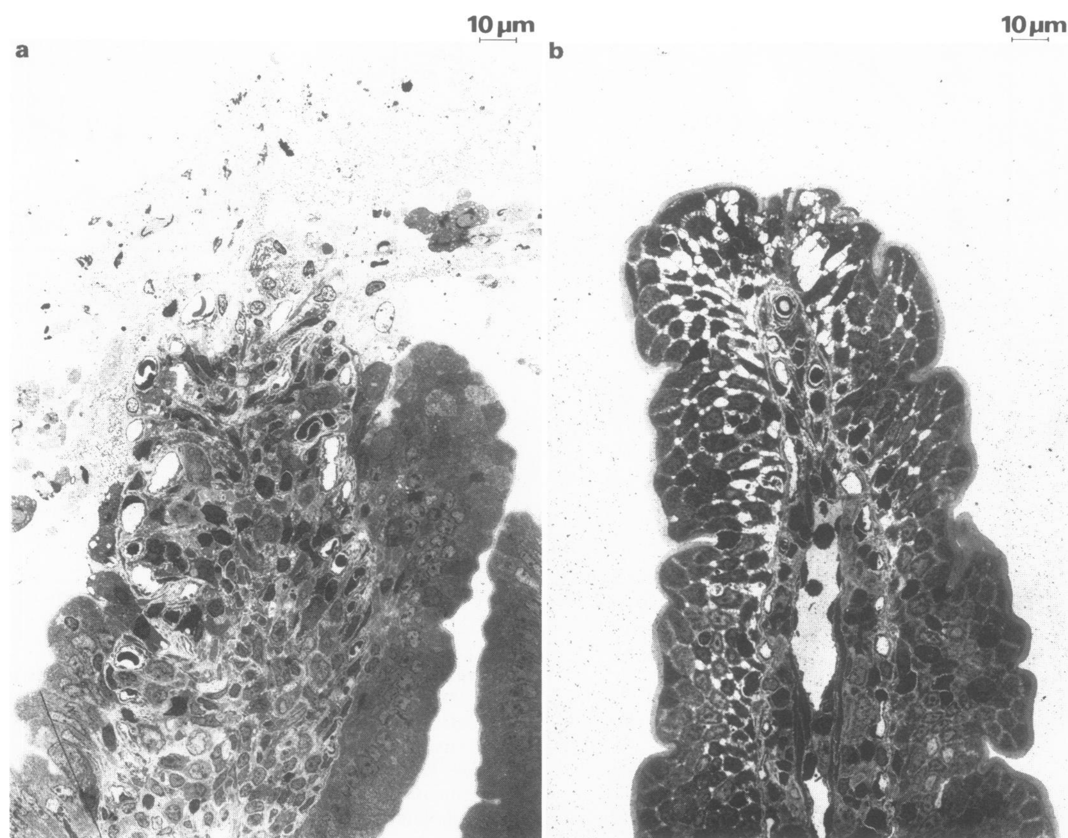

Figure 4 Electron micrographs of mucosal tissue taken from the proximal part of the rat small intestine following perfusion for 45 min with (a) 5 mM chenodeoxycholate in saline or (b) saline alone to demonstrate the extent of damage to the epithelial cells at the tips of the villi caused by the bile salt.

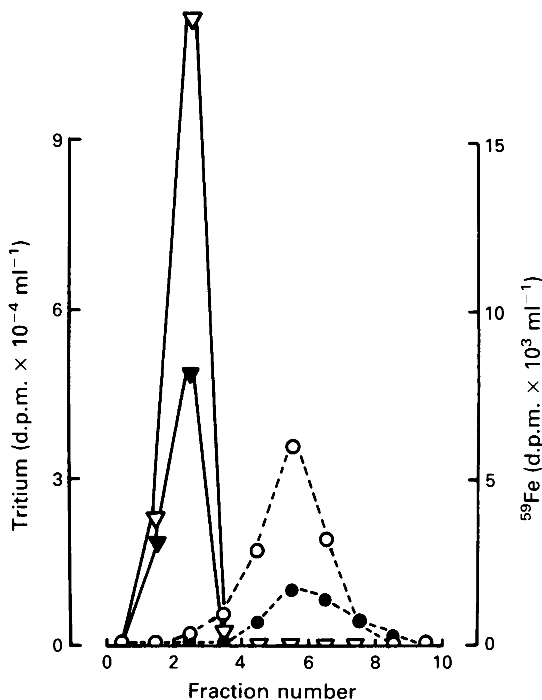

Figure 5 Gel filtration profiles of ^{59}Fe (▼, ▽) and of tritium (●, ○) in plasma samples taken 5 min (closed symbols) and 60 min (open symbols) after intraduodenal administration [^{59}Fe]-ferric [^{3}H]-maltol at a dose of 7 mg elemental iron into anaesthetized rats previously perfused for 45 min with 5 mM chenodeoxycholate in saline/10 mM glucose; 100 μl volumes of plasma were applied to 10 ml columns of Sephadex G-25 and 1 ml fractions eluted. Values are shown as d.p.m. of radioactive material in each fraction. Data presented are from one of the three separate experiments.

^{59}Fe uptake from ferric maltol and from ferrous sulphate/ascorbate into isolated intestinal fragments following perfusion

Rates of ^{59}Fe uptake were increased significantly at all concentrations of iron both from ferric maltol and from ferrous sulphate/ascorbate in fragments taken from bile salt perfused intestines (Figure 6). However, ^{59}Fe uptake from ferric maltol still remained largely saturable with only a small diffusional component being evident. By contrast, ^{59}Fe uptake from ferrous sulphate/ascorbate showed a large diffusional component and this was dramatically enhanced by bile salt treatment.

Discussion

Twice daily oral administration of high doses of ferric maltol to rats over the course of two weeks was sufficient to bring about reduced iron uptake from a single dose of ^{59}Fe but produced no obvious signs, either morphological, biochemical or physiological, of intestinal damage. Each dose used i.e., 7 mg elemental iron would be equivalent to 2 g in a 70 kg human subject. It is well known that iron absorption decreases when body iron stores are raised and increases during periods of iron deficiency and that these alterations are effected by mechanisms within the duodenum (Forth & Rummel, 1973). Indeed even single doses of iron insufficient to alter body iron stores may bring about decreased absorption of subsequent doses (O'Neil-Cutting & Crosby, 1987). The results with ferric maltol are thus entirely in keeping with the idea that ferric maltol donates its iron to normal regulatory uptake pathways in the intestine.

The mechanisms concerned with this regulation are not entirely elucidated but are most likely multifactorial, not only

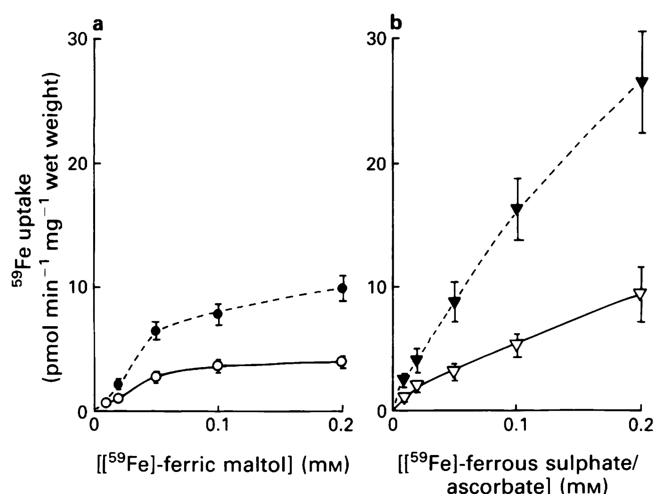

Figure 6 The effect of concentration on the initial rates of uptake of ^{59}Fe from [^{59}Fe]-ferric maltol (a, ●, ○) and from [^{59}Fe]-ferrous sulphate/ascorbate (b, ▼, ▽) into fragments isolated from the proximal part of the small intestine from rats following 45 min perfusion of the intestines with 5 mM chenodeoxycholate in saline (closed symbols) or with saline alone as control (open symbols). Each value represents the mean of data from four separate experiments, quadruplicate determinations being made in each experiment; s.e.mean shown by vertical bars. Values are given as pmol of iron min^{-1} mg^{-1} wet weight of tissue.

alterations in demand e.g. via erythropoeisis modifying iron transfer from serosal surface to plasma but also changes taking place at the luminal surface of the duodenum modifying initial uptake (Muir & Hopfer, 1985; Raja *et al.*, 1988). In the present study, significantly lower rates of iron uptake from ferric maltol were observed in duodenal fragments compared with ileal fragments isolated from animals pretreated for two weeks with ferric maltol. This contrasts with the similar rates of ^{59}Fe uptake observed in duodenal and ileal fragments taken from control animals and with the higher rates of uptake seen in the duodenal fragments isolated from iron-deficient animals. It is probably not valid to compare directly the absolute uptake rates between animals of different iron status since values are given with reference to the wet weight of the tissue and structural changes in the shape and dimensions of the epithelial cells are known to occur with altered dietary regimens (Callingham & Barrand, 1987; Powers *et al.*, 1988).

Differences in intracellular processing of iron within the intestinal mucosa may provide further regulatory control of iron absorption (Topham *et al.*, 1985). Mucosal ferritin levels are known to vary with body iron status and defects here have been identified in hereditary haemochromatosis where iron absorption is inappropriately high (Cook & Skikne, 1987). Previous studies have shown that ^{59}Fe taken up from ferric maltol into intestinal fragments isolated from normal iron-replete animals is largely sequestered by ferritin in the duodenal mucosa but that in fragments isolated from iron-deficient animals the ^{59}Fe from ferric maltol is passed on to mucosal transferrin (Callingham & Barrand, 1987).

From the results obtained so far there is no evidence to suggest that iron taken up from ferric maltol can in any way by-pass the normal regulatory mechanisms of intestinal iron absorption. Even under conditions where the intestines had been rendered more permeable by treatment with chenodeoxycholate, ^{59}Fe absorption from ferric maltol was not greatly enhanced, tissue accumulation of ^{59}Fe being not significantly higher than in control animals. As seen from the ^{59}Fe uptake studies into isolated intestinal fragments, initial rates of ^{59}Fe uptake from ferric maltol were increased after bile salt treatment. Thus factors involved in subsequent transfer of iron to the plasma were still able to control overall iron absorption from ferric maltol. Undissociated ferric maltol was not found in the plasma either 5 min or 60 min after administration even with the more permeable intestines.

Saturable uptake kinetics were still evident with ferric maltol in the intestinal fragments treated with the bile salt. What seemed to account for the increased iron uptake was the appearance of a small diffusional component. This component was present even in normal intestinal fragments during iron uptake from ferrous sulphate/ascorbate and was predominant during uptake into the bile salt-treated intestinal fragments. Several pathways have been suggested for transfer of iron from the intestinal lumen into the mucosa (Dorey *et al.*, 1988; Peters *et al.*, 1988) including a saturable carrier-mediated transfer and an intercellular permeability pathway. It is possible that the diffusional component seen in the present studies represents iron passing between cells. This is more noticeable with ferrous sulphate than with ferric maltol and increases with damage to the intestinal mucosa.

It is concluded that iron uptake and absorption from ferric maltol in the rat is probably under regulatory control. Even where there is intestinal cell damage, and increased intestinal permeability, ferric maltol does not cross the intestinal barrier as the complex. Diffusional entry of iron into the intestinal epithelial cells still appears to be limited and subsequent steps in iron transfer to the plasma still control the overall absorption. It is therefore possible that the use of ferric maltol in human subjects as an oral agent in the treatment of iron deficiency will not provide any greater potential danger of iron overload than with iron preparations currently used in clinical practice.

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